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# Investigation of Plant Water Relations with Divided Root Systems of Soybean[1]

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## ABSTRACT

**Soybean (*Glycine max*) was grown with root systems divided between adjacent cartons containing nutrient solution or soil. By adding polyethylene glycol (Carbowax 6000) to reduce solute potential or withholding water to reduce soil matric potential until water absorption from that side stopped, the root xylem water potential could be ascertained. Carbowax appeared to increase root resistance. An imbalance technique is described with which soil moisture contents of adjacent containers were followed individually. The patterns of water absorption obtained following repeated additions of water or addition of $CaCl_2$ solutions to one side indicated soil hydraulic conductivity became limiting at a soil water potential of −2 bars. A high concentration of $CaCl_2$ added to one side greatly reduced transpiration and produced severe plant injury. With part of the root system developing in nutrient solution, growth of roots into and water absorption from soil were slow; however, reduction of solute potential in the solution side greatly increased water absorption from the soil side.**

Divided root systems have been grown in solutions to investigate the uptake of mineral elements (8, 9, 21, 23), to check the effects of solute concentration on salt and/or water absorption (5, 7, 21) and on water potential ($\psi$) of leaves and solute potential ($\psi_s$) (19), to compare the effects of inorganic and organic solutes (21), and to determine average (day + night) $\psi$ of roots (7). Micropotometers have been devised and used on various sections of isolated single roots grown in solution to investigate both water and ion absorption and the effects of various osmotica on these processes (3–5, 10–12, 25).

Divided root systems in soil have been used to study cross transfer of minerals (1) and flooding injury (16); however, most work has been directed toward investigation of root growth into, water loss from roots to, and mineral absorption from dry soil (2, 13, 15, 26, 27).

The use of divided root systems in determining root $\psi$ and following patterns of water absorption in different situations is described here.

## MATERIALS AND METHODS

**Plants and Chamber.** Primary roots were removed from sand-grown seedlings of soybean (*Glycine max* (L.) Merr. Bragg). Divided root systems were allowed to grow in nutrient solution or soil contained in adjacent 1-quart polyethylene freezer cartons (Fig. 1).

The plants were grown in a Sherer CEL 512-37 growth chamber with 22 F96T12-CW-VHO fluorescent plus 6 60-w incandescant lamps without barrier. The bench was 108 cm below the lamps. Light intensity, measured with an Eppley pyrheliometer and expressed as cal $cm^{-2}$ $min^{-1}$, ranged from 0.22 in the center to 0.16 in the corners at bench height and from 0.31 in the center to 0.13 in the corners at 65 cm below lamp level. Photoperiod was 14 hr with a temperature of 25 C. Dark temperature was maintained at 25 C in an effort to minimize soil temperature gradients which would influence soil moisture movement. The level of $CO_2$ was not controlled or measured; however, earlier measurements indicated that levels usually would have been in excess of 0.04%.

**Solution Culture.** The nutrient solution was No. 2 of Hoagland and Arnon (14) except that 10 $\mu$g of Fe per liter were supplied as the EDTA chelate. Solutions were aerated with humid air introduced through fine capillary tubes. Volume was maintained by graduated constant level tubes supplying water. These also permitted measurement of fluid absorbed. Pharmaceutical grade Carbowax 6000 (Union Carbide Corporation) was added to reduce $\psi$. Values for $\psi$ were obtained by thermocouple psychrometry, courtesy of Dr. H. D. Barrs, Commonwealth Scientific and Industrial Research Organization, Griffith, NSW, Australia. In an attempt to prevent damage to roots, solutions to be replaced were drained by siphoning through a tube kept in place throughout the growth period; however, some bruising occurred between the tube and the carton wall during handling.

**Soil Culture.** The soil in a carton was 1500 or 1300 g of air-dry (0.5% $H_2O$) Cecil sandy clay loam with 1 g of dolomitic limestone, 0.2 g of $NH_4H_2PO_4$, and 0.1 g of $KNO_3$ added per kg. Moisture percentages at various matric potentials ($\psi_m$) taken from the desorption curve were 10.9% at −0.3 bar, 7% at −2.0 bars, and 4.1% at −15.0 bars. Until experimentation started, soil moisture usually was kept between 14 and 7%, with water added both to the surface and through 100 mesh stainless steel irrigation tubes (Fig. 1) extending a little more than halfway to the bottom. Water loss was followed gravimetrically. At harvest, 1.27-cm diameter cores, spaced as in Figure 1 and with the total depth divided into thirds, were used to sample moisture content. Root systems were washed for photographing and determination of fresh, dry, and ash weights, the latter used to correct for retention of soil particles.

