Transpiration, a prerequisite for long-distance transport of minerals in plants?

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The major ''benefit'' alleged to accrue from transpiration (the evaporative loss of water from plant surfaces) is that it is essential for the long-distance transport of mineral ions, but the possible interrelation between these two processes has rarely been tested. Transpiration was experimentally dissociated from mineral supply by growing sunflowers (*Helianthus anuus*) in hydroculture and providing mineral nutrients only during the nights. These plants grew as well as a control group that received nutrients only during the day and transpired 12–15 times more water during the exposure period. It thus appears that convective water transport in the xylem, brought about by root pressure and the resultant guttation, ''growth water,'' and Münch's phloem counterflow is in itself sufficient for long-distance mineral supply and that transpiration is not required for this function.

Although there is no experimental evidence to support the general proposition, it is commonly believed that transpiration, the evaporative loss of water from plant leaves, is required for the long-distance transport of inorganic nutrients in the xylem of higher plants (1, 2). Of course, there is no dispute that the increased flow of water during transpiration elicits a corresponding increase in the rate at which dissolved solutes move upward in the xylem elements. The specific question we address is whether this acceleration, mediated by transpiration, is essential for plant growth. We argue that other forces, which result in solute movement upward in the xylem, are adequate for the delivery of nutrients and that transpiration, *per se*, is not necessary for this or indeed any vital function in plants. The concept concerning the role of transpiration in plant nutrition goes back to Julius Sachs (3), who explained the enrichment of minerals within plants as compared with their concentration in soil water simply by analogy to distillation. It is now recognized that the metabolic uptake of ions and the passive uptake of water are independent processes. Nevertheless, over the years, the view had developed that some useful function must be fulfilled by the large amount of water moving through plants because of transpiration, and long-distance transport of mineral nutrients has received the most attention. Strong views questioning such a role for transpiration have occasionally been expressed (4, 5), but these have not been supported by experiment. It has been shown by growing plants under high humidity that transpiration could be reduced by >60% without any effect on growth or mineral content (6, 7), but the suggestion that transpiration was unimportant did not go unchallenged (8). The major difficulty in studying the contribution of transpiration to long-distance transport of minerals is that it is not possible strictly to maintain 100% relative humidity (R.H.) around plant leaves in the light. The unavoidable temperature difference between a leaf absorbing light and the water-saturated air of a growth chamber prevents the maintenance of 100% R.H. in the intercellular spaces of the leaf and the immediate neighborhood of its surface. Water loss due to transpiration cannot be reduced by more than about 70% (7). We now describe a different experimental approach to address this issue. Transpiration and mineral uptake were temporally dissociated by growing sunflower plants in a 12 h light/12 h dark regime under which minerals were supplied only in the dark, during which time the growth chamber was maintained at 100% R.H. The water loss during the night exposure to nutrients was reduced by a factor >10 over a control group receiving nutrients only during the light, yet the growth and mineral content of the plants was unaffected.

Materials and Methods

Growth of Plants. When about 15 cm high, sunflower plants (*Helianthus anuus*) were transferred from soil to hydroculture. The 10 liters of mineral medium contained 2 mM $Ca(NO_3)_2$, 0.55 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 0.04 mM $FeCl_3$, 0.04 mM NA_2EDTA, 0.01 mM H_3BO_3, 0.5 μM $MnCl_2$, 0.5 μM $ZnSO_4$, 0.2 μM $Cu(NO_3)_2$, 0.01 μM $(NH_4)_6Mo_7O_{24}$. After 1 week in the hydroculture medium, the actual experiment with plants of about 30 g fresh weight was started. One group of plants was kept in deionized water containing 0.3 mM $CaCO_3$ during the 12-h day and in mineral medium during the 12-h night. The control group experienced the opposite regime. All vessels were continuously aerated. The 10-liter medium and the deionized water plus 0.3 mM $CaCO_3$ were renewed in 3- to 4-day intervals. The plants were grown in a growth chamber (type 6′SD/+ 22 JU-Pa-5), from Weiss (Reiskirchen, Germany); light source: HQI-R 250-W NDL lamps; 100 μmol of photons per m^{-2} per s at 1 m above the growth chamber floor; other conditions specified in the text.

