

Effect of Mineral Nutrient Sprays on Photosynthesis and Stomatal Opening of Water-stressed and Unstressed Apple Seedlings II. Potassium Sulfate Sprays¹

Dariusz Swietlik, R. F. Korcak, and Miklos Faust²

U.S. Department of Agriculture, Agriculture Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705

Additional index words. mineral nutrition, plant water consumption, polyethylene glycol, transpiration, *Malus domestica*

Abstract. 'York Imperial' apple seedlings (*Malus domestica* Borkh.) grown in nutrient solution cultures with decreased water potential to -1.0 bar by polyethylene glycol (PEG) increased water consumption, photosynthesis rate (P_n), and stomatal conductance (C_s). High light preconditioning of the plants used in this experiment was probably the reason why -1.0 bar water potential in the nutrient solution was not low enough to induce apple seedling responses typical of water-stressed plants. However, application of PEG stress (-1.0 bar), to K-sprayed (K_2SO_4 , -0.5%) trees lowered seedling water consumption P_n , and C_s . Potassium sprays alone did not significantly affect water consumption, P_n or C_s . When the water potential of the nutrient solution of PEG stressed plants was further decreased to -2.5 bars, unsprayed trees started to wilt within 2 days while sprayed trees did not. It is proposed that earlier stomatal closure of K-sprayed trees when stressed, already at low level of water stress (-1.0 bar), prevented plant water depletion when stress level was increased. This in turn delayed commencement of plant wilting. Potassium sprays also increased root:shoot ratio and root K concentration in PEG-stressed plants. These responses of K-sprayed trees could also contribute to greater tolerance to higher levels of water stress.

Apple seedlings grown under PEG-induced water stress have shown reduced water consumption, growth, and stomatal conductance and lowered photosynthesis rate (18). Spraying water-stressed seedlings with complete nutrient solution further decreased water consumption, net photosynthesis, and stomatal conductance.

It is well-documented that stomatal opening requires K influx to increase guard cell turgor (4, 6, 8, 13, 17). Potassium deficiency has been reported to increase stomatal diffusion resistance in different plant species (6, 11, 14).

The aim of the present study was to determine the effect of K sprays on water consumption, growth, photosynthesis, and stomatal diffusion conductance of apple seedlings grown under PEG-induced water stress.

Materials and Methods

Growth and nutrient uptake determinations. Open-pollinated 'York Imperial' apple seedlings were grown in flats of sand in the greenhouse. When approximately 40 cm in height, plants were selected according to uniformity and transferred to 1-liter, wide-mouth, brown plastic bottles (1 plant per bottle) filled with continuously aerated nutrient solution. The plants were allowed

to grow for 3 weeks in the greenhouse with supplemental light [$800 \mu\text{Em}^{-2}\text{s}^{-1}$ photosynthetically active radiation (PAR)] for 8 hr to ensure undamaged roots prior to PEG additions. Nutrient solution and PEG type were the same as described by Gergely et al. (5). Eight plants were exposed to a PEG-induced water solution potential of -1.0 bar (stressed), and an additional 8 plants (unstressed) were grown in regular nutrient solution. One-half of either the stressed or unstressed trees were sprayed daily (in the morning) with $0.5\% K_2SO_4 + 0.1\% v/v$ Tween 20³ (pH = 6.5); the other half were left unsprayed. The duration of the experiment, consisting of 4 treatments, was 15 days. After day 8, nutrient solutions were changed and the osmotic potential of nutrient solutions of the stressed plants was further reduced to -2.5 bars.

Daily water consumption and leaf area were determined as reported previously (18). Increase in plant fresh weight was calculated from the difference at the beginning and end of experiment. Plants were harvested after day 15 and divided into roots, stems, and leaves, oven-dried (48 hr at 70°C), weighed, and then ground to pass a 20-mesh sieve. Root to shoot ratio (dry weight/dry weight) was calculated. Tissue samples (0.500 ± 0.005) were dry-ashed at 500° overnight and the residue was dissolved in 6N HCL. K, Ca, and Mg concentrations were determined by atomic absorption spectrophotometry and phosphorus was determined colorimetrically by the molybdo-vanado-phosphoric method (9).

Photosynthesis determinations. The photosynthetic determinations were conducted in growth chambers with 12 hr of light at $1000 \mu\text{Em}^{-2}\text{s}^{-1}$ (PAR). Day/night temperatures and humidities were $26^\circ/20^\circ\text{C}$ and 60/90%, respectively. Treatments, plant material and nutrient solution were the same as those in the growth studies. However, nutrient solution osmotic potential for the stressed plants was kept at -1.0 bar throughout the entire experiment, and each treatment consisted of 2 plants. From each plant, the youngest fully expanded leaf was selected for photosynthesis and stomatal conductance determinations.

¹Received for publication Sept. 21, 1981.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

²Visiting Research Horticulturist, Research Institute of Pomology, Skierniewice, Poland; Soil Scientist and Plant Physiologist, respectively, Fruit Laboratory, Horticultural Science Institute. The authors wish to express their gratitude to James Bunce for his assistance and thoughts on the photosynthesis portion of this work.

³Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

Initial net photosynthesis rate (P_n) and stomatal conductance (C_s) measurements were made on day 0. Nutrient solutions were then changed and PEG (-1.0 bar) was added. K sprays were applied on day 1 and daily for the entire length of the experiment. Sprays were applied always in the morning about 2 hr before the start of photosynthetic measurements. Net photosynthesis rates, transpiration rates, and leaf temperatures were measured in an open system previously described (18). Air flow through the system was $4 \text{ liters min}^{-1}$. Stomatal conductance (C_s) to water vapor was calculated from the transpiration rate and the difference between water vapor pressure inside the leaf and ambient air.

The values of P_n and C_s obtained at day 0 were used to calculate the relative (Day 0 = 1.0) net photosynthesis rate and stomatal conductance.

Results

Growth and nutrient uptake determinations. There existed a significant PEG \times K-spray interaction on daily water consumption during the first 8 days (Fig. 1 and Table 1). Unsprayed trees stressed with PEG showed increased water consumption while sprayed trees had decreased water consumption when PEG-stressed (Fig. 1). When the nutrient solution osmotic potential of stressed plants was decreased to -2.5 bars, these responses were the same on days 9 and 10, although the PEG \times spray interaction was nonsignificant (Table 1). PEG at -2.5 bars (treatments 3 and 4) significantly reduced daily water consumption during days 11–15 compared to unstressed trees (treatments 1 and 2) (Fig. 1, Table 1). The PEG-stressed trees (treatment 3) displayed severe wilting symptoms (varying from marginal burn to total leaf necrosis). However, plants which were treated with PEG and sprayed (treatment 4) did not wilt. These plants consumed less water than control plants, but the magnitude of reduction was comparable to that when root medium water potential was -1.0 bar.

Water stress tended to reduce fresh weight increase of unsprayed trees. Fresh weight increase was 18.7 and 17.2 g from stressed plants which were sprayed or unsprayed, respectively. Fresh weight increase of unstressed seedlings which were sprayed or unsprayed was 18.7 and 18.4 g, respectively.

The PEG-induced water stress and K sprays applied together increased root:shoot ratio (dry weight/dry weight), whereas water stress alone caused an opposite effect (Table 2). Treatment with PEