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The Effect of Molecular Size, Concentration in Nutrient Solution, and Exposure Time on the Amount and Distribution of Polyethylene Glycol in Pepper Plants[1, 2]

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BYRON E. JANES
Department of Plant Science, University of Connecticut, Storrs, Connecticut 06268

ABSTRACT

Pepper plants *Capsicum annuum L.* var. California Wonder were grown in nutrient solutions of either −3.0 or −5.0 bars osmotic potential, using polyethylene glycol with molecular weights of 400, 600, 1000, 1540, or 4000 as osmotica. Polyethylene glycol with molecular weights of 1000 or 1540 proved most satisfactory as osmotica to decrease the water potential of nutrient solutions.

There was no relationship between the small amount of polyethylene glycol accumulated in the plants and the amount of water transpired. The concentration of polyethylene glycol in the expressed sap of the leaves and the total accumulated was inversely related to molecular weight of polyethylene glycol, was greater at lower osmotic potential of nutrient solution, and increased with time in solution. Except for plants grown in polyethylene glycol 4000, there was more polyethylene glycol in leaves than roots. The indications were that, when the concentration of polyethylene glycol reached a value of 1 to 2 mg per ml, any additional quantity absorbed was transferred to the leaves. The major proportion of polyethylene glycol 4000 absorbed was retained in the roots.

The results of Sephadex gel chromatographs showed that the passage of polyethylene glycol through the plants did not alter the average molecular weight. This indicated that there was no selective absorption of small molecules that might be present as contaminates in the commercial product.

The use of PEG[3] to reduce the water potential of nutrient solutions has become an accepted technique to create water stress in plants. The majority of researchers report satisfactory results and indicate that PEG is usually superior to salts, sugars, or other organic compounds (5, 7–9, 11, 13, 16). However, there are reports of deleterious or toxic effects resulting from use of PEG as osmotica (10, 12, 15). The work of Lagerwerff (10), Lawlor (11), and Michel (13) and in this laboratory has shown that there may be toxic substances in some PEG formulations, most likely resulting from the manufacturing process. The indications are that some of the variation in plant response to PEG may result from the use of different size molecules.

The data presented here were obtained over a period of time to answer the following questions which have arisen from the use of these polymers. (*a*) What is the relationship between rate of transpiration and absorption of PEG? (*b*) What is the possibility that the small amount of PEG absorbed by the plants is due to traces of some low molecular weight material in the samples? (*c*) What is the effect of molecular weight on the distribution within the plant of the small amount of PEG absorbed? (*d*) What are the locations and something of the nature of the filtering mechanisms that serve as a barrier to the entry of PEG into the roots?

MATERIALS AND METHODS

Growth and Treatment of Plants. Pepper plants (*Capsicum annuum* L. var. California Wonder) were grown in Hoagland's nutrient solution in an environment controlled as follows: temperature 26 C, relative humidity 60 to 70%, 18 hr per day of light from fluorescent and quartz iodine lights giving radiant energy of 1.05×10^5 ergs cm^{-2} sec^{-1} at plant height. Plants were approximately 4 weeks old at test maturity, with a leaf surface of 2 to 3 dm^2. The data were obtained from two different experiments. In the first experiment the plants were grown for 1, 3, or 7 days in solutions of either −3.0 or −5.0 bars osmotic potential using PEG with average molecular weights of 400, 600, 1000, 1540, or 4000 as osmotica. There were two plants per treatment (12 plants for each polymer). These plants were used to determine the rate of accumulation, distribution of PEG within the plant, and the relation between uptake of PEG and the amount of water transpired. In the second experiment the plants were grown for 7 days in solution with −5.0 bars OP using each polymer as the osmoticum. These plants were used to determine the effect of molecular size on transpiration, growth, leaf water potential, and selective absorption of molecular size. At the start of the treatment the plants were transferred to 1.8 liters of fresh nutrient solution containing the PEG polymer to be tested. The weights of the several PEG polymers added to 100 ml of nutrient solution to produce either −3.0 or −5.0 bars OP are given in Table I. Toxic impurities were removed from PEG 4000 by passing a concentrated solution through a column of standard Bantam demineralizing resin. The OP of the solutions was checked by determining the freezing point depression with a Precision Systems osmette.

The loss of water and change in weight of plants was determined once a day. The entire culture was weighed, the plant and cover of the vessel were removed, roots were allowed to

[1] This paper is dedicated to the memory of Solon A. Gordon, a fellow graduate student at the University of Michigan, who distinguished himself as an enthusiastic and creative plant physiologist.