Determination of Water Loss, Total Ion Uptake, and Cation Content of Plant Tissues. The water lost by transpiration (and guttation) was determined by weighing the vessels and plants each time the medium and water was changed. Control vessels without plants were treated identically to account for water loss due to aeration. Total ion uptake was determined by following the decrease in conductivity with a Digital-Konduktometer CG 855 (Schott–Geräte, Hofheim, Germany) and by correcting for the conductivity change because of the loss of water. At the end of the experiment plant fresh and dry weights were determined. Dried leaves (5 g) and upper halves of stems were ashed (500°C for 12 h) and K^+, Ca^{2+}, and Mg^{2+} content was determined with an inductively coupled plasma (ICP) instrument (Jobin Yvon, Paris; JY 70 Plus) either from HCl-solubilized ash (Table 3) or from roots macerated in 1 M HCl (Table 4). K^+ uptake per plant per day (Table 4) was determined in the same way directly from aliquots of the medium.

Results

Growth of Sunflower Plants Supplied with Minerals Only During the Night. Four experimental plants and four control plants (variety ''Albenga''), with an initial fresh weight of 30 ± 2 g each, were

Abbreviations: R.H., relative humidity; MD-plants, plants exposed to mineral solution during the day; MN-plants, plants exposed to mineral solution at night; MDN-plants, plants exposed to minerals day and night.

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Table 1. Water loss, total ion uptake, and growth of sunflower plants at 52% R.H. during the day and >98.5% R.H. during the night period

	H_2O loss, liters		Ion uptake, ($\Delta\mu$S/10 liters)		Weight, g	
Plants*	Day	Night	Day	Night	Fresh	Dry
MN-plants†	13.62	**1.18** (8%)	<100	**1997** (90%)	607 (109%)	50.1 (94%)
MD-plants†	**13.90** (100%)	1.03	**2225** (100%)	<50	557 (100%)	53.6 (100%)
MDN-plants‡	19.9 (143%)		2,970 (136%)		802 (144%)	77.1 (144%)

*Plants were grown for 30 days in hydroculture.
†Average values of 4 plants.
‡Average values of 2 plants.

grown in hydroculture at 23°C during the day and the night. The R.H. during the night was kept at >98.5% and during the day at 52%. Experimental and control plants were exposed to mineral solution during the night (MN-plants) and day (MD-plants), respectively. In the second 12-h period both groups were supplied with deionized water that contained a small amount (0.3 mM) of $CaCO_3$. The latter had to be included because otherwise the roots, and particularly those of the MN-plants, appeared brown and deformed after 8–10 days, a phenomenon observed in 1966 by S. Veirs (22), who first investigated plants growing under these conditions. Because of this root defect, plant growth was severely affected and the work of Veirs was never published. Two further control plants obtained mineral solution day and night (MDN-plants). The experiment was terminated after 30 days. At that time all plants were flowering; there was no time difference in the beginning of flowering between the three groups. Table 1 lists the average fresh weight and dry weight per plant (variety "Albenga") in each group, as well as the average amounts of water lost and ions taken up during the 30-day period. Ion uptake was followed by the decrease in conductivity of the medium, corrected for by the volume change and summed up over the whole experimental period. As can be seen from the data presented, the MD-plants, transpiring 13.9 liters of water, took up an equivalent of 2,225 μS/10 liters ions. The ion uptake of MN-plants was 10% less, whereas an amount of only 1.18 liters of water was lost by transpiration and guttation during exposure to nutrients. This result confirms that under physiological conditions of low ion concentration, ion uptake is independent of water uptake and water loss (9–11). The main result of the experiment was that, despite the more than 10-fold difference in the amount of water passing through the plants during the time they were supplied with mineral nutrients, the fresh and dry weight increases did not differ significantly. It is important to note that the control plants supplied with minerals both day and night yielded an increased growth of almost 50%. This result shows that mineral supply was growth limiting in MD- and MN-plants (see ref. 8).

