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## DIURNAL FLUCTUATION IN ROOT PRESSURE[1]

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(WITH FOUR FIGURES)

Diurnal cycles in root activity under constant conditions seem to have been first described by HOFMEISTER in 1862 (7). Other investigators have confirmed his findings (1, 11), but in recent years it has been concluded that cycles in pressure and exudation were due to temperature fluctuations (HEYL, 5). On the other hand, studies in this laboratory (9) have substantiated HOFMEISTER'S original results, and WHITE (10) has reported a cycle in exudation of excised tomato roots (though he did not control the conditions under which he was working). Complete proof of the existence of autonomic cycles has recently been obtained by the author with plants grown and tested under controlled conditions (4).

Several theories have been developed to explain the general phenomena of root pressure and exudation. For the purpose of this discussion it is sufficient to point out that osmotic mechanisms have generally been used. SABININ (8) holds the view that a simple osmotic mechanism is sufficient.

The general problem is confused by the controversy over the parts played by the phloem and xylem in translocation and root pressure. Since these measurements extended over periods of several days it seems probable that sieve tubes were plugged (CRAFTS, 3) and that the phloem played little part directly in maintaining the observed pressures. Here it is assumed that the hydrostatic-pressure system involved is the xylem-manometer system.

In these studies, plants (*Helianthus*) were grown in culture solution with forced aeration, in a greenhouse. The solution concentration was low, $\frac{1}{4}$ H— (see explanation later) and typically not renewed, so that the plants were vigorous and healthy at the time of experimentation and were comparable to the "low salt plants" of HOAGLAND and BROYER (6), with root systems capa-

[1] The experimental work on which this paper is based was carried out at the University of California (at Berkeley) through the cooperation of the Department of Botany and the Division of Plant Nutrition, during the summer months of 1937.

ble of very active absorption. On several occasions such root systems maintained pressure for 14 days, indicating that they were quite healthy, had large food stores, and were "starved" only in the technical sense of having a low salt content.

A fairly satisfactory experimental technique has been developed: Plants were grown in two-quart jars fitted with sintered glass aerators. Small bore (1-mm.) u-tube manometers were built so that they might easily be fastened to the side of the jar with heavy rubber bands (fig. 1). The small

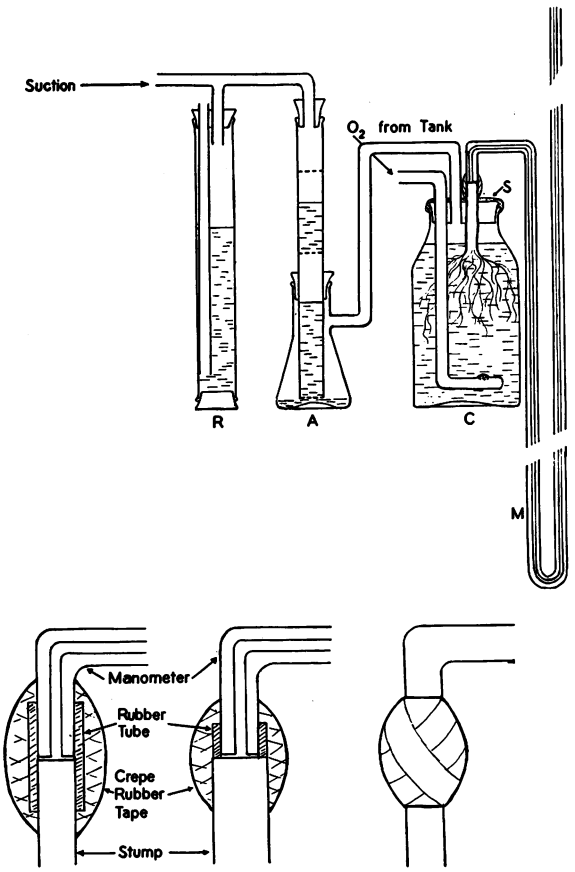


FIG. 1. Apparatus used to measure root pressure and respiration; R, regulator on suction line; A, absorption column; C, culture vessel (in which the plant was grown); S, paraffine seal; M, small bore mercury manometer.

bore and double action of the u-type manometer gave a convenient method of measuring pressure changes with only small change in volume of liquid in the xylem-manometer system (0.004 cc./cm. Hg). Manometers were attached to squarely cut stumps with the aid of rubber tubing and crepe rubber tape

