

Effect of Mineral Nutrient Sprays on Photosynthesis and Stomatal Opening of Water-stressed and Unstressed Apple Seedlings I. Complete Nutrient Sprays¹

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Additional index words. mineral nutrition, polyethylene glycol, transpiration, *malus domestica*

Abstract. Solution cultures altered with polyethylene glycol to induce a water stress of -1.0 bar decreased net photosynthesis (P_n) and stomatal conductance (C_s) in the leaves of 'York Imperial' apple seedlings (*Malus domestica* Borkh.). Sprays with a complete nutrient solution produced similar responses and further decreased P_n and C_s in water-stressed plants. It appeared from the mesophyll conductance (C_m) calculation that reduction in P_n rate in water-stressed, as well as sprayed trees, was not caused solely by a stomatal factor. It is not known, however, whether nonstomatal limitation of P_n reflects a decreased capacity for CO_2 fixation or increased light respiration, since mesophyll conductance was calculated assuming constant (zero) sink CO_2 concentration in the leaf. In another experiment, decreasing to -1.0 bar water potential of nutrient solution reduced daily water consumption and fresh weight of 'York Imperial' apple seedlings. Sprays with complete nutrient solution also tended to decrease daily water consumption of plants. Water stress decreased leaf Ca and root K and Mg concentrations, indicating that water stress may lower the absorptive capacity of the roots. However, water-stressed plants contained more Ca in the roots than unstressed plants, suggesting that lower leaf Ca concentration in stressed plants was caused by suppressed transport. Leaf sprays with complete nutrient solution increased Mg concentration in the leaves and stems and Ca concentration in the leaves.

Accumulation of mineral nutrients in plants is frequently reduced by water stress (8, 11, 17). It is not clear whether low soil moisture affects nutrient uptake by lowering availability of nutrients through decreasing diffusion and/or mass flow to the root or by changing the absorptive capacity of the roots (18). The use of polyethylene glycol (PEG) as an osmotic modifier in solution cultures offers the possibility of separating the effect of water stress on root absorptive capacity from that related to nutrient availability.

The response of various plants to water stress can depend on their nutritional status (11, 12, 13). Lahiri (11) reported that adequate mineral nutrition stabilized the growth and yield of cluster bean, pearl millet, and wheat exposed to sporadic drought.

In this paper, we examine the effects of applying complete nutrient sprays during a PEG-induced water stress on maintaining physiological processes and growth of apple seedlings.

Materials and Methods

Growth and nutrient uptake study. This experiment was carried out in a growth chamber with 12 hr of light at $450 \mu E m^{-2} s^{-1}$

¹Received for publication Sept. 21, 1981.

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$250 \mu E m^{-2} s^{-1}$ photosynthetically active radiation (PAR). The light source consisted of fluorescent and incandescent lamps. Temperature was a constant $22^\circ C$ and relative humidity was not controlled, although it was monitored. Open-pollinated 'York Imperial' apple seedlings (about 30 cm in height), grown in flats of sand in the greenhouse, were transferred to nutrient solution and allowed to grow for 2 weeks in order to obtain undamaged roots prior to treatment. Uniform plants were then selected and placed in 1-liter, wide-mouth, brown plastic bottles (2 plants per bottle) filled with nutrient solution (800 ml/bottle). The composition of nutrient solution was the same as reported by Gergely et al. (5). Solutions were changed weekly and continuously aerated.

Water-stressed plants (6 bottles \times 2 plants = 12 plants) were exposed to a nutrient solution of 1.0 bar lower water potential by dissolving 35.2 g of PEG (Carbowax 4000³, MW = 3500-3700) in 800 ml of nutrient solution (4). The same number of control (unstressed) plants were grown in regular nutrient solution. Half of the unstressed and stressed plants (3 bottles \times 2 plants = 6 plants in each group) were sprayed daily (in the morning) with complete nutrient solution. The spray solution was a modification of the nutrient solution used in the bottles. It was 8 times more concentrated than the nutrient solution and KNO_3 and $Ca(NO_3)_2$ were replaced by equivalent amounts by urea, K_2SO_4 and $CaCl_2$ respectively. Surfactant (Tween 20)³ was added to give a concentration of 0.1% v/v.