[2] Scientific Contribution No. 588, Storrs Agricultural Experiment Station.

[3] Abbreviations: PEG: polyethylene glycol; OP: osmotic potential.

Table I. *Grams of Polyethylene Glycol Required to Depress the Osmotic Potential of 100 Ml of Nutrient Solution from −0.5 to −3.0 or −5.0 Bars*

Mol Wt	−3.0 Bars	−5.0 Bars
	g	
400	4.0	6.9
600	5.0	8.6
1000	6.7	10.7
1540	8.0	12.3
4000	9.9	14.1

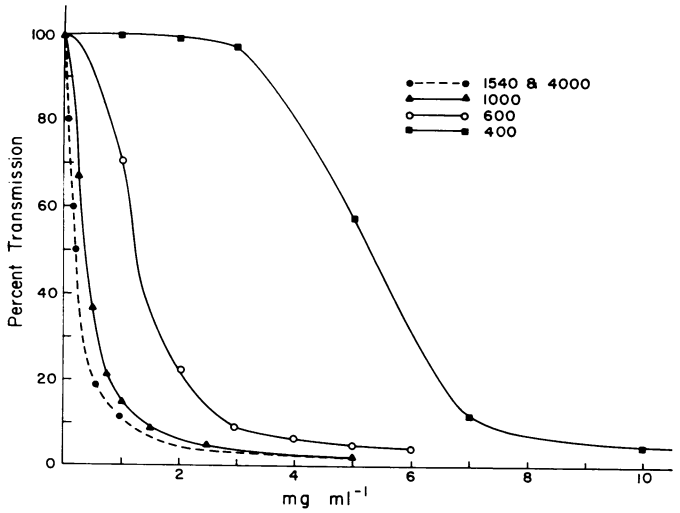

FIG. 1. Relationship between concentration of PEG and turbidity of the trichloroacetic acid precipitate of different size molecules.

drip for 1 min, and the culture without the plant was weighed. The volume of the culture solution was restored with deionized water. Using these data, an estimate of the leaf area of intact plants was obtained from the relationship between leaf area and plant weight previously established (6). At the end of the desired treatment period, the plants were harvested and separated into leaf, stem, and roots. The leaf area was determined photometrically. The roots were thoroughly washed in running warm water and blotted dry. After weighing, the separate plant parts were placed in plastic bags and frozen. Samples of sap obtained by application of pressure to thawed material were analyzed for PEG content.

Analysis of PEG in Plant Material. The following procedure was adopted from the method described by Hyden (4). The expressed sap was centrifuged for 5 to 10 min at 11,500*g*. A 1.0-ml aliquot of suitable dilution was pipetted into a centrifuge tube, and 0.6 ml of 10% (w/v) $BaCl_2 \cdot 2H_2O$, 0.6 ml of saturated $Ba(OH)_2$, and 0.6 ml of 5% (w/v) $ZnSO_4 \cdot 7H_2O$ were added with shaking between each addition. The tube was allowed to stand at least 5 min before centrifuging at 7,600*g*. The supernatant liquid was poured into a colorimeter cuvette, and 3 ml of trichloroacetic acid solution were added (450 g of trichloroacetic acid and 50 g of $BaCl_2 \cdot 2H_2O$ in 1,000 ml of H_2O). The cuvette was turned upside down several times, and after standing 5 min the turbidity was measured as the percentage of light (600 nm) absorbed. The relationship between turbidity and PEG content was obtained by constructing a calibration curve for each molecular size (Fig. 1). The larger the mol wt of PEG the lower concentration at which precipitation occurred. The precipitation of PEG 400 was more sensitive than the larger molecules to the concentration of the reagents. Accurate and standardized procedures were required for all determinations as the relationship between turbidity and PEG concentration varied appreciably with change in concentration of trichloroacetic acid.

Lawlor (11) reported the presence of interfering substances in some plant material. A test for interfering substances in peppers indicated that this was not a problem in the sap from frozen tissue. Better than 95% of all the molecular sizes was recovered from sap to which known amounts were added. This test was made numerous times throughout the study.