("Sterilastic tape" carried by most drug stores). Such attachments could be made to withstand more than two atmospheres of pressure and resulted in a minimum of crushing or injury to stems. Except as designated, all solutions were balanced nutrient solutions, HOAGLAND'S solution ($KH_2PO_4$ 0.001M; $MgSO_4$ 0.002M; $KNO_3$ 0.005M; Ca $(NO_3)_2$ 0.005M) served as a base. Concentrations are expressed as $\frac{1}{4}$ H, 1 H, and 4 H, representing fractions or multiples of the original solution. During the growing period additions of iron and supplementary solution (boron, etc.) were made as needed. Plants grown in a greenhouse were transferred to a thermostatically controlled darkroom, and pressures were recorded at short intervals (usually 3 hours) under constant conditions of light, temperature, and aeration.

One of the most pronounced effects observed was a diurnal fluctuation in pressure which could not be correlated with any change in the environment of the plant during the test period. Careful study of thermograph records showed that there were slight shifts in temperature during extended periods but no diurnal fluctuation could possibly be read into the record for many of the days when root pressures showed such fluctuations.

When it became evident that root pressure did not reach an equilibrium under experimental conditions, considerable effort was made to secure as constant an environment as possible. Tanks of compressed oxygen were used to furnish a uniform supply of oxygen for the aeration of solutions. Artificial light at several intensities was used and remained as constant as commercial electrical service. These improvements did not measurably affect results and it is concluded that these plants were capable of maintaining a diurnal cycle in root pressure which was not directly related to current conditions but possibly controlled by a physiological cycle set up during the growth period in the greenhouse.

## Experimentation

Considerable variability was observed and not all plants showed such fluctuations (of special interest was a group of plants which had symptoms probably resulting from boron deficiency and which did not show a diurnal cycle). Data from three experiments are presented to illustrate some of the typical responses observed:

### EXPERIMENT I

"Pure line" seed obtained from Cornell (S-24–150) was used. Plants were selected twice during their five-weeks growing period, and were quite uniform and vigorous. Setting up of the experiment began at 3 P.M. The solutions were changed to $\frac{1}{2}$ saturated $CaSO_4$ (*i.e.* 0.005M±) and $\frac{1}{2}$ H, manometers attached, and aerators connected (tank $O_2$), by 7 P.M. Absorption towers for $CO_2$ were connected at 8 P.M. thus allowing a full hour for the solutions to come into equilibrium with the gas stream (the rate of gas flow was well above one liter per hour). Pressures were recorded every three

hours and respiration determined for six-hour (later twelve-hour) intervals. Data are presented graphically in figure 2, each curve representing the average

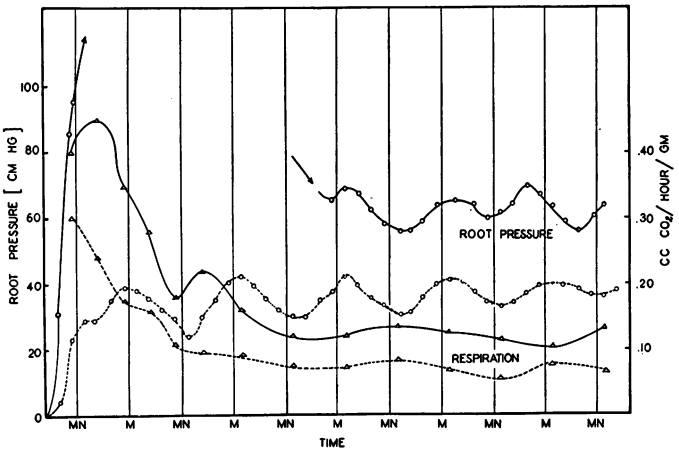


FIG. 2. Root pressure and respiration of 5-week-old sunflower plants. Solid lines represent averages obtained in balanced nutrient solution; broken lines represent averages obtained in dilute solution of $CaSO_4$.

for five plants. Values for individual plants were determined and plotted, but showed the same trends as the averages.