The duration of the experiment was 14 days. Daily net water consumption by the plants was determined by weighing the bottles at 24-hr intervals and calculating the water loss. Loss of water due to evaporation was obtained by weighing bottles without plants. Water consumption was expressed on a leaf area basis (in $ml/dm^2 \cdot day$). Leaves present on the trees in the first and second week of the experiment were marked and their total area was measured at the end of the experiment using a LI-COR LI-3000 leaf area meter with a LI-05A transparent belt conveyer.

Increase in plant fresh weight and height was calculated from the difference at the beginning and end of the experiment. After harvest, roots, stems, and leaves were dried at 70°C for 48 hr, weighed, and ground to pass a 20-mesh sieve. Tissue samples, 0.500 ± 0.005 g, were ashed at 500° overnight and the residue was dissolved in 6N HCl. Potassium, Mg, and Ca were determined by atomic absorption spectrophotometry using standard methods and phosphorus was determined colorimetrically by the vanado-molybdo-phosphoric method (9).

Photosynthetic study. Plant material, treatments, and nutrient solutions used were the same as in the growth and nutrient uptake study. Seedlings were grown in 1-liter water culture bottles (1 plant/bottle) in a growth chamber with 12 hr of light at $1000 \mu\text{E m}^{-2}\text{s}^{-1}$ (PAR) with day/night temperature of 26°/20°C and humidities of 60/90%.

There were 2 trees in each treatment, and from each tree, the youngest fully expanded leaf was selected for photosynthetic and transpiration measurements. The day before the first measurement, the nutrient solution in the bottles was changed and PEG (– 1.0 bar or 35.2 g/800 ml of nutrient solution) and/or leaf spray with complete nutrient solution was applied. The sprays were repeated at daily intervals, always in the morning about 2 hours before photosynthetic measurements. Water loss from the bottles was replaced daily. The photosynthetic measurements were performed over 5 consecutive days.

Leaf net photosynthesis (P_n) and transpiration (E) were measured by placing the leaf in a climate-controlled cuvette through which air was passed. Dew points of the airstream entering and leaving the cuvette were determined (0.05°C accuracy) with a General Eastern 1100 AP dewpoint hygrometer, and the difference in CO_2 content of the input and output airstream was recorded with a Beckman 865 differential CO_2 analyzer. The flow of the air through the cuvette was $3.2 \text{ dm}^3 \text{ min}^{-1}$. Leaf temperature (0.1°C accuracy) was measured with a thermocouple attached to the lower surface of the leaf. Leaf temperature was maintained at $26^\circ \pm 0.5^\circ$ by passing water of desired temperature through a heat exchanger placed in the cuvette. The air in the cuvette was mixed by a mini-fan. Stomatal diffusion resistances to water vapor were calculated from transpiration rate and water vapor pressure of the leaf and cuvette air. It was assumed that the air within the leaf was saturated with water at the measured leaf temperature. Mesophyll resistance to CO_2 assimilation was calculated as a difference between total resistance to CO_2 assimilation and stomatal resistance. Total resistance was calculated from photosynthesis rate and from the difference between CO_2 concentration outside the leaf and sink CO_2 concentration in the leaf. Sink CO_2 concentration was assumed to be zero. Reciprocals of stomatal resistance and mesophyll resistance to CO_2 assimilation are given hereafter as a stomatal conductance (C_s) and mesophyll conductance (C_m), respectively.

