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Physiological and Nutritive Effects of K-Pretreatment and KCl Sprays on Water-stressed and Unstressed Apple Seedlings[1]

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Abstract. **Low- and high-K pretreated 'York Imperial' apple seedlings (*Malus domestica* Borkh.) were grown in nutrient solution cultures. Addition of polyethylene glycol (PEG) to the nutrient solution to reduce water potential to −1.0 bar reduced water consumption, fresh weight, specific leaf weight (SLW), and leaf water potential and increased the amount of water consumed per unit of fresh weight gain. High-K pretreatment increased water consumption of unstressed seedlings but decreased water consumption of PEG-stressed plants. Daily sprays with 0.5% KCl applied in early afternoon had no effect on water consumption rate in apple seedlings. However, sprays probably induced wider stomatal opening, since K-sprayed trees had lower leaf water potential when measured at noon than unsprayed trees. This effect was not observed when water potential was measured in the morning (0800 HR). High-K plants had higher leaf water potential than low-K plants in the morning. Potassium pretreatment and PEG stress as well as K-sprays had numerous effects on plant mineral composition. The K-pretreatment or K-sprays did not alleviate the detrimental effects of PEG-induced water stress despite the effects of K-pretreatment and K-sprays on mineral composition and leaf water potential.**

Potassium has a strong influence on plant-water relations. Reduced transpiration under conditions of K deficiency has been reported for McIntosh apple trees (1) as well as for other plant species (8, 9). The effect has been shown to be due to greater stomatal resistance of these plants (7, 8, 9). There is considerable evidence that increased stomatal opening is induced by higher guard cell turgor pressure due to K influx (5, 11). However, it was found that high-K tea plants had higher cuticular resistance to water vapor than low-K plants (7).

Root permeability to water was found to be greatly decreased in sugar beets due to K deficiency (5). We have shown that water stress can lower apple seedling root K concentration in a relatively short period of time (16).

Water stress induced by polyethylene glycol (PEG) in nutrient solutions decreased water consumption, stomatal conductance, and net photosynthesis rate (P_n) in apple seedlings (16). Later, we examined the effect of K_2SO_4 sprays on water consumption, stomatal performance and P_n in water-stressed and unstressed apple seedlings (17). The results showed that K_2SO_4 sprays had no effect on unstressed plants but caused stomatal closure and reduced P_n in water stressed plants. Recently, it was reported

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(10) that the positive effect of K on stomatal opening depended on the accompanying anion. Chloride anion along with K^+ cation increases the turgor of guard cells (10) and acts as a counterion for K ions (12) as well.

The aim of the present study was to examine the effect of K pretreatment and KCl sprays on apple seedling performance under water stress conditions induced by addition of PEG to the nutrient solution.

Materials and Methods

The experiment was conducted in a greenhouse. Day temperature maxima varied between 23 and 37°C, and air relative humidity maxima varied between 34 and 70%.

Three-month-old 'York Imperial' apple seedlings, germinated in sand flats, were cut back and placed in 1-liter brown plastic bottles (2 plants/bottle) filled with nutrient solution. Half of the plants were transferred, when about 15 cm in height, to low-K nutrient solution (low-K pretreatment), and half received the original solution (high-K pretreatment). Nutrient solution compositions are presented in Table 1. Plants were allowed to grow under these K pretreatment conditions for 2 weeks. The effect of the low or high K pretreatments on nutritional status of apple seedlings was checked by harvesting 6 plants from each treatment (Table 2). No visual symptoms of K deficiency were noted. After pretreatment, uniform low-K plants (12 bottles × 2 plants/bottle) and high-K plants (12 bottles × 2 plants/bottle) were placed in high-K solution and treated as follows: 1) control, untreated; 2) sprayed with KCl (0.5%) everyday in early afternoon; 3) water-stressed by addition of polyethylene glycol (PEG) to the nutrient solution to create an osmotic potential of −1.0 bar (i.e., 35.2 g Carbowax[4] 4000, MW 3500–3700 per 800 ml of nutrient solution); 4) same as treatment 3, but sprayed with KCl (0.5%) every day in early afternoon. Nutrient solutions were changed weekly and continuously aerated in all phases of growth.

Daily (day and night) water consumption rate of the plants was measured during 15 consecutive days by weighing the bottles at 24-hr intervals and calculating water loss. Evaporative loss was obtained by weighing bottles without plants every day. The values obtained were used to calculate plant net water consumption rate, expressed on a leaf area basis. 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 conveyor.