Estimates of Molecular Size. The following procedure was used to determine if there was any change in the distribution in the range of molecular size of each polymer by selective absorption or degradation. A 1-ml sample of plant sap or 1-ml of nutrient solution containing PEG was treated as indicated in the first step of procedures for analysis of PEG. The supernatant liquid was applied to the top of a 0.9 × 20.5 cm column of Sephadex G-100 or G-25 and eluted with deionized water. The eluted material was collected in approximately 1.5-ml samples and analyzed for PEG. Three ml of trichloroacetic acid solution were added to each sample, and turbidity was measured as previously indicated. The volume of solution eluted before appearance of PEG and the volumes containing PEG for each sample was recorded. The larger the molecules the more rapid the passage through the column.

The ratio of Stoke's radii of the different polymers to the pore radius of the two Sephadex columns was estimated from the elution volumes according to the formula proposed by Ackers (1). Since the pore radius was constant, the values obtained are apparent Stoke's radii × a constant (K).

RESULTS

Toxic Effects. The lot of PEG 4000 used for the first set of experiments contained a very toxic substance. Within 24 hr after placing roots in a solution of this material, they were brown and appeared flaccid. Roots of plants grown for 7 days in solutions of this same PEG 4000 after it had been dissolved in water and passed through a column of standard Bantam demineralizing resin showed no signs of injury. Two other lots of PEG 4000 have been used in this laboratory; one of these showed no signs of toxicity and the other had only a slight toxic effect. Similar results have been obtained with PEG 6000. There were no visible signs of root damage when PEG with a mol wt of 1540 or smaller was used.

Concentration of PEG in Expressed Sap. A small amount of PEG was absorbed by the roots and accumulated in the roots and leaves (Figs. 2 and 3). There was little or no PEG found in the sap of the stems. Therefore, only data on PEG content of roots and leaves are presented. The smaller the molecules the greater the concentration of PEG in the expressed sap of the leaves (Fig. 2).

Except for plants grown in −5.0 bars solution of PEG 400 or −5.0 bars solution of PEG 600 for 3 or 7 days, the concentration of PEG was between 0.5 and 2.0 mg ml^{-1} in the expressed sap of roots. It was interesting to note that the concentration of PEG in roots grown in PEG 4000 was with one exception greater than the concentration in roots grown in PEG 1000 and was 0.5 to 1 mg/ml more than in leaves of plants grown in PEG 4000.

Accumulation in Leaves and Roots. The values for accumulation of PEG presented in Figure 3 were obtained by multiplying the concentration (mg/ml) in expressed sap by the water content of the plant part. It was assumed that all PEG was soluble in the expressed sap and that 90% of the fresh weight was water. PEG accumulation in the plants was inversely related to the molecular size and directly related to the time of

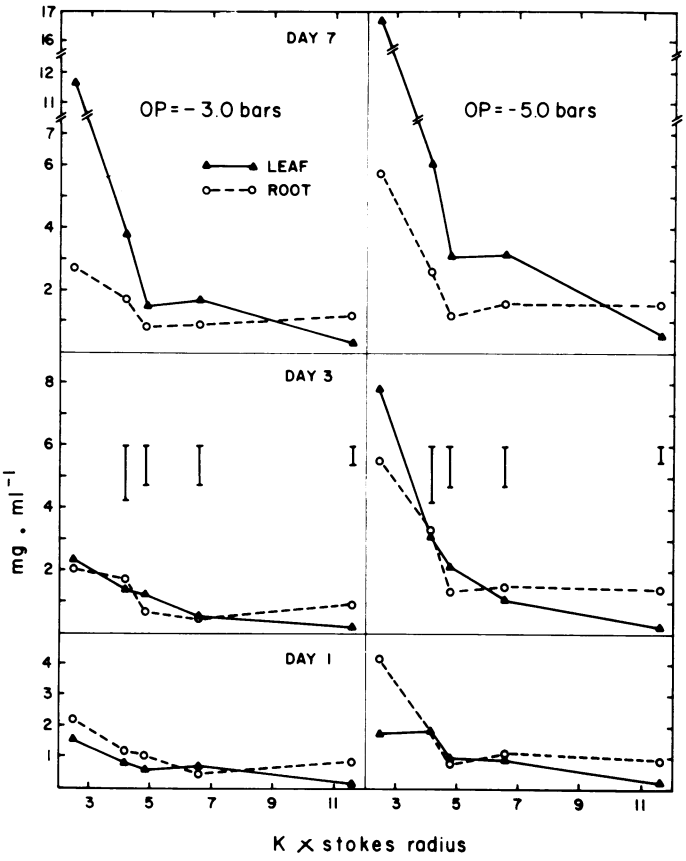

FIG. 2. Concentration of different polyethylene glycol polymers in sap of leaves and roots of pepper plants grown for 1, 3, or 7 days in nutrient solutions at either −3.0 or −5.0 bars osmotic potential. Bars in center represent least significant difference for values for particular polymer.

