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Movement of Tritiated Water in the Root System of Helianthus annuus in the Presence and Absence of Transpiration [1]

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Ordin and Gairon (7) have studied the effect of osmotic substrate on the diffusion rate of tritiated water into root sections of *Vicia* and *Zea*. They found that while osmotic stress administered with mannitol had no effect on the equilibration of tritiated water in *Zea* an enhancement was observed in *Vicia* roots. Since the above results were obtained with excised roots the objective of this paper was to establish the pattern with which tritiated water equilibrates in root systems in the presence and absence of transpiration from the shoots.

Materials and Methods

Plant Preparation. The plants were grown and treatments carried out in a controlled-environment chamber equipped with a ceiling bank of 14 high-intensity, power-groove, fluorescent tubes 2 m long and spaced at intervals of about 13 cm, with nine 100-w incandescent bulbs interspersed to furnish red and yellow spectral wavelengths.

All plants were grown at 20°, 3000 ft-c of light, with 12-hour days and 12-hour nights.

The nutrient solution used, half-strength Hoagland's No. 2 (3), included the A–Z micronutrient solution except that 1 ml of sodium iron sequestrene (21 g/liter) instead of iron tartrate was used per liter of nutrient solution.

Tritium was supplied as liquid tritiated water (THO), with a specific activity of 0.1 μc/ml.

Seeds of sunflower (*Helianthus annuus*, Advance) were selected for uniform size and barely covered with distilled water in the dark for 24 hours. Seeds with a visible radicle were then planted in vermiculite in a porous clay pot on a bench in the controlled-environment chamber. The pot was irrigated daily with nutrient solution and allowed to drain. When the cotyledons were fully expanded (about 7 days), the seedlings were reselected for uniformity, maximum size, deep-green color, and morphological similarity of roots and shoots.

The root-stem transition was marked with indelible red ink, and each seedling was transferred to a 1-liter aluminum-foil wrapped glass jar containing nutrient solution. The seedlings were secured by a wad of unwettable cotton in a slot in a paraffin-coated cork fitted snugly into the top of the jar. Two holes in the cork permitted gas exchange between the solution and the atmosphere. The root-stem transition was held about 2 cm above the surface of the solution. Solution volume was restored daily with distilled water.

Plant Selection. At time of treatment (15–30 days) the plants were selected for morphologic uniformity. Water use by each plant was determined by measuring weight loss over a 12-hour light period

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in the controlled-environment chamber. The plants were then grouped by rate of water loss.

Plant Harvest. The sunflower root systems were handled in the following manner. A group of root systems (with or without attached shoots) was fastened to the edge of a board spanning a tray containing the same solution in which the plants had been growing. While they were being attached, the roots were submerged in the solution with the root-stem transition zone about 2 cm above the water surface. After the plants were attached, the board was lifted and suspended until the roots had ceased to drip. Then the board was lowered onto the edge of a similar tray containing a solution of THO.

Periodically, 1 root system was removed from the holding board, blotted dry with filter paper, and segmented simultaneously into pieces 2 cm long.

Comparison of blot-drying with rinsing of *Helianthus* roots showed that in roots which had been submerged in THO, rinsing with distilled water allowed back exchange of tissue THO and prevented root tissue water from reaching the specific activity of the external THO solution (fig 1). Another experiment was conducted to assess the importance of vapor exchange between roots grown in THO and a saturated HHO atmosphere. Figure 2 shows that such exchange can be quite rapid, with a half-time between 0.5 and 5 hours. This suggests that tritium exchange between root tissue and the atmosphere after equilibration with a THO solution and before lyophilization occurs will reduce the concentration of tritium in tissue. To minimize these effects, root tissue was tightly corked in 15 × 45 mm vials immediately after segmentation, and the vials were frozen in dry ice for storage.

Recovery of Tritium. Tritiated water (THO) in the plant tissue was recovered by freeze-drying. Since the structure of the tissue remaining was not an object of interest, a maximum rate of water removal short of combustion was desired.