To reduce soil $\psi$ at high soil moisture, $CaCl_2$ solutions of −5, −10, and −15 bars were used. Solutions were added at the end of a photoperiod to permit equilibration overnight. Assuming complete mixing with soil water and disregarding original soil $\psi_s$, dilution produced soil $\psi_s$ values of −3.7, −7.4, and −11.1 bars

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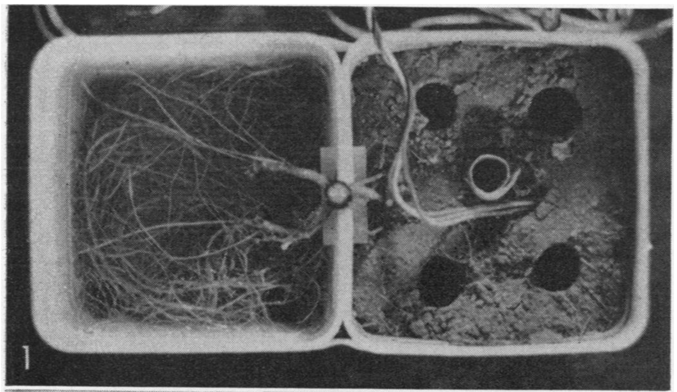

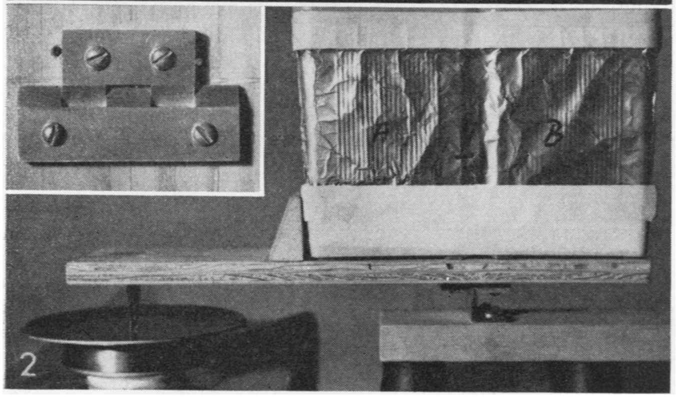

FIG. 1 (upper). Top view of adjacent cartons holding a divided root system of soybean in solution and soil. Note stainless steel screen irrigation tube, holes from which soil cores were removed, and wires from fiberglass resistance cell.

FIG. 2 (lower). View of apparatus used for imbalance technique, with aluminum foil covered adjacent cartons in place for weight measurement. Insert shows square-toothed bevels that rest on the knife edge pivot.

An imbalance technique was developed to follow the water losses from soil on both sides independently (Fig. 2). Total weight loss in combination with any difference in balance across a central pivot made possible the calculation of weight loss from each side. A platform, with stop to permit exact placement, held the adjacent cartons centered over the meshed, square-toothed bevels (Fig. 2, insert) resting on a knife edge. One arm of a sheet metal angle rested on the pan of a direct reading, top loading balance. The distance from pivot to arm was four times that from pivot to carton center of gravity. To prevent plant growth from creating imbalance, the upper portions of rapidly growing soybean shoots were removed. Plants were held in place by cord between stainless steel rods. Prior to starting, the plants were allowed to approach wilting or become slightly wilted to assure even distribution of water between the two sides. Equal volumes of liquid were then added to both sides. The cartons were placed carefully, the balance was read, and the procedure was repeated one or more times to obtain a mean value. The cartons were reversed and a second mean was obtained. Differences between the two initial means and subsequent means were summed and then added to and subtracted from half the total weight loss to find the weight losses from the individual cartons.

Although not considered adequate for following the water losses from both sides with sufficient speed or accuracy to replace the above technique, calibrated fiberglass resistance cells were sometimes placed in the soil to help determine when water loss from one side stopped.

**Premises and Calculations.** The basic premise on which the validity of the divided root system technique depends is that the resistance in the xylem interconnecting the roots and the stem base is insufficient to permit the development of appreciable $\Delta\psi$ in the xylem among various parts of the root system. Slavíková (26) found only small differences in $\psi$ between branches of a single root system when one branch was in dry and the other in damp soil. Resistances and $\Delta\psi$s between the root xylem and root surface may be much greater; however, should the water movement into a portion of the root system cease, the $\Delta\psi$ between root surface and xylem must become zero. Measurement of the $\psi$ at the external surface of the root ($\psi_e$) at this time would indicate the $\psi$ within the root xylem ($\psi_i$); and, if $\psi_e$ and the flow rate ($F$) of the other side can be measured, the resistance of the root system exterior to the xylem ($r$) can be calculated by the equation, $r = (\psi_e - \psi_i)/F$.