In four additional experiments with sunflowers (variety "Helene ZS" or variety MRS37) carried out in a similar way to the studies presented in Table 1, the MN-plants reached average fresh weights of 98% ± 5% and dry weights of 97% ± 11% (MD-plants taken as 100% in each experiment). The amount of water lost during the night period in the four experiments amounted to 7% as compared with the water transpired during the 12-h day. The control plants of the four experiments with continuous mineral supply (MDN-plants) reached an average fresh weight of 161% ± 19%. MD- and MN-plants of one such experiment are shown in Fig. 1.

Plant Growth Under High R.H. During the Day. An experiment with the additional goal of minimizing transpiration also during the light period (>98% R.H.), and in this way possibly uncovering a contribution of transpiration for MN-plants (ions taken up in the night might partly be translocated vertically during the day), did not yield a different result (Table 2). The difference in the amount of water lost during the particular mineral exposure time under these conditions is only 5-fold; again, mineral uptake and growth yield are comparable, whereas growth was stimulated by more than 50% when minerals were supplied both day and night.

Content of Ash and Main Cations. Ash content and the content of the main cations of the plants in the experiments of Table 1 and 2 are given in Table 3. In no case were the values for MN-plants significantly lower than either control group. The higher content of Mg^{2+} in MDN plants correlated, to some extent, with a lowered Ca^{2+} content, and thus may be related to a competition between these ions (possibly in the apoplast). As mentioned above, MD- and MN-plants were provided with a 0.3 mM $CaCO_3$ solution for the 12-h interval during which nutrients were being withheld.

Changes in the Main Cation Content of Roots in a Single Night and Day Period. Although the outcomes of the experiments presented in Tables 1 and 2 do not differ, the possibility that MN-plants take up ions under nontranspiring conditions during the night, but store them in the root system and translocate them vertically the next day, was tested. Sunflower plants with 40–50 g fresh weight were kept as MD- and MN-plants and the $K^+/Ca^{2+}/Mg^{2+}$ content of their total roots was measured at the end of the mineral supply interval (beginning of the water interval) and at the end of a water interval (beginning of mineral supply). In addition the K^+ uptake per plant per day was determined for MN- and MD-plants by following the amount of K^+ in the medium. The results from these experiments are given in Table 4.

Such studies clearly showed that the amount of K^+ decreased as expected within the roots of both plant groups during the time they were kept in water. However, the decrease was more or less the same in MN- and MD-plants, and accounted for only 17% and 21%, respectively, of the amount of K^+ taken up from the medium in the corresponding 12-h interval of mineral supply. The amount of K^+ absorbed from the medium, being on the order of 40 mg/day (Table 4), exactly matched the K^+ required for the increased fresh weight of 10 g/day of these plants. Taken together, the results prove that plants taking up ions under conditions in which transpiration is reduced to <10% are translocating these ions from the roots to the growing parts of the shoot; storage of ions in roots, until a period of increased transpiration is experienced again, does not play a role.

The data of Table 4 do clarify another point. During the water interval—in these experiments, no $CaCO_3$ was added to the water to also follow changes in Ca^{2+} content—the divalent ions are decreasing significantly more than K^+, which, at least in the case of Ca^{2+}, could be explained by the fact that it is localized to a large extent in the extracellular space, the apoplast (12). By the same reasoning it is understandable that MN-plants, kept in pure distilled water during the day, loose more Ca^{2+} from their roots than do MD-plants (Table 4), because the former flush

Fig. 1. Plantlets of *Helianthus anuus* (variety MRS37, 28 days old, initial fresh weight 50 ± 5 g) were grown for 22 days in a growth chamber at 23°C with a R.H. of 70% during the day and >98% during the night. MN-plants in the front row; MD-plants in the back row.

their apoplast with 10–15 times more deionized water. Thus, not the time spent in water—which is the same, of course, for MN- and MD-plants—but the amount of deionized water passing through the plant is the reason why roots of MN-plants after several days become brown and distorted (see above). This root-browning effect was completely avoided by the addition of 0.3 mM $CaCO_3$ to the water.