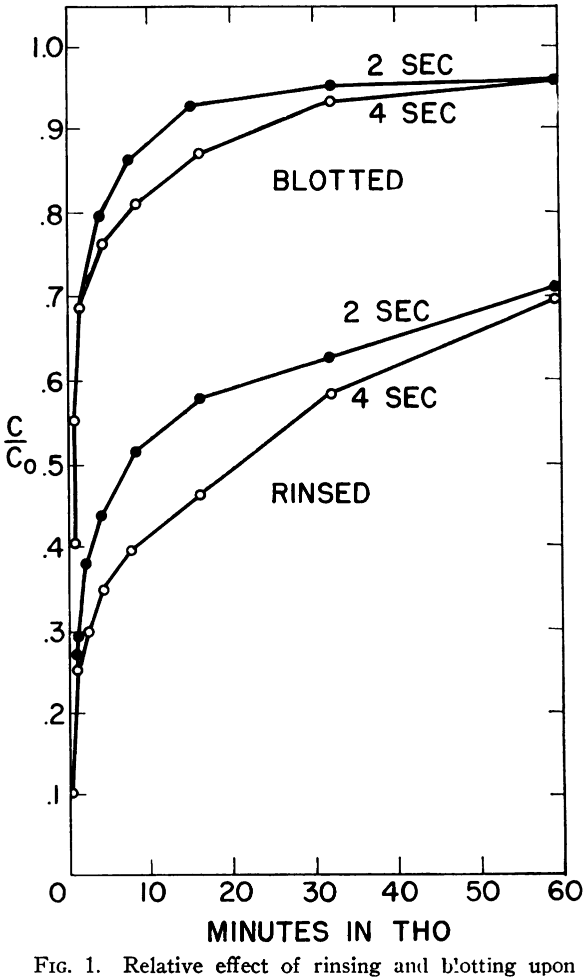

Fig. 1. Relative effect of rinsing and blotting upon rate of gain of THO by detopped sunflower root systems which have been submerged in tritiated water. C = Tritium concentration in root tissue water. C_0 = Tritium concentration in external solution.

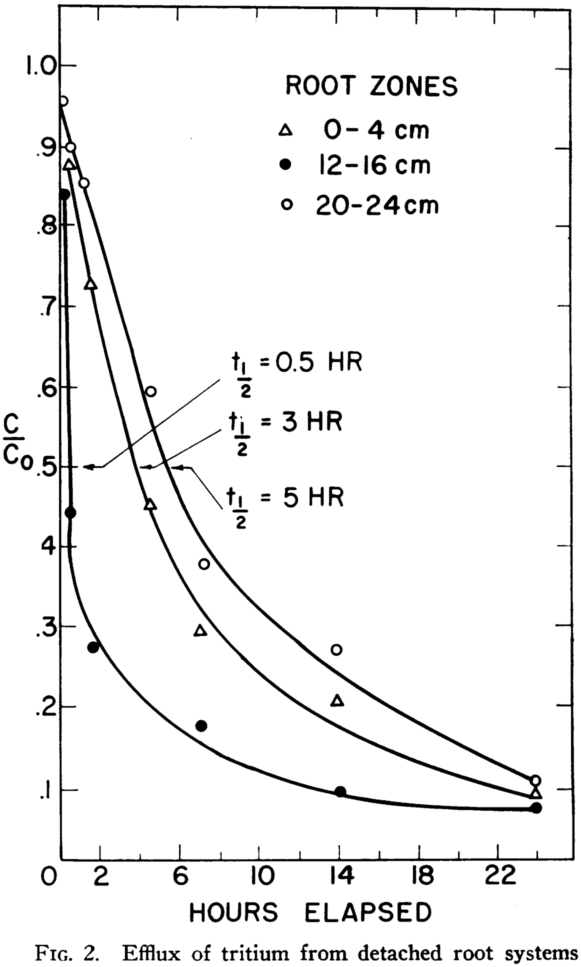

Fig. 2. Efflux of tritium from detached root systems of THO-grown sunflower plants into saturated HHO vapor. C = Tritium concentration of root tissue water at various times. C_0 = Tritium concentration of root tissue when efflux began.

An occasional report (6) has indicated that partitioning of oxides of hydrogen isotopes may occur during freeze-drying. However, since the specific activity of captured water did not vary significantly between one-half minute after drying began and 15 minutes later when the tissue had reached constant weight, isotope fractionation appeared to be insignificant in our studies.

A further test showed that the residue left after complete freeze-drying of leaf tissue contained about the same amount of moisture as tissue oven dried at 105° for 24 hours.

Results

Equilibration of THO in Detached Roots of HHO-grown Plants. Sunflower plants grown 2 weeks in a nutrient solution containing only HHO were detopped at the cotyledonary node, and the root system was submerged in a large volume of THO. At various times, root systems were removed from the solution, blotted, and analyzed for tritium.