In solutions stirred sufficiently well that $\psi_s$ is uniform, $\psi_s$ should equal $\psi_e$. In soil from which water is being absorbed, $\psi_e$ will be different from the average $\psi$ of the soil; however, water absorption from soil should cease only when $\Delta\psi$ between soil and root is zero. At such time, $\psi_i = \psi_e =$ average $\psi$ of soil. If other roots of the same system are in different soil with sufficiently high $\psi$ and if $F$ is measured, a reasonable estimate of $r$ for the absorbing portion of the root system can be calculated by the above equation.

For plants grown in solution, estimates of the root system resistances at other times were calculated on the basis of two assumptions: (*a*) that the $\psi_i$ of an untreated control plant subjected to unchanging environmental conditions would be nearly constant and (*b*) that the growth and resistance of the untreated portion of roots of an experimental plant would be similar to the control. Kirkham *et al.* (19) reported little change in bean leaf $\psi$ values during constant growing conditions and found the control side of a split-root plant to have nearly the same apparent total resistance as the control plant.

## RESULTS AND DISCUSSION

**Solution Culture.** Columns 2 to 5 of Table I present known and measured values of $\psi_e$ and $F$ for soybean plants with both sides of divided root systems in solutions.

Using the premises and calculations previously described, start with a day when absorption from one side was essentially zero—day 7.

Table I. *Effect of Changing the External Water Potential of Side B of Divided Soybean Root Systems with Carbowax on Flow Rate, Root Resistance, and Internal Root Water Potential*

| Day | External Water Potential ($\psi_e$) | | Flow Rate[1] ($F$) | | Root Resistance ($r$) | | Internal Water Potential ($\psi_i$) Both sides |
|---|---|---|---|---|---|---|---|
| | Side *A* | Side B | Side *A* | Side *B* | Side *A*[2] | Side *B* | |
| | *bars* | | *ml hr*$^{-1}$ | | *bar hr ml*$^{-1}$ | | *bars* |
| 1 | −0.7 | −0.7 | 34.7 | 32.7 | 0.074 | 0.080 | −3.3 |
| 2 | −0.7 | −0.7 | 47.7 | 43.9 | 0.064 | 0.071 | −3.8 |
| 3 | −0.7 | −1.8 | 60.7 | 30.5 | 0.056 | 0.075 | −4.1 |
| 4 | −0.7 | −1.8 | 66.2 | 31.4 | 0.048 | 0.067 | −3.9 |
| 5 | −0.7 | −3.2 | 99.8 | 18.2 | 0.040 | 0.082 | −4.7 |
| 6 | −0.7 | −3.2 | 112.2 | 17.3 | 0.039 | 0.110 | −5.1 |
| 7 | −0.7 | −5.0 | 110.1 | 1.2 | 0.039 | ... | −5.0 |
| 8 | −0.7 | −5.0 | 81.9 | 0.0 | 0.039 | ... | −3.9 |
| 10 | −0.7 | −0.7 | 98.3 | 33.6 | 0.039 | 0.113 | −4.5 |

[1] Means from two plants. RH of chamber was 50% days 1 to 6 and 58% days 7 to 10.

[2] $r$ for side $A$ was determined on day 7 with other values calculated from total water loss of a control plant (see text).

For side $B$ on day 7, since $F_B \simeq 0$ ml hr$^{-1}$, $\psi_e \simeq \psi_i \simeq -5.0$ bars.

For side $A$ on day 7, $F_A = 110$ ml hr$^{-1}$; $\psi_e = -0.7$ bar; and $\psi_i \simeq -5.0$ bars, because $\psi_i$ of side $A \simeq \psi_i$ of side $B$.

$\therefore$, using the equation $r = (\psi_e - \psi_i)/F$, $r_A$ on day 7 $= (-0.7 - (-5.0))/110 = 0.039$ bar hr ml$^{-1}$.

Because of continuing growth of roots, $r$ on day 7 would be less than on day 1. To obtain $r_A$ on day 1, compare with control plant. Because $\psi_e$ was kept constant and $\psi_i$ was assumed to remain unchanged, changes in $r$ would have been proportional to changes in $F$.

For control plant (not shown in Table I) on day 7, $F = 147$ ml hr$^{-1}$ and on day 1, $F = 77$ ml hr$^{-1}$.

$\therefore r$ on day 1 $= 147/77 \times r$ on day 7.

Assuming $r_A$ changed as did $r$ of control, $r_A$ on day 1 $= 147/77 \times 0.039 = 0.074$ bar hr ml$^{-1}$.

From these starting points, other values found in Table I were calculated in similar manner.

As expected, reduction of $\psi_e$ around a portion of the root system caused a reduction in $\psi_i$. Of great interest is the increase in $r$ developed in the presence of Carbowax.

Janes (17) reported increased internal resistance to water flow in pepper plants with roots exposed to Carbowax. He suggested the most likely location of the increase to be the roots. Kirkham *et al.* (19) reported increased resistance in those portions of split-root systems exposed to additions of NaCl. With decapitated root systems under hydrostatic pressure, Mees and Weatherley (24) found that both mannitol and $KNO_3$ increased root system resistance. On the other hand, results from exposure to osmotica have been interpreted to indicate decreased resistance of exposed roots (3–5, 18). Decreasing root resistances with increasing transpiration rates have also been indicated (6, 22).

Some localized drying and browning of leaves indicated Carbowax movement to them, probably primarily through injured roots (20). Such injury and the possibility of additional effects (22) suggest need for caution in the use of Carbowax for reducing $\psi_e$ around roots.

**Soil Culture.** Figure 3 illustrates use of the imbalance technique with water content cycled between specified limits on one side while water was withheld from the other side until water absorption from it stopped. At this time the minimal water content of the wet side was permitted to drop another 2% for four cycles. Close inspection of the results shows that the dropping of the minimal water content of the wet side was preceded by a slight additional reduction in moisture of the dry side; therefore, the latter could hardly have resulted from the former and may have occurred because of overnight moisture redistribution within the soil of the dry side. It is also possible that the small apparent drop in moisture content of the dry side reflected an inaccuracy of the imbalance technique employed.

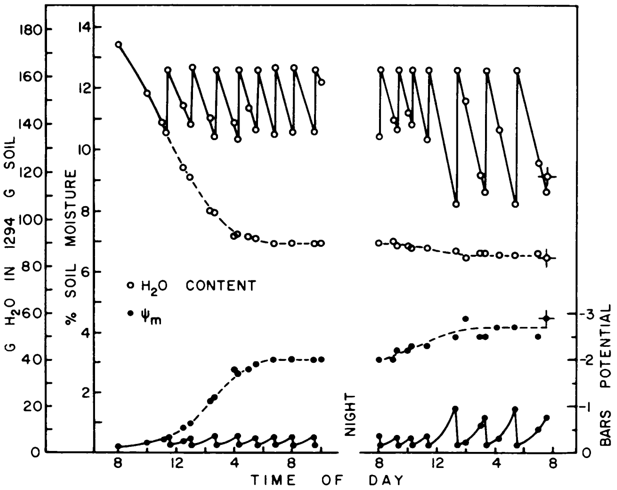

FIG. 3. Changing soil water content and matric potential on each side of a soybean divided root system as water content was cycled between limits on one side (solid line) and water was withheld from the other (broken line). Points marked by vertical and horizontal lines represent values determined at harvest.

With the equation $r = (\psi_e - \psi_i)/F$, and, for the wet side, $\psi_e = -0.8$ bars, $\psi_i = -2.8$ bars, and $F = 23$ ml hr$^{-1}$, $r = 0.087$ bar hr ml$^{-1}$. This value for $r$ is somewhat higher than found for a half root system in solution culture. At harvest the fresh weights of the half root systems were 29.0 g from solution and 22.8 g from soil.

Figure 4 illustrates use of the imbalance technique after adding $CaCl_2$ solution to the soil of one side and water to the other, then following water losses from both sides without further additions. Because an initial saline soil $\psi_s$ of $-3.7$ bars nearly stopped water absorption, the $\psi_i$ of the soybean roots must have been near $-4$ bars most of the 1st day. This seems reasonable in view of the high water content of the nonsaline side and the values reported in

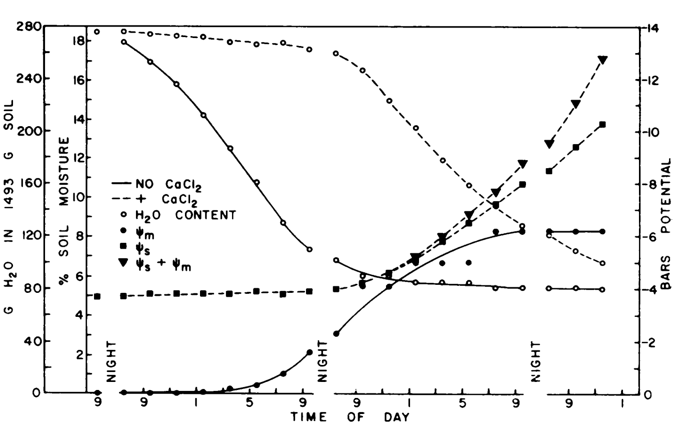

FIG. 4. Changing soil water content and water potential components on each side of a divided root system of soybean following addition of water to one side and $CaCl_2$ solution to the other. Soil solute potential ($\psi_s$) was assumed to be zero in absence of added $CaCl_2$.