Results

Growth and nutrient uptake study. Apple seedling water consumption (Fig. 1) fluctuated from day to day despite stable temperature and light conditions kept in the growth chamber. The only environmental factor not controlled was relative humidity. It can be seen from Fig. 1 that there was an inverse relationship between plant water consumption and daily maximal air relative humidity. Day-to-day changes in water consumption were generally parallel in all treatments, although the plants with PEG-induced water stress (treatments 3 and 4) were somewhat

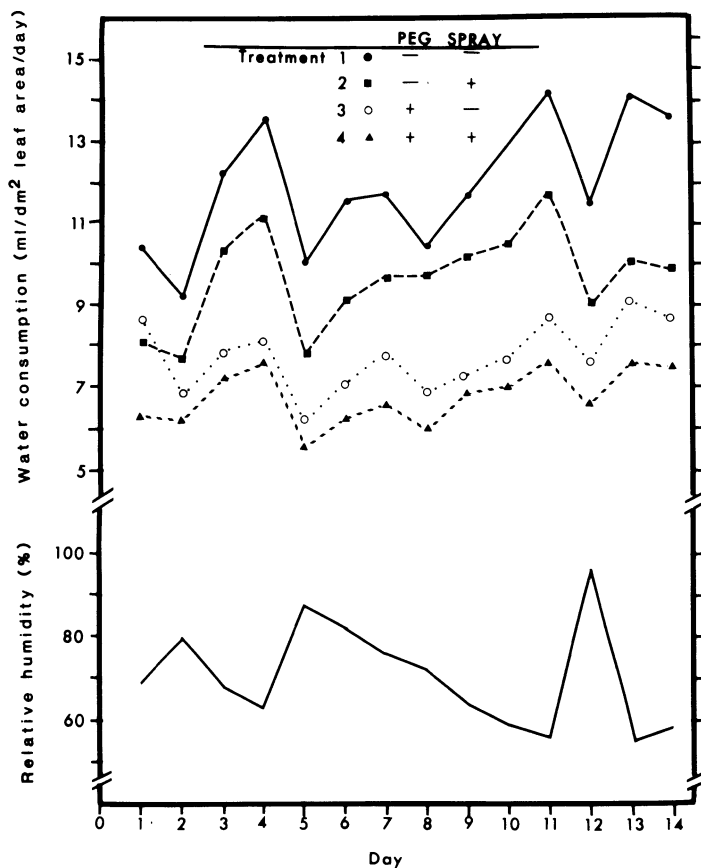


Fig. 1. Daily water consumption of 'York Imperial' apple seedlings and relative humidity maxima. Seedlings were grown in solution cultures with or without PEG and with or without daily sprays of complete nutrient solution on the leaves.

less able to respond to humidity fluctuations. There was a significant PEG treatment \times day interaction (Table 1).

Water stress induced by PEG decreased plant water consumption significantly regardless of spray treatment (Fig. 1, Table 1). It is noteworthy that the complete nutrient solution sprays tended to decrease water consumption of stressed as well as unstressed plants (Fig. 1).

Shoot extension was reduced in response to spray and/or water stress but the differences obtained were not statistically significant (data not shown). Increase in plant fresh weight per 1 dm^2 of leaf area was diminished ($P < 1\%$) by PEG-induced water stress. Increase in plant fresh weight per 1 dm^2 of leaf area was 1.1 g and 2.1 g in stressed (treatments 3 & 4) and unstressed (treatments 1 & 2) plants, respectively.

Plants growing under PEG-induced water stress (treatments 3 and 4) used ($P < 1\%$) more water per unit of increase in plant fresh weight than those not exposed to stress (treatments 1 and 2). The values obtained were 87 ml and 66 ml of water consumed per 1 g of increase in plant fresh weight, respectively.

The influence of PEG-induced water stress and sprays with complete nutrient solution on mineral content of apple seedlings is summarized in Table 2. Water stress lowered leaf Ca, root Mg and K but increased root Ca. Leaf sprays with a complete nutrient solution increased Mg concentration in the leaves and stems and Ca concentration in stems.

There existed a significant ($P < 5\%$) interaction between PEG and spray treatments on root phosphorus level. In unsprayed trees, root phosphorus levels were 0.58 and 0.38% with and

Table 1. Analysis of variance for apple seedlings water consumption.

Source of variation	df	MS	Significance
PEG	1	514	**
Spray	1	110	NS
PEG × Spray	1	17	NS
Day	13	---	---
PEG × Day	13	2.3	**
Spray × Day	13	0.4	NS
PEG × Spray × Day	13	0.5	NS

NS, **Not significant (NS) or significant at the 1% level (**).