exposure and the decrease in OP of the nutrient solution. During the first 24 hr, except for plants in PEG 400, the accumulation of PEG was essentially the same in all plants (15–30 mg per plant). As the length of exposure was increased, the accumulation of PEG was similar in plants with roots in PEG 1000, 1540, and 4000. However, the distribution between roots and leaves was different. Except for day 1 when leaves and roots had approximately the same amount, there were more of the smaller molecules in the leaves than in the roots. This would indicate that for these polymers the roots had a limited holding capacity and that any amount absorbed in excess of threshold concentration was transferred to the xylem and transported to the leaves. The major portion of PEG 4000 was found in the roots with very small quantities in the leaves.

Relation between Transpiration and Accumulation of PEG. The values relating amount of water transpired and PEG accumulated in plants (Table II) are approximations based on the assumption that all plants growing in a particular solution absorbed PEG at the same rate. Estimates of accumulation during days 2 and 3 were obtained by subtracting the value for concentration of PEG in plants sampled on day 1 from that for plants sampled on day 3. Similarly, values were figured for accumulation during the period day 4 through day 7 by subtracting value for day 3 from value for day 7. Because of plant variability this procedure indicated no absorption of PEG 1540 during days 2 and 3. Thus, plants harvested on day 1 accumulated more PEG 1540 during day 1 than plants harvested on day 3 did in 3 days.

The uptake of PEG per ml H_2O transpired was greatest during the first 24 hr and least during days 2 and 3. The differences between μg PEG g^{-1} H_2O transpired during days 2 and 3 and days 4 to 7 were not large but with one exception the values for the latter period were greater than for the former. It would seem probable that the greater accumulation per g H_2O transpired during the first 24 hr resulted from a rapid uptake shortly after roots were placed in solution of low osmotic potential plus a reduced rate of transpiration during the period. The initial reaction to the reduced water potential undoubtedly was a reduction in turgidity which could have altered the root permeability, allowing for a surge of PEG into the roots. In these experiments, the plants were transferred to the PEG solutions while they were rapidly transpiring. It seems likely that the shock to the plants and the accompanying surge of PEG into the roots would have been less if the transfer had been made while plants were in the dark or if the PEG had been added in small increments during light periods.

Growth and Transpiration. The similarity of changes in rate of growth and transpiration in the plants grown in solutions of the different molecular sizes indicate that the source of the response was primarily the osmotic potential of the nutrient solution and not molecular size (Table III). The rate of transpiration of all plants expressed as g H_2O dm^{-2} hr^{-1} gradually decreased as new leaves partly shaded the older ones. The rate of transpiration from plants in nutrient solution with −0.5 bar OP was always greater than from plants in solutions with −5.0 bars OP. The size of the molecule apparently had no effect on the passage of water through the plants. Leaf water potentials, ψ_L, estimated from balancing pressures in a pressure bomb, were 2.5 to 3.9 bars lower in plants in solutions with −5.0 bars OP than ψ_L of plants grown in solution with −0.5 bar OP.