Table II. *Patterns of Soil Moisture Distribution at Harvest*

| Treatment during Experiment | Upper Third | Middle Third | Lower Third |
|---|---|---|---|
| | | *% dry wt* | |
| Water added | 18.6 | 19.0 | 19.9 |
| Water withheld | 12.9 | 11.7 | 9.9 |
| Water added (cores from regular position) | 16.0 | 17.3 | 19.1 |
| Water added (cores adjacent to irrigation tube) | 16.5 | 18.0 | 21.3 |
| Water withheld | 6.2 | 5.9 | 6.0 |
| Water added (system of Fig. 3) | 10.7 | 9.0 | 7.5 |
| Water withheld (system of Fig. 3) | 6.3 | 6.4 | 6.7 |
| Water withheld without $CaCl_2$ addition (system of Fig. 4) | 5.5 | 5.1 | 5.0 |
| Water withheld after $CaCl_2$ addition, initial $\psi_s = -3.7$ bars (system of Fig. 4) | 7.1 | 6.9 | 7.0 |
| Water withheld without $CaCl_2$ addition | 5.3 | 5.2 | 5.0 |
| Water withheld after $CaCl_2$ addition, initial $\psi_s = -7.4$ bars | 10.3 | 9.8 | 9.9 |
| Water withheld without $CaCl_2$ addition | 6.6 | 6.5 | 6.7 |
| Water withheld after $CaCl_2$ addition, initial $\psi_s = -11.1$ bars | 15.5 | 17.4 | 18.8 |

Table I. Water absorption from the nonsaline side began to slow appreciably and from the saline side, to increase rapidly as the nonsaline soil $\psi_m$ approached $-2$ bars. This must mark the point in these experiments when soil hydraulic conductivity became limiting. From this point on, even though the soil $\psi$ of the saline side remained lower than the average $\psi_m$ of the nonsaline soil, water absorption from the nonsaline soil was slower than from the saline. Under these circumstances, the $\psi_e$ is not indicated by average values.

Values of $\psi_s$ after addition of $CaCl_2$ are calculated and may be in error because of ion exchange and absorption by roots; nevertheless, the limitations imposed by soil hydraulic conductivity are demonstrated clearly.

Sufficient $CaCl_2$ to give a starting saline soil $\psi_s$ of $-7.4$ bars gave results comparable to those presented in Figure 4; however, sufficient $CaCl_2$ to give a starting saline soil $\psi_s$ of $-11$ bars produced salt injury to soybean shoots. Even though the salt was added to only one side and adequate water was present in the other, transpiration in one plant dropped to about 10% of normal and continued nearly uniformly throughout the 2.5-day experiment. Without drooping or appearing wilted, many leaves dried in place. The second plant transpired at about 30% of normal rate the 1st day, increased to about 60% the 2nd day, and dropped to about 15% the 3rd, when it appeared water began to be absorbed from the saline side. During earlier experiments, the $\psi_s$ of $CaCl_2$ had reached less than $-10$ bars and $-13$ bars without apparent ill effect, other than the plants showing signs of incipient wilting, with vertical alignment of the leaflets becoming striking in the latter instance.

One of the problems encountered was the unequal distribution of roots in soil. Though roots seemed to permeate the soil, they were concentrated at the sides and bottom. Patterns of soil moisture distribution found at harvest are reported in Table II. When added in excess of field capacity (10.9%), water accumulated in the lower part of the carton. It was removed fastest from that region; but, as low $\psi_m$ values were reached, water distribution became quite uniform. These root distribution and water absorption patterns do not invalidate the results presented above.

**Solution and Soil Culture.** An experiment in which one container held solution and the other soil (Fig. 1) was a limited success because roots developed so much faster in aerated nutrient solution than in damp soil. Root growth in and water absorption from soil were increased upon reduction of the $\psi_s$ of the solution by Carbowax addition. At harvest the fresh weight of roots from soil was 13 to 21% of those from solution.

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