The THO content of the tissue water (C) from sunflower roots detached from the shoot before treatment tended toward equality with the external solution (C_0) with a half-time of 30 seconds and closely approached the external value after about 1.5 hours (fig 3). The shape of the efflux curves was the same regardless of how long the roots had been in THO before efflux was initiated by transferring the root system to distilled water.

Thirty-day old sunflower plants were used to investigate the effect of transpiration upon equilibrium half-times and the pattern of tritium distribution in the root. Certain plants remained intact, and others were detopped before the root systems are submerged in THO. Some of the intact plants were exposed to light and others were kept in the dark during submergence of the root system in tritiated nutrient solution. At intervals, root systems were removed, blotted, and cut into 2-cm segments, and each segment was analyzed for THO.

The pattern of THO influx into tissue water of sequential 2-cm segments of these sunflower root systems is shown in figure 4 (detached roots), figure 5 (attached roots, darkness), and figure 6 (attached roots, light).

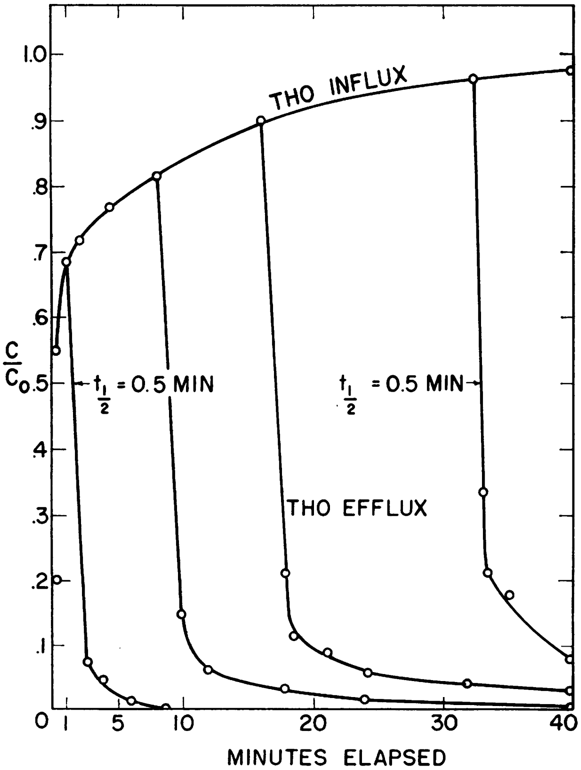

FIG. 3. Influx and efflux of liquid THO in root systems of HHO-grown, detopped sunflowers 2 weeks old exposed to liquid THO for various periods. C = Tritium concentration in tissue water. C_0 = Tritium concentration in external solution. t½ = Time required to reach tritium concentration equal to one-half of that in the external solution.

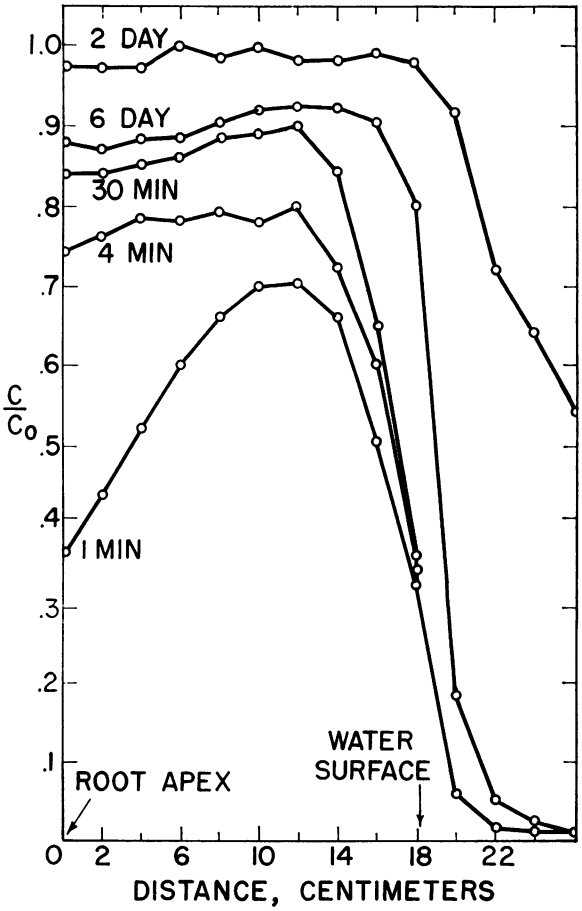

FIG. 4. Absorption of liquid THO by sunflower roots detached from the shoot. C = Tritium concentration in root tissue water. C_0 = Tritium concentration in external solution.