Table 2. The effect of PEG-induced water stress or leaf sprays on mineral content of 'York Imperial' apple seedlings (% dm).

PEG treatment	Leaves		Stems		Roots		
	Ca	Mg	Ca	Mg	Ca	Mg	K
<i>PEG treatments</i>							
Unstressed	1.16'	---	---	---	0.67	0.21	1.17
Stressed	0.97	---	---	---	0.82	0.17	0.96
<i>Spray treatments</i>							
Unsprayed	---	0.31	0.49	0.11	---	---	---
Sprayed	---	0.39	0.56	0.14	---	---	---

'Numbers within each column differ significantly at the 5% level.

without PEG, respectively. However, the sprayed plants produced root phosphorus levels of 0.59% and 0.63% with and without PEG.

Photosynthetic study. Day-to-day changes in P_n rate showed different patterns for sprayed (treatments 2 and 4) and unsprayed (treatments 1 and 3) trees (Fig. 2). There was a significant spray × day interaction (Table 3). Over a period of days, the PEG, as well as sprays, lowered P_n rate (Fig. 2, Table 3). The effect of sprays was more pronounced than that of PEG.

Stomatal conductance (C_s) data showed similar patterns to those of the P_n data (Fig. 3). Also, the spray × day interaction was significant (Table 3). Over a period of days, sprays, as well as PEG-induced water stress, decreased C_s (Fig. 3, Table 3). However, stomatal conductance of the plants treated only with PEG (treatment 3) on days 4 and 5 almost equaled the C_s from unstressed and unsprayed trees (treatment 1). Regression analysis between P_n and C_s produced a significant positive correlation (Fig. 4).

Sprays, as well as PEG-induced water stress, decreased mesophyll conductance (data not shown). Regression analysis between P_n and C_m also showed a significant positive correlation (Fig. 5).

Discussion

PEG-induced water stress at - 1.0 bar diminished plant water consumption (Fig. 1) and plant fresh weight gain and also diminished C_s (Fig. 3) and P_n rate (Fig. 2). These responses are often observed on plants suffering from water stress (2, 5, 6, 8). Sprays with complete nutrient solution caused similar responses, and as far as C_s and P_n are concerned, the effect of sprays was even more pronounced than that of PEG. The question remains as to why the complete nutrient solution sprays reduced stomatal conductance and as a consequence, lowered photosynthesis. Since the spray solution had a pH of 4.4, it

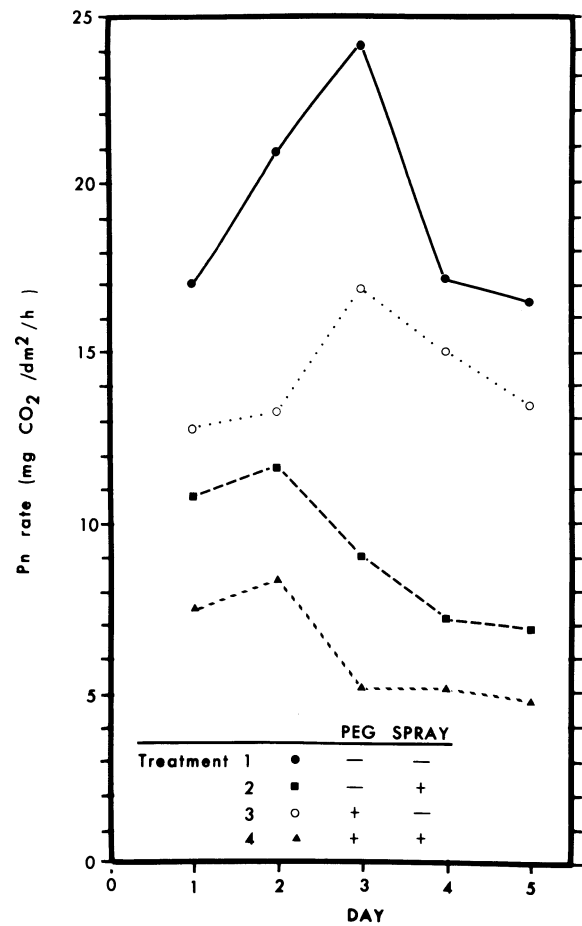


Fig. 2. The effect of PEG-induced water stress (— 1.0 bar) and sprays with complete nutrient solution on P_n rate in leaves of 'York Imperial' apple seedlings.