Leaf water potential (γ_w) was measured on 4 detached medium leaves from each of 4 seedlings using a pressure bomb (14). Leaf water potential measurements were made twice, noon of day 13 and morning (0800 HR) of day 14. After γ_w measurements were made, leaf area and dry matter (80°C for 48 hr) were determined. Specific leaf weight (SLW) was calculated as the ratio between dry matter to leaf area (mg/cm^2).

After day 15, seedlings were harvested and the increase in fresh weight was calculated from the difference of seedling weight at the beginning and end of the experiment. Leaves were washed in the solution of 0.1 N HCl + 0.1% Tween 20[4] and rinsed twice with demineralized water. Roots and leaves were oven-dried (80°C for 48 hr), 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 6 N HCl. K, Ca, and Mg concentrations were determined by atomic absorbtion spectrophotometry using standard methods and P was determined colorimetrically by the vanado-molybdo-phosphoric method (6).

Table 1. Composition of nutrient solutions.

Element	High-K	Low-K	Nutrient Source
N	5 mM	5 mM	$NH_4H_2PO_4$ $Ca(NO_3)$ $4H_2O$
P	1 mM	1 mM	$NH_4H_2PO_4$
K	2 mM	0.2 mM	K_2SO_4
Ca	3.3 mM	3.3 mM	$Ca(NO_3)_2$ $4H_2O$ $CaCl_2$ $2H_2O$
Mg	1.5 mM	1.5 mM	$MgSO_4$ $7H_2O$
B	0.55 ppm	0.55 ppm	H_3BO_3
Fe	5.83 ppm	5.83 ppm	Na-Fe EDTA
Cu	0.25 ppm	0.25 ppm	$CuSO_4$ $5H_2O$
Mn	0.50 ppm	0.50 ppm	$MnSO_4$ H_2O
Mo	0.50 ppm	0.50 ppm	H_2MoO_4
Zn	0.50 ppm	0.50 ppm	$ZnSO_4$ $7H_2O$

Table 2. Mineral composition of low-K and high-K 'York Imperial' apple seedlings after K-pretreatment period. Each value is an average of 3 replications (2 plants per replication).

K pretreatment	Mineral composition (% dry wt)			
	P	K	Ca	Mg
	Leaves			
Low	0.32 ± 0.03	0.66 ± 0.21	0.99 = 0.23	0.30 ± 0.03
High	0.35 ± 0.05	1.24 ± 0.24	0.76 ± 0.03	0.21 ± 0.02
	Roots			
Low	0.76 ± 0.18	0.35 ± 0.03	0.90 ± 0.20	0.13 ± 0.01
High	0.81 ± 0.09	0.72 ± 0.07	0.83 ± 0.16	0.16 ± 0.02

Results and Discussion

Throughout the course of the experiment, polyethylene glycol-induced water stress (−1.0 bar) reduced water consumption of apple seedlings, irrespective of K-pretreatment and K-sprays (Table 3, Fig. 1). Similar responses to PEG-induced water stress at −1.0 bar were observed in previous studies (4, 16). There was also a significant K-pretreatment × PEG × day interaction on apple seedlings' water consumption rate (Table 3). High K-pretreatment as compared to low K-pretreatment had a stimulative effect on water consumption in unstressed plants, but the

Table 3. Analysis of variance of apple seedling water consumption rate data from the low- or high-K-pretreated seedlings treated with PEG or treated with PEG and KCl-sprayed.

Source of variation	df	MS	Significance
K-pretreatment (K)	1	17.3	NS
Spray	1	0.1	NS
PEG	1	1656.7	**
K × Spray	1	5.1	NS
K × PEG	1	11.5	NS
Spray × PEG	1	13.5	NS
K × Spray × PEG	1	70.1	NS
Day	14	——	——
K × Day	14	0.7	NS
Spray × Day	14	0.2	NS
PEG × Day	14	20.6	**
K × Spray × Day	14	0.3	NS
Spray × PEG × Day	14	0.5	NS
K × PEG × Day	14	2.2	**
K × Spray × PEG × Day	14	0.4	NS