Various parts of a detached root system approached external tritium concentration (C_0) at differing rates (fig 4). The maximum rate of gain of tritium activity over shorter intervals occurred in the middle sections of the root system of sunflower. At first, the tips of the root system and the root segments near the water surface gained radioactivity the most slowly. After about 30 minutes, the apical third of the root systems was gaining activity at the same rate as the middle third. The upper third of the root system, near the surface of the solution, was still lagging.

Root systems of shoots held in darkness (fig 5) acquired tritium from the external solution with a pattern very much like that of detached root systems (fig 4). Before treatment with THO the intact plants were held in the dark for 6 hours after a preceeding 12-hour light period in order to minimize xylem tension developed during the light period. Even so, apparently some tension remained, since the tissue above the water surface gained activity more rapidly than when the shoot was absent.

Strikingly different results were obtained when the influx of tritium was determined while the root systems of intact plants were exposed to THO during a light period of 12 hours. During the first 2 hours the root system approached equality with tritium in the external solution much more slowly when the shoot was transpiring (fig 6) than when the root was detached from the shoot (fig 4) or when the intact plant was in the dark (fig 5). Again, the root tips and the root zone near the surface of the solution lagged far behind for the first 2 hours.

Discussion

The pattern for tritium influx from a THO solution into submerged whole sunflower root systems was related to the position of the tissue along the axis of the root system (fig 4, 5, 6). The average half-time to equality with the external medium was about 0.5 of a minute for detached roots (fig 4). However, equilibration was generally slower in both the basal regions and in regions close to the root apex. The slow equilibration of the more basal regions may be attributed either to the larger diameter of roots or to greater suberization in this region, which may alter

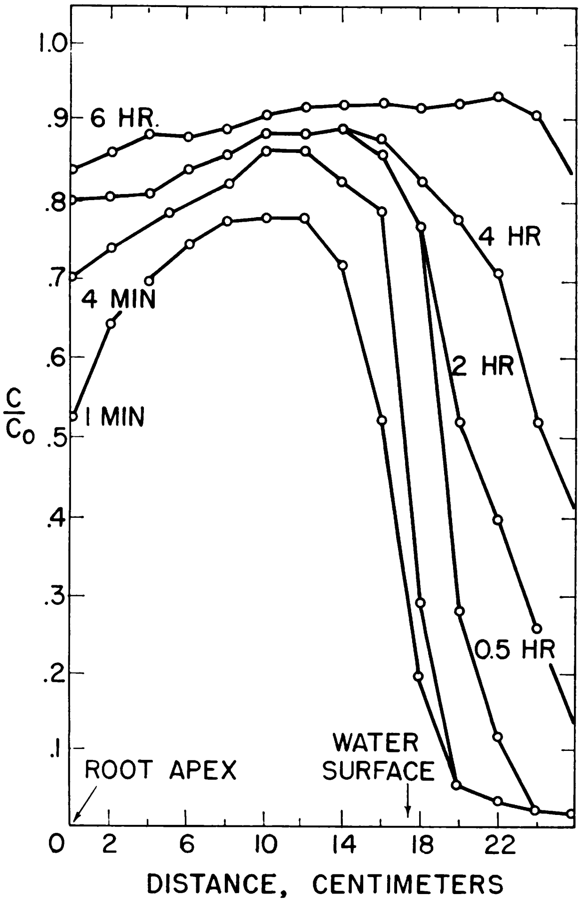

Fig. 5. Absorption of liquid THO by sunflower roots attached to the shoot in darkness. C = Tritium concentration in root tissue. C_0 = Tritium concentration in external solution.