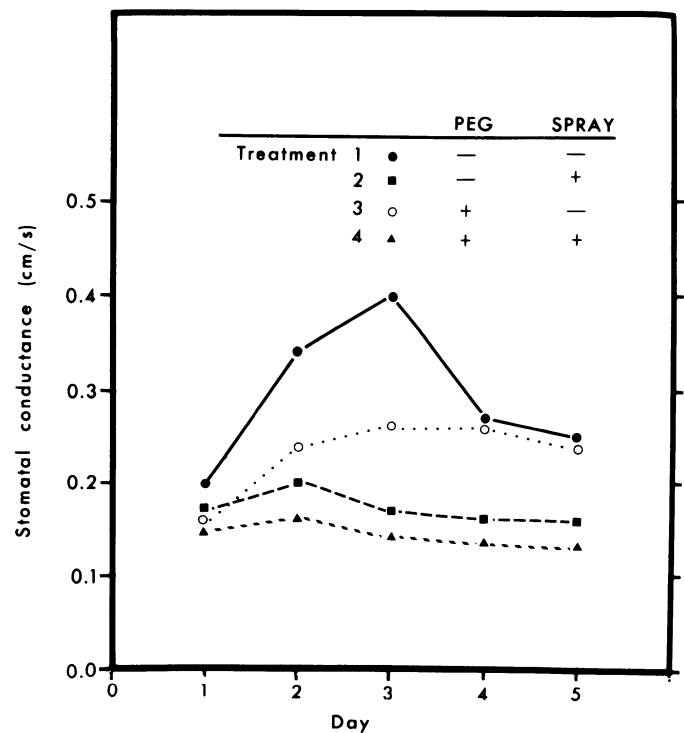


Fig. 3. The effect of PEG-induced water stress (— 1.0 bar) and sprays with complete nutrient solution on stomatal conductance in leaves of 'York Imperial' apple seedlings.

Table 3. Analysis of variance of P_n rate and stomatal conductance (C_s).

Source of Variance	P_n			C_s	
	df	MS	Significance	MS	Significance
PEG	1	160	*	12	*
Spray	1	85	**	54	**
PEG × Spray	1	12	NS	0.2	NS
DAY	4	---	---	---	---
PEG × Day	4	6.3	NS	0.3	NS
Spray × Day	4	21	*	2.0	*
PEG × Spray × Day	4	2.8	NS	0.3	NS

NS, *, **Nonsignificant (NS) or significant at 5% (*) or 1% (**) level.

could be due to acidity (10). It is also possible that stomatal closure was stimulated by the presence of Ca and/or surfactant in the spray solution since these have been reported to reduce stomatal aperture (16). Stomatal closure due to nutrient sprays cannot be linked with damage caused by sprays, since no injuries were observed.

It is noteworthy that sprays applied to stressed plants further decreased water consumption, P_n , and C_s (Fig. 1, 2, and 3). One can speculate that lower stomatal conductance and lower water consumption might allow plants to maintain a better balance between water uptake and loss, thus avoiding depletion of water under stress conditions (15). It has to be emphasized, however, that sprays applied to unstressed apple seedlings were not beneficial in terms of plant growth and photosynthesis.

The lower P_n of stressed and sprayed trees can be attributed to the lower stomatal conductance and possibly to lower mesophyll conductance. Net photosynthesis positively correlated to C_s and C_m over the treatments applied (Fig. 4 and 5). These data are consistent with previous reports for species other than fruit trees that water stress increases both stomatal and nonsto-