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

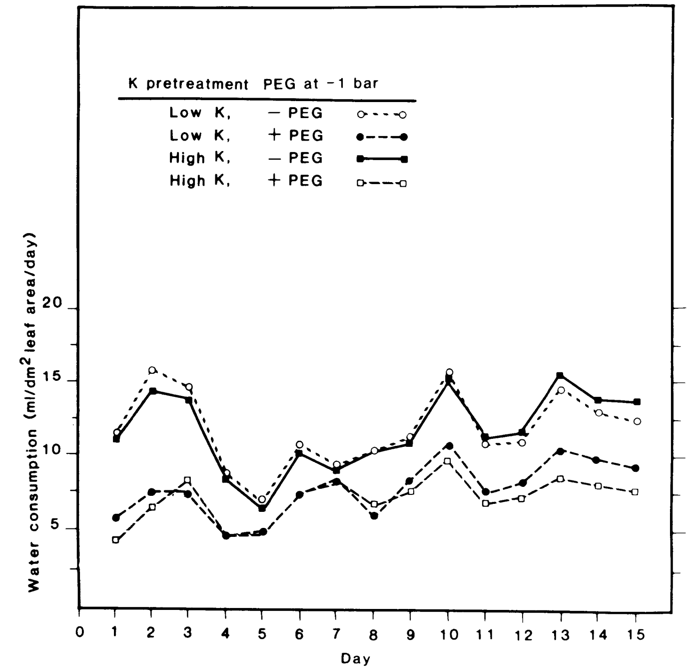

Fig. 1. Daily water consumption rates of 'York Imperial' apple seedlings pretreated by growing in either low or high K nutrient solution which were unstressed or stressed (− 1.0 bar) by addition of polyethylene glycol (PEG).

opposite effect was observed when plants were PEG-stressed (Fig. 1). This phenomenon was only observed in the last 5 days of the experiment (Fig. 1). Enhanced water consumption of high-K plants might be attributed to wider stomatal opening (5, 9). High-K apple trees and tea plants were reported to have higher transpiration rates than K-deficient plants (1, 8). However, it is not known why high-K pretreatment lowered water consumption compared to low K plants in PEG-stressed plants (Fig. 1). This result is also in contrast with that obtained by Nagarajah and Ratnasurija (8) for tea, which showed that the magnitude of reduction in transpiration due to water stress was higher in low- vs. high-K plants.

The effect of K-pretreatment on water consumption of unstressed and PEG-stressed plants was noted only in the last 5 days of the experiment (Fig. 1). No definitive explanation for this phenomenon can be offered. However, it can be suggested that small differences in water consumption due to K-pretreatment during the first 20 days were caused by limited precision in measuring total leaf area. Total leaf area on each tree was measured at the end of the experiment. Leaf growth is a dynamic process, and it is impossible to overcome errors. Thus, total leaf area measured at the end of the experiment more accurately reflects the actual leaf area of the seedlings during the last days of the experiment.

Reduction in leaf γ_w in stressed compared to unstressed plants in the morning and at noon (Table 4) indicates that the level of water stress applied was high enough to affect plant water balance. At noon, however, the magnitude of reduction in leaf γ_w due to PEG stress depended on K-pretreatment and K-spray treatment. This was reflected by significant K-pretreatment × PEG and spray × PEG interactions (Table 4). The most pronounced effect of PEG was observed in unsprayed low-K plants (Table 4).

Leaf γ_w of high-K plants was higher compared to low-K plants when measurements were performed in the morning (Table 4).

Table 4. The effect of K-pretreatment, PEG, and K-sprays on leaf water potential of apple seedlings measured at noon on day 13 and in the morning (0800 HR) on day 14.

Treatment		Leaf water potential (bars)			
		Day 13		Day 14	
		K-sprays		K-sprays	
K	PEG	−	+	−	+
Low	−	− 13.0	− 18.0	− 14.5	− 15.0
	+	− 19.5	− 19.0	− 16.5	− 16.0
High	−	− 16.0	− 19.0	− 13.5	− 12.5
	+	− 17.0	− 20.0	− 15.0	− 16.0

ANOVA table

Source of variation	df	Day 13 MS	Day 13 Significance	Day 14 MS	Day 14 Significance
K-pretreatment (K)	1	1.1	NS	18.8	**
Spray	1	60.5	**	1.3	NS
PEG	1	40.5	**	27.2	**
K × Spray	1	0.5	NS	0.6	NS
K × PEG	1	12.5	*	4.9	NS
Spray × PEG	1	24.5	**	0.1	NS
K × Spray × PEG	1	21.1	**	4.9	NS

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

Table 5. Stomatal resistance of 'York Imperial' apple seedlings as affected by sprays with different potassium salts. Stomatal resistance was measured on 9 replications (3 plants) with a diffusive resistance porometer, LI-60, in growth chamber at 400 $\mu E\ m^{-2}\ s^{1}$ photosynthetically active radiation and day/night temperatures of 25/20°C and 65% relative humidity.