It is apparent from the curves that plants transferred to a fresh dilute, but balanced, nutrient solution respired appreciably more and developed much more root pressure than those transferred to dilute $CaSO_4$. In fact the manometers used did not have sufficient capacity (1 meter Hg) to measure the pressure produced by four of the five plants, and no significant measurement was made for a period of about forty-eight hours while mercury was being forced back into the manometers and allowed to come to equilibrium again. Subsequent readings may have been affected by the loss from the root systems of a considerable volume of exudate immediately following the "blowing out" of the manometers. Even with this treatment a fairly distinct diurnal fluctuation is evident in the root pressure values. Plants in dilute $CaSO_4$ had lower root pressures than those in complete nutrient solution and they maintained a diurnal fluctuation well into the sixth day. In neither case did the plants show a consistent relation between root pressure and respiration. Respiration values for both groups dropped rapidly during the first twenty-four hours and then leveled off with the complete-solution group maintaining a 20 to 50 per cent. higher rate.

## EXPERIMENT II

Groups of three similar plants five weeks old were treated in the following manner, five days before the experiment was set up: The first group

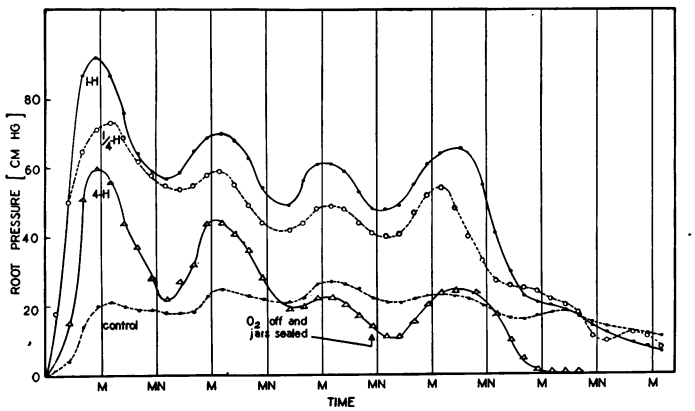


FIG. 3. The effect of concentration of balanced nutrient solution and oxygen supply on root pressure.

was left undisturbed (*i.e.*, the solution contained the unused portion of ¼ H solution in which the plants were grown), while other groups received additional nutrient salts equivalent to ¼ H, 1 H, and 4 H. Those treated with full strength solution showed the highest pressure. The untreated ones gave the lowest and most constant readings while those treated with 4 H developed a fairly high pressure but failed to maintain it. All four groups showed diurnal fluctuations.

After three days the oxygen was turned off (all groups of plants) and the jars sealed with paraffine. The curves (fig. 3) show that root pressure was definitely affected in about one day. By taking values from the previous respiration curve it was calculated that the root systems were using about 25 cc. of oxygen per day. The solution contained about 12 cc. (assuming saturation), and a small space between the solution and cork was filled with oxygen which, however, could move into the solution only very slowly, as there was no stirring, and convection currents would be greatly reduced in a thermostated room. Thus it is indicated that under these conditions *Helianthus* roots may maintain root pressure until the rate of oxygen supply is greatly reduced, but that the pressure falls when oxygen becomes too low or the carbon dioxide partial pressure too high.

## EXPERIMENT III

The root pressure of four groups of three plants each were measured for two days and then additions were made equivalent to ¼ H, 1 H, and 4 H, and readings taken at short intervals (fig. 4). It should be noted that the time scale is greatly increased, and the changes are rapid. In the three cases in which additional salts were used, a sharp decline in pressure was observed; its magnitude, however, was not quantitatively related to the osmotic value of added salts. In less than two hours all pressures were rising rapidly.

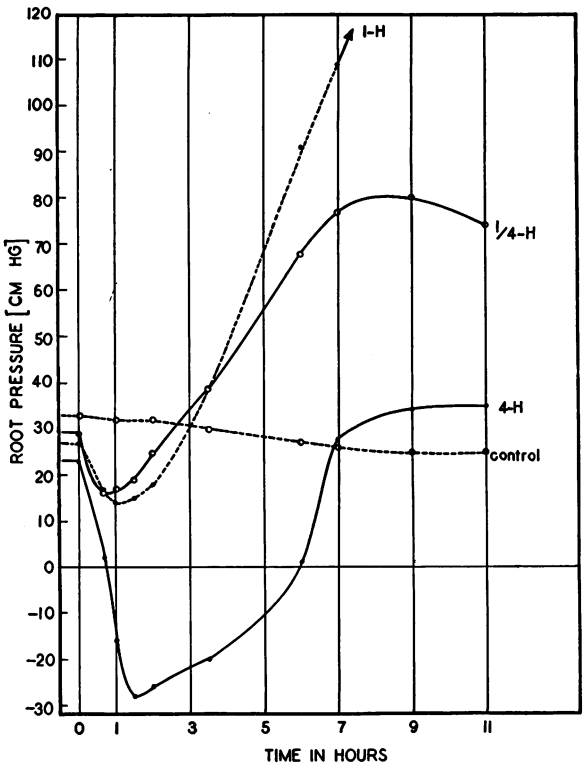


FIG 4. The effect of addition of nutrient salts on root pressure (addition made two days after manometers were attached).

In seven hours all reductions had been completely overcome. Again full strength solution gave maximum response, forcing the mercury out of the manometers. The 1 H solution used has an approximate osmotic value of 0.7 atmosphere. If we assume for convenience that its actual value is 50 cm. of Hg we see that the reduction produced by the $\frac{1}{4}$ H solution was in the same order as that expected as a result of simple osmosis but that the reductions produced by either 1 H or 4 H solution was only $\frac{1}{4}$ of that expected.

## Discussion

An explanation of root pressure in terms of a *simple* osmotic mechanism seems impossible in the presence of a diurnal cycle because: first, it would be necessary to assume that solutes (organic or inorganic) were periodically released into and removed from the sap; second, since the volume of liquid in the xylem-manometer system changes in the same way that the pressure changes, it follows that water must be entering and leaving at the same time solutes do; third, to be effective osmotically, changes in solutes must occur in the same regions in which water enters and leaves.

When electrolytes are added to the external solution (exp. III) there is an apparent osmotic effect (the sudden decrease in pressure). A stimulating effect of the added salts soon overcomes this in the case of active plants and builds the pressure up again. The first response may in reality be simply osmotic. The subsequent rapid rise in pressure must be the result of water entering the xylem system, and if this water enters in response to osmotic force it must be entering while the salts are becoming continuously more concentrated.

From these considerations it seems clear that if we assume a *simple* osmotic mechanism to be responsible for these changes in root pressure we are forced to admit:

(1) that water and solutes must enter and leave the xylem system together, *i.e.*, as a solution.
(2) That such movement cannot be due to osmotic force and hence our original premise is incorrect.

Root pressure, then, is not caused simply by the difference in osmotic values of the xylem sap and the external solution, but must be caused by some other forces in the root system which control and activate this movement. Evidently the mechanism of the diurnal cycle is in some way related to the mechanism responsible for root pressure. Yet, it has long been established that under many conditions there is a relation between root activity and respiration. Several such correlations are apparent in these experiments but the diurnal cycle of root pressure was not shown to be paralleled by a cycle in respiration. If such a cycle exists, its magnitude is probably less than that of root pressure.

Changes in permeability might explain fluctuations in exudation alone (**8**) but will not explain pressure fluctuations in a simple osmotic system since they would affect only the rate of change of pressure and not its equilibrium values.

If, however, a complex osmotic system be postulated the above reasoning breaks down. It may be argued that with increasing pressures solutes are released into the xylem sap in localized areas (cells or parts of cells) and that water enters in response to osmotic force in the immediate vicinity, while with decreasing pressure solutes could either be reabsorbed or merely diffuse out. In this case the cycles could be explained as a shifting balance between the two opposing processes. This is not, however, a simple osmotic mechanism and it would be impossible to speak of the osmotic value of the internal solution as it would in reality consist of a whole series of concentration gradients being constantly upset by diffusion and mass flow of sap resulting from movement of water and changes in pressure. It would be exceedingly difficult to obtain direct evidence to prove or disprove such a theory.

BENNET-CLARK, GREENWOOD, and BARKER (**2**) and others have concluded

that in potential growing tissues there is "positive water secretion" from the protoplasm into the vacuole in addition to the "osmotic pressure of the vacuole." Whatever the mechanism of such secretion is, it is becoming increasingly clear that some such process must be involved in many phases of the movement of water in living tissues. In root pressure it seems probable that such a force may operate in either direction and will, in addition to the osmotic forces involved, determine the pressure.

It would be pretentious to list those who have in some way contributed to this brief paper, but I wish to thank them for their help, especially for criticism of the discussion.

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