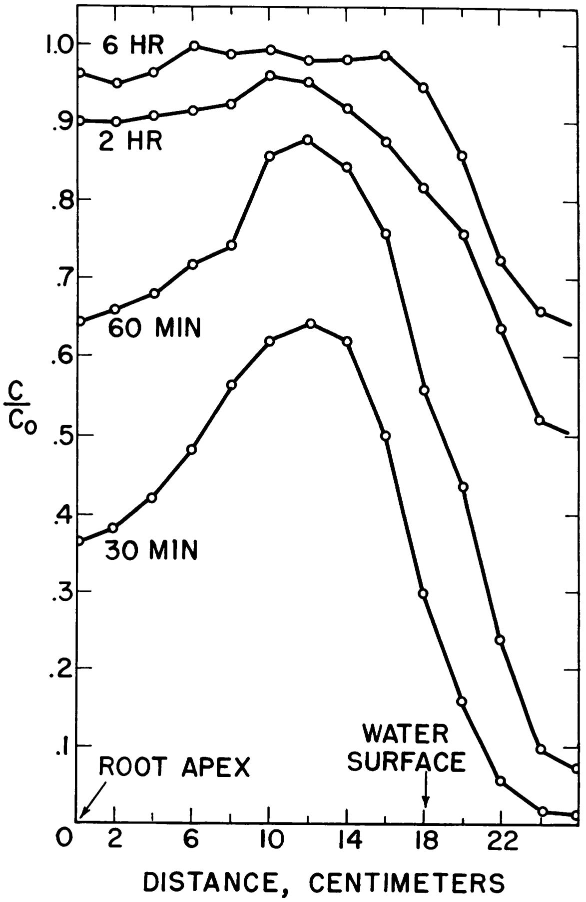

Fig. 6. Absorption of liquid THO by sunflower roots attached to the shoot in light. C = Tritium concentration of root tissue water. C_0 = Tritium concentration of external solution.

the diffusion coefficient. The slower equilibration near the root apex cannot be attributed to root size, which was generally smaller than in sections nearer the shoot end of the root. It is possible that the diffusion coefficient of THO may be altered near the root apex in this plant material. From studies on root sections, Ordin and Gairon (7) showed that the diffusion coefficient for THO in *Vicia* and *Zea* roots varied between 5 and 20 % of the value reported by Wang et al. (10) for the free-diffusion coefficient of THO in HHO.

Philip (8) has shown that the half-time to equilibrium of tissues of the same shape but differing in volume will vary as the square of their dimensions. Therefore, the half-time for diffusion into a tissue of a given shape will be 4 times that of another tissue with half these dimensions. The significance of these relationships must await further search for the reasons for our observed departure of THO equilibration rates from those expected on the basis of a cylindrical model for diffusion into a root. The data in figures 4, 5, and 6 seem to support the hypothesis of Brouwer (2) and Hylmo (4) that the path of water movement from the external solution to the xylem can be influenced by transpiration. Under hydrodynamic conditions of transpiration pull most of the water flow would occur in larger pores of the conducting medium. On the other hand, diffusion is not as dependent on pore size. The larger pores of the root system are probably in the cell walls. The fact that the tritium equilibrium half-time in the root system of a transpiring sunflower plant (fig 6) was much longer than in a nontranspiring plant (fig 5) indicates not only that in a transpiring plant the THO moved primarily in preferred channels but also that lateral diffusion from these channels may have, in some way, been restricted. The reason for such a hypothetical reduction in lateral diffusion from conducting channels is not obvious but it is of great interest that it must be assumed to be present during transpiration and absent in nontranspiring plants. Strugger (9) has long argued for considerable movement in the cell walls, primarily from interpretation of the movement of dyes.

The results presented here are in an apparent conflict to those of Ordin and Gairon (7) who observed enhancement of the diffusion rate of tritiated water into *Vicia* root sections due to osmotic stresses. However, the results should not be considered comparable since the effects of transpiration pull on the water relations of root cells may be qualitatively different than those of an osmotic substrate. A possible hypothesis to explain the results obtained here is that under rapid transpiration the diffusion permeability of root cells decreases. The nature of such a decrease is still unknown. The reasons for inequalities in the rates of equilibrium between tracers like THO and various plant tissues in relation to theoretical models merits further investigation.

Summary

In a controlled-environment chamber at 20° the movement of tritiated water was followed in 2 to 4 week old root systems of *Helianthus* plants grown in nutrient solution containing tritiated water (THO). Tritium did not uniformly enter entire root systems detached at the cotyledonary node and exposed to THO for varying periods. Influx of THO was most rapid in the middle segments of the root system, and about equally slow in the apical and basal root segments.

The pattern of tritium flux into entire root systems attached to the shoot in the dark was similar to flux into detached root systems. In the light, however, entire root systems attached to the shoot took up THO at much slower rates.

Acknowledgments

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