Treatment	Stomatal resistance (s/cm) Before spray	Time after spray 2 hr	Time after spray 24 hr
Control-unsprayed	4.10 ± 1.3ᶻ	3.5 ± 0.9	4.6 ± 0.4
KCl	4.10 ± 1.1	2.8 ± 1.3	3.5 ± 0.6
KNO_3	4.00 ± 1.3	3.7 ± 1.5	4.1 ± 1.2
K_2SO_4	4.10 ± 1.8	3.6 ± 1.2	6.0 ± 1.6

ᶻ± SD.

It must be added, however, that K-pretreatment did not affect leaf γ_w at noon.

The K-sprays lowered leaf γ_w when measurements were taken at noon, except for stressed low-K plants (Table 4). However, K-sprays did not affect γ_w when measurements were performed in the morning (Table 4). This suggests that K-sprays could induce stomatal opening at full sunlight, except for water-stressed, low-K plants, and consequently could reduce γ_w. Since we did not measure diurnal stomatal resistance in the present study, we cannot confirm this supposition. However, in a separate experiment, we found that KCl sprays lowered stomatal resistance of apple seedlings, whereas K_2SO_4 tended to show an opposite effect (Table 5).

Potassium-pretreatment had numerous effects on seedling mineral composition. High-K pretreatment increased K in leaves and roots and decreased P, Ca, andMg in leaves compared to low-K pretreatment (Table 6). PEG-induced water stress decreased the level of Ca and Mg in the leaves and Mg in the roots compared to unstressed trees (Table 6). There was a significant ($P < 1\%$) PEG × K-pretreatment interaction on root K. Low-K plants which were unstressed and stressed contained 0.57 and 0.59% respectively, whereas unstressed and stressed high-K plants contained 0.84 and 0.70% K, respectively. This result implies that root K was reduced by PEG stress but only in high-K plants. In our previous work (16) we also found that PEG stress lowered root K in apple seedlings. The apple seedlings used in that experiment can be considered here as high-K plants. The above data indicate that PEG-induced water stress decreased the absorption capacity of the roots (4, 16, 17, 18), and this effect can be noted in as short a period of time as 2 weeks. Clark (2) reported that an osmotic water stress (− 1.0 bar) decreased N, P, and Rb concentrations in wheat within 24 hr. This was before dry matter decreased due to the applied stress. It must be emphasized, however, as reported previously (16), that water-stressed seedlings had higher Ca content in roots than unstressed seedlings (Table 6). This indicates that the lower leaf-Ca level was a result of supressed Ca transport from roots and/or decreased absorption capacity of the roots. Independent of the reason of low Ca content in leaves, it is obvious that water stress can have a negative effect on Ca levels in apple trees and consequently may decrease storage quality of fruits.

PEG treatment increased K level in the leaves (Table 6). Water stress also increased root P level (Table 6). A similar effect of − 1.0 bar PEG-stress on root P level was obtained previously (16). The effect of PEG-induced water stress on P concentration in plants depends on the magnitude and intensity of the applied stress (2). Resnick (13) showed that when PEG is used as an osmoticum, nutrient solution water potential has to be reduced below −5.0 bars to decrease P uptake by maize. However, much higher water potentials of root medium reduced upward translocation of P within the plant. Thus, it is possible that suppressed upward translocation of P, due to the water stress applied in the present study, caused accumulation of P in roots which resulted in elevated concentrations. If this is true, lower P concentration in the leaves should be expected. This was not found, probably because of reduced growth of the PEG-stressed plants. More studies are needed to explain the effect of various levels of PEG-induced water stress on P and K uptake and translocation in apple trees.

There was a significant ($P < 1\%$) K-pretreatment × spray interaction on root K. Root K levels in sprayed and unsprayed low-K plants were 0.64 and 0.52%, respectively, whereas sprayed and unsprayed high-K plants contained 0.77 and 0.76% K, respectively. Therefore, potassium sprays increased root K level only in low-K plants. This suggests that in high-K plants, K absorbed from sprays by the leaves was not transported to the roots but stayed in aboveground tissues. This is supported by the fact that, independent of K-pretreatment, K-sprayed trees contained more K in their leaves compared to unsprayed trees (Table 6). Potassium is readily absorbed by apple leaves from sprays (15).