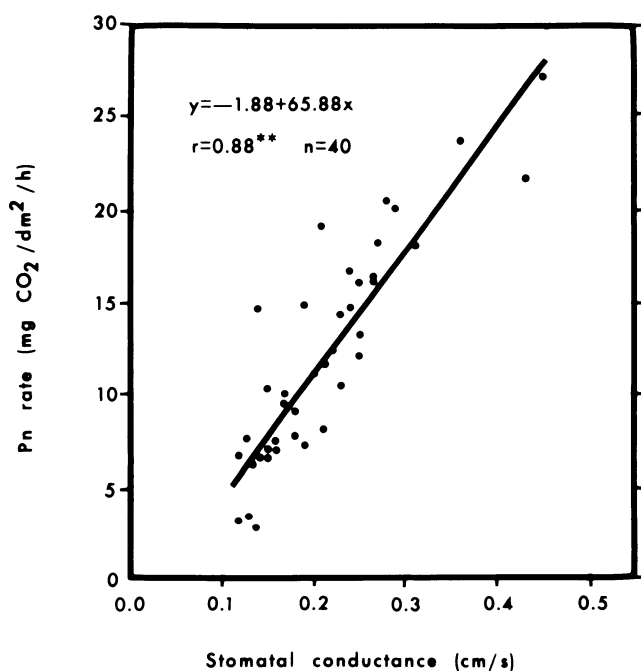


Fig. 4. Regression analysis of P_n rate on stomatal conductance for leaves of 'York Imperial' apple seedlings.

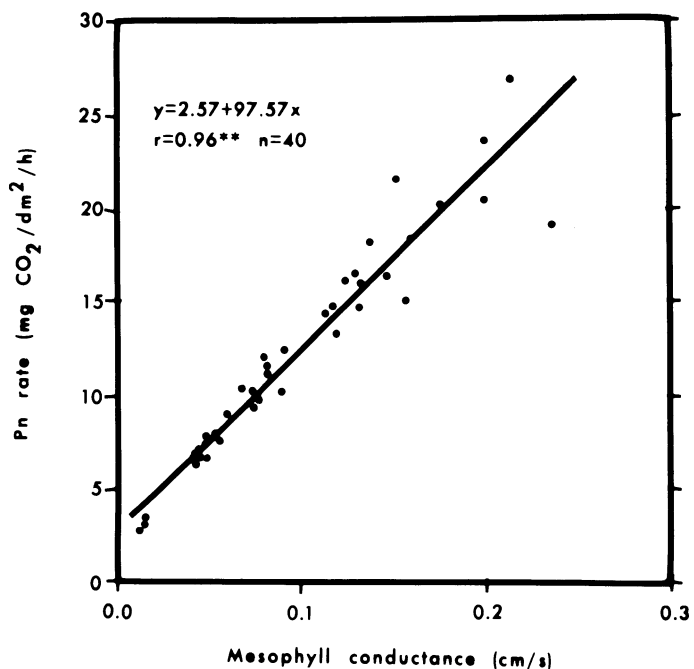


Fig. 5. Regression analysis of P_n rate on mesophyll conductance for leaves of 'York Imperial' apple seedlings.

mal limitations of photosynthesis (7, 8, 14). It has to be stressed, however, that since the C_m was calculated assuming constant (zero) sink CO_2 concentration in the leaf, certain reservations must be considered as far as C_m values are concerned. Our C_m values are lower compared to values calculated using CO_2 compensation point. Moreover, it is not known whether lower C_m in stressed and/or sprayed plants was caused by a lower capacity for CO_2 fixation, or by higher light respiration (1). Our C_m values, however, allow us to conclude that stomata was not the sole cause of P_n reduction in stressed and/or sprayed plants.

Although the duration of the experiments was only 2 weeks, it was shown that water stress decreased the concentration of leaf Ca and root K and Mg (Table 2). This supports the view that water stress may lower the absorptive capacity of the roots (18). It was found before (6) that PEG-induced water stress decreased both the uptake of ^{45}Ca and the total amount of ^{45}Ca transported toward the top of the plant. Moreover, photosynthetic inhibitors were also reported to decrease Ca uptake and transport within plants (3, 4). In the present studies, the total uptake of Ca was not measured, but increased concentration of this element in the roots and decreased levels in the leaves (Table 2) support the idea that its upward transport can be strongly suppressed in response to water stress. The question whether lowered Ca concentration in the leaves was fully compensated for by its higher concentration in the roots remains unanswered.

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