Discussion

The main open question is, how do plants manage their long-distance mineral transport under conditions when the transpiration stream is reduced to very low levels? It is obvious, in the first place, that the same amount of ions can be translocated only with 1/10 of volume flow if the ion concentration in the xylem is increased accordingly. Because the actual mineral uptake is not affected by the decrease in transpiration, as shown previously (9–11), and also as shown for the growth conditions applied here (Tables 1 and 2), this assumption is unavoidable and has indeed been experimentally demonstrated (13). Second, however, there exists transpiration-independent water flow in the xylem. Growth water (14) and "Münch's counterflow" (replacing, within the xylem, the water exported from source leaves by way

Table 2. Water loss, total ion uptake, and growth of sunflowers at >98 R.H. during the day and night period

	H_2O loss, liters		Ion uptake, ($\Delta\mu$S/10 liters)		Weight, g	
Plants*	Day	Night	Day	Night	Fresh	Dry
MN-plants	4.49	**0.72** (18%)	ND	**1603** (114%)	450 (120%)	37 (106%)
MD-plants	**3.93** (100%)	0.52	**1409** (100%)	ND	375 (100%)	35 (100%)
MDN-plants	8.25 (210%)		3,000 (213%)		702 (187%)	55 (158%)

ND, not determined.
*Plants were grown for 23 days in hydroculture.

Table 3. Ash and main cation content of the sunflower plants of the experiments of Tables 1 and 2

	Content, mg/g dry weight							
	Leaves*				Stems*			
Plants	Ash	K^+	Ca^{2+}	Mg^{2+}	Ash	K^+	Ca^{2+}	Mg^{2+}
Exp. 1								
MN-plants	177	50	42	2.8	110	37	15	1.8
MD-plants	180	49	42	4.9	85	32	9	1.2
MDN-plants	159	45	32	5.6	93	34	10	3.9
Exp. 2								
MN-plants	236	72	34	5.4	172	76	12	3.3
MD-plants	201	65	30	5.6	160	75	8	3.4
MDN-plants	232	71	25	9.3	159	67	14	6.7

*Five-gram (dry weight) aliquots of leaves and stems (upper halves) were ashed, and the cation content was determined.

of the phloem, "Saftzirkulation"; ref. 15), are minor fractions in heavily transpiring plants, but constitute a significant portion of water when transpiration is reduced.

Applying the same criteria as we did previously (7), we can estimate the amount of water moving independently of transpiration. In Table 5 this is done for the MN-plants of Table 1. The increase in fresh weight of the shoot amounted to 450 g; 90% of it was taken as growth water. The estimation of Münch's counterflow is based on the assumption that two-thirds of the dry weight increase (root and 50% of the shoot) plus an additional 20% used up by respiration is photosynthesized in the top part of the plant and transported downward. Assuming further an average sucrose concentration of 10% in the sieve tubes, the volume flow in the phloem amounts roughly to 400 ml. This has to be compensated for by a corresponding water volume flowing upward in the xylem. Finally, the water movement caused by root pressure is taken as 150 ml. This is based on the fact that guttation drops appeared only on MN-plants during the night, and the difference in the water lost during the night between MN- and MD-plants was 150 ml (Table 1). Thus, a volume of close to 1 liter would be transported independently of transpiration equaling approximately the amount of water moving because of residual transpiration in MN-plants during the night (Table 5). Therefore, these 2 liters of water of MN-plants transport the same amount of minerals as the 15 liters of water (13.9 liters by transpiration and ≈1 liter by transpiration-independent water flow) in the MD-plants.

It has to be pointed out that transpiration-independent acropetal water flow in the xylem has recently been elegantly demonstrated with submerged plants by Pedersen and Sand-Jensen (16, 17). These authors also demonstrated that this water transport was not solely because of root-generated pressure, because submerged shoots—the root being cut off—show this phenomenon, too (17). This is most likely caused by negative water potentials arising because of growth ("growth water"). Finally, H_2O circulation in the sense of Münch's proposal was proved by NMR using intact *Ricinus* plantlets (18). Half of the water moving acropetally was shown to recirculate under the experimental conditions applied.