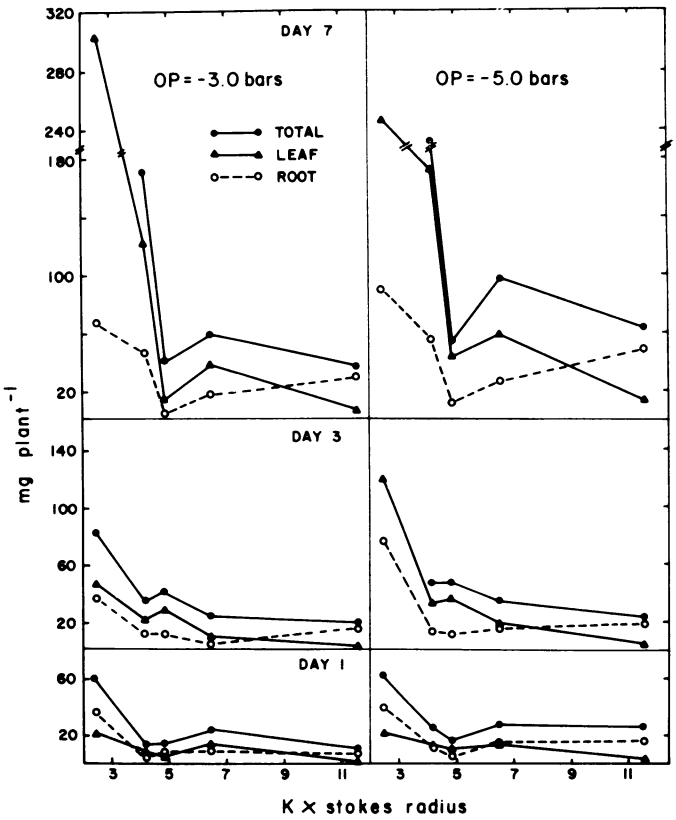

FIG. 3. Amount of different polyethylene glycol polymers in leaves and roots of pepper plants grown for 1, 3, or 7 days in nutrient solutions at either −3.0 or −5.0 bars osmotic potential.

Filtering Capacity of Roots. The PEG formulations are mixtures of different molecular sizes with the average approximately that designating the particular material. For example, PEG 400 has a range in mol wt from 380 to 420 and PEG 4000 ranges from 3000 to 3700. It seemed possible that the PEG found in the plants was a trace of low mol wt material, mixed with the predominately larger size molecules, which was being selectively absorbed by the roots. To test this hypothesis comparisons were made of the elution patterns from gel chromatographs, using Sephadex G-100 or G-25, of nutrient solutions containing PEG polymers and expressed sap of treated plants (Fig. 4). The larger the molecule the more rapid the passage through the column. Thus, the presence of any appreciable concentration of smaller molecules in the plant extract would have resulted in higher peak volume in the plant extract than in the nutrient solution. The peak volumes represented by cross lines indicate that there was no difference in average mol wt before and after passing through the plant roots. This would suggest that the PEG molecules entered the roots in a random manner and that the number of molecules entering the roots was related to the sizes of the pores or passages in the filtering membrane. There was an appreciable number of pores of a size to permit passage of PEG 400 but fewer pores large enough for PEG 4000 to enter the roots.

Table II. *Effect of Time and Concentration of Different Molecular Weights on the Accumulation of PEG*

Mol Wt	Day 1		Days 2 and 3		Days 4 to 7	
	−3.0 bars	−5.0 bars	−3.0 bars	−5.0 bars	−3.0 bars	−5.0 bars
	μg PEG g per H_2O transpired					
400	660	1070	128	610	278	633
600	200	602	90	79	155	247
1000	140	171	7	15	18	2
1540	140	256			72	137
4000	67	154	32	3	38	68

Table III. *Effect of Molecular Weight of PEG on the Growth, Transpiration, Leaf Water Potential, and PEG Content of Pepper Plants*

Values are averages of four plants.

Mol Wt	Leaf Area		Root Wt	Transpiration			Leaf Water Potential	PEG Concn Day 7	
	Day 0	Day 7	Day 7	Day 0	Day 7	Difference 0–7	Day 7	Leaves	Roots
	dm^2		*g*	*g H_2O dm^{-2} hr^{-1}*			*bars*	*mg per ml sap*	
400	2.6	6.1	15.9	1.4	0.5	0.9	−8.8	10.2	5.0
600	2.6	7.4	18.4	1.4	0.6	0.8	−9.1	5.4	1.2
1000	2.4	7.7	17.6	1.6	0.7	0.9	−8.5	4.5	1.2
1540	2.3	7.1	19.4	1.6	0.7	0.9	−8.3	3.3	1.4
4000	2.8	7.7	19.2	1.6	0.6	1.0	−7.7	0.4	1.7
Control	2.5	9.8	22.3	1.4	0.9	0.5	−5.2	0.0	0.0
LSD 5%				0.04	0.12	0.04	0.6		

DISCUSSION

Polyethylene glycol with a mol wt of 1000 or 1540 proved most satisfactory as an osmoticum to decrease the water potential of nutrient solutions to produce a water stress in plants. The smaller molecules of PEG 600 and 400 were absorbed in appreciable amounts, and in experiments lasting more than 24 hr this may result in disruption of normal plant processes. Commercial samples of PEG with a mol wt of 4000 or larger may contain toxic impurities, apparently residues left from the manufacturing process. These toxic substances can be removed by passing solutions of the polymers through deionizing or Sephadex columns. Even when purified, the solutions of these larger polymers, because of the increased viscosity and tendency to foam, create problems of aeration.