PEG-induced water stress reduced fresh weight gain of plants expressed per unit of leaf area (Table 7). High K-pretreatment increased fresh weight gain of unstressed seedlings having no effect on PEG-stressed plants (Table 7).

High K-pretreatment lowered the amount of water used per 1 g of fresh weight increase in unstressed plants but had no effect in stressed seedlings (Table 8). Unstressed seedlings used significantly less water per 1 g fresh weight gain compared to stressed plants (Table 8). Irrespective of K-pretreatment and K-sprays, PEG stress decreased ($P < 1\%$) specific leaf weight (SLW). SLW was 7.0 and 6.5 mg/cm^2 from unstressed and stressed plants, respectively.

In view of the above discussion, it can be concluded that plant K status or K-sprays did not alleviate the effects of PEG-induced water stress in terms of water consumption, fresh weight increase, water use per unit of fresh weight gain, or SLW, despite the effects of K-pretreatment and K-sprays on mineral composition of the plants and γ_w. It must be emphasized, however, that low-K level in unstressed plants was a limiting factor for growth, since unstressed high-K plants had higher fresh weight gain (Table 7) and used less water per unit of fresh weight gain (Table 8) compared to unstressed low-K plants. Thus, in terms of plant growth and water use per unit of fresh weight gain, the effects of PEG-stress and low-K pretreatment were not additive.

Table 6. The effect of K-pretreatment, PEG, and K-sprays on mineral content of 'York Imperial' apple seedlings.

	Mineral content (% dry wt)							
	Leaves				Roots			
Treatment	P	K	Ca	Mg	P	K	Ca	Mg
			K-pretreatment					
Low-K	0.30	0.93	1.31	0.36	0.56	0.58	0.59	0.14
High-K	0.27	1.40	1.05	0.27	0.54	0.77	0.62	0.13
	*	**	**	**	NS	**	NS	NS
			PEG-treatment					
Unstressed	0.29	1.06	1.27	0.33	0.48	0.70[z]	0.43	0.14
Stressed	0.28	1.27	1.08	0.30	0.62	0.64	0.77	0.12
	NS	**	**	**	**	**	**	*
			K-spray treatment					
Unsprayed	0.29	1.04	1.19	0.32	0.52	0.64[y]	0.55	0.13
Sprayed	0.28	1.29	1.16	0.32	0.57	0.70	0.65	0.14
	NS	**	NS	NS	NS	**	NS	NS

[z]See text for PEG × K-pretreatment interaction on root K levels.
[y]See text for K-pretreatment × spray interaction on root K levels.
NS, *, **Nonsignificant (NS) or significant at the 5% (*) or 1% (**) levels.

Table 7. Fresh weight increase of apple seedlings per unit leaf area as affected by K-pretreatment and PEG treatment.

PEG treatment	Fresh wt increase (g/dm^2) K pretreatment Low	High	Mean
−	2.0	2.6	2.3
+	1.0	1.0	1.0

ANOVA table

Source of variation	df	MS	Significance
K-pretreatment (K)	1	0.3	NS
Spray	1	0.1	NS
PEG	1	10.5	**
K × Spray	1	0.0	NS
K × PEG	1	0.6	*
Spray × PEG	1	0.0	NS
K × SPRAY × PEG	1	0.2	NS

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

Table 8. The effect of K-pretreatment and PEG on amount of water used per 1 g fresh weight increase in apple seedlings.

PEG treatment	Water used (ml/g)		
	K pretreatment		
	Low	High	Mean
–	84	67	76
+	105	112	109

Source of variation	ANOVA table		
	df	MS	Significance
K-pretreatment (K)	1	117	NS
Spray	1	330	NS
PEG	1	6567	**
K × Spray	1	402	NS
K × PEG	1	950	*
Spray × PEG	1	0	NS
K × Spray × PEG	1	0	NS

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

The measurements of γ_w suggested that the KCl sprays might induce stomatal opening of apple seedlings at full sunlight. However, more research is needed to evaluate the effect of different forms of K-sprays on stomatal opening of apple seedlings in different light conditions in order to confirm this supposition. Such knowledge could provide a valuable tool for controlling the physiological processes in plants.

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