One may even question, therefore, whether the very low amount of transpiratory water loss, by way of transpiration, that took place in our experiment during the night is essential for

Table 4. K^+, Ca^{2+}, and Mg^{2+} content of roots at the end of a single mineral supply period and at the end of a single period in deionized water (without $CaCO_3$)

	Content, mg/g fresh weight			K^+, mg/root
	K^+	Ca^{2+}	Mg^{2+}	
Content in MN-plants*				
a (morning)	3,740	240	78	56.1
b (evening)	3,180	170	55	47.7
a − *b*	560	70	23	
	(15%)	(29%)	(30%)	
a − *b* (per total root)				8.4
Content in MD-plants*				
a (evening)	3,750	230	75	56.3
b (morning)	3,300	183	58	49.5
a − *b*	450	47	7	
	(12%)	(20%)	(22%)	
a − *b* (per total root)				6.8
Content in MDN-plants*				
a (evening)	3,400	230	80	
b (morning)	3,400	210	80	
Uptake, mg/plant/day				
MN-plants	39.0 ± 5.0	ND	ND	
MD-plants	40.3 ± 2.4	ND	ND	

ND, not determined.

*Plants with an average fresh weight of about 90 g were used for the experiment. The roots of one plant each (approximately 15 g fresh weight) were macerated with 40 ml of 1 M HCl. Percent values represent the relative decrease in ion content during the water period.

Table 5. The extent of residual transpiration and transpiration-independent water flow in the MN-plants of Table 1

Water Flow	Volume, ml
Transpiration	1030
Growth water	405
Münch's counterflow	400
Guttation	150
Σ Transpiration-independent	955

plants in the context earlier discussed by Smith (8). Clearly, the transpiration-independent volume flow observed and a concomitant increase in ion concentration by a factor of about 2 would completely suffice for optimal supply of mineral nutrients, and such an increase is not unreasonable. Thus Allen *et al.* (19) found that K^+, the main osmotically active ion in the xylem, amounts to about 1 mM in a transpiring plant. In our MN-plants we have to assume, therefore, that the concentration of K^+ increased to about 7.5 mM, because 2-liter volume flows in MN-plants bring about the same result as 15 liters in MD-plants. Extrapolating to a complete absence of transpiration—just as a ''Gedanken experiment''—a rise in the K^+ concentration to about 15 mM would be required. This is less than 20% of the K^+ content of typical plant cells. It would not be expected, therefore, that such an increase in concentration would create an osmotic problem, although living cells of the stem would lose some water to establish a new equilibrium.

It could be argued that what may be possible for sunflowers does not hold for trees, because root pressure is by far too low for trees 100 m high. As can be seen in Table 5, even for sunflowers water flow because of root pressure (guttation) is the least important component of transpiration-independent convective water transport. Based on a photosynthesis rate of 15 mg of CO_2 fixed per dm^2 of leaf area per hour and on the assumption that a mature leaf exports half of the photosynthate as sucrose solution by way of the phloem, one can estimate that within less than 4 days a water potential of less than -2.0 MPa would arise in the absence of any transpiration, a value large enough to pull water up a 100-m-tall tree.

Would the results reported herein have been the same if the experiments had been conducted in soil? Accessibility of ions for roots has been claimed to be positively affected by transpiration (12). However, it has been calculated that mass flow of water to roots as compared with diffusion affects only the availability of phosphate, and even for this anion, the contribution of mass flow has been estimated to be less than 10% (20, 21).

After reducing the rate of transpiration by more than 90% without observing so much as a hint of an adverse effect on growth, and pointing out that forces other than transpiration are fully capable of moving solutes up the xylem, we conclude that transpiration is not essential for long-distance mineral transport. Only a convincing experimental demonstration that the remaining 7% of transpiration is of some special significance would now sustain the contrary view.

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