Lawlor (11) showed that when cotton plants had healthy roots only small amounts of PEG 1000, 4000, or 20,000 were absorbed. When roots were injured there was a rapid uptake of all molecular sizes which accumulated in the leaves and

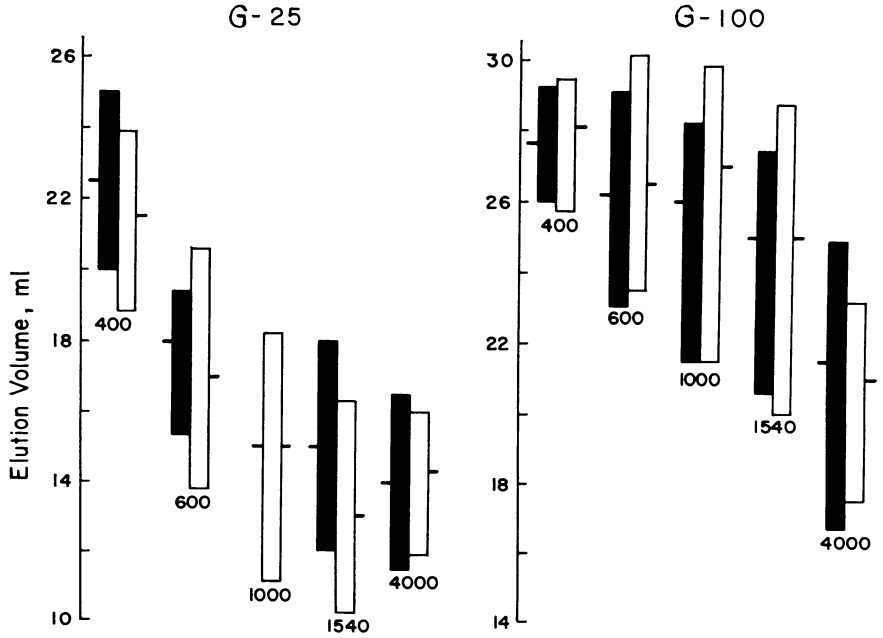

FIG. 4. Elution volumes from Sephadex G-25 and G-100 columns. Solid bars: elution volumes for nutrient solutions; open bars; elution volumes for plant extracts; center line in each bar: peak concentration.

resulted in severe injury. Using radioactive PEG 4000, Lawlor (11) showed that the material that entered through injured roots accumulated in the mesophyl of the margins of the leaves. He did not report on the distribution in other parts of the plants. The data in Figures 2 and 3 show that the amount accumulated and the distribution within the plant varies with the size of the molecule. There was much more of the PEG 400 and 600 in the leaves than in the roots. On the other hand, much of the small amount of the PEG 4000 absorbed was retained in the roots.

The following evidence would indicate that the PEG entered the plant through intact roots with a reflection coefficient of approximately 1. The amount of PEG accumulated in the leaves was only a small fraction of that present in the nutrient solution. There was no relationship between the amount of PEG accumulated and the rate of transpiration. There was no change in molecular distribution of each polymer as it moved from the solution to the leaves. There was no evidence that there was any absorption which could be associated with the mass flow of water.

The studies of Crossett (2) and Emmert (3) indicate that the centripetal passage of ions involves a series of steps and that the patterns displayed suggest that ions pass through a "pool" as they move from the nutrient solution to the xylem. The pattern of accumulation of PEG would suggest that the centripetal passage of this substance is similar to that of ions. However, it seems likely that different mechanisms and pathways are involved. It would appear that there are two semipermeable membranes or barriers between the epidermis and xylem of the root with the pool located between them. PEG passed the first barrier and reached a critical concentration before moving through the second membrane and entering the xylem. Once in the xylem it was carried along by the transpiration stream and eventually accumulated in the leaves. The critical concentration in the roots was between 1 and 2 mg/ml. After several days in solution, the concentration of PEG 400 and 600 in the roots does go above these values. This may be a result of accumulation in root tissue outside the pool.

The existence of the second barrier is also demonstrated by the lack of movement of PEG 4000 out of the root. The concentration of PEG 4000 in the roots is the same or slightly higher than for the smaller PEG 1540 and 1000. However, the concentration of PEG 4000 in the leaves is much lower than that of other polymers, indicating that PEG 4000 is trapped in the roots by an inner barrier that does not permit passage of larger molecules out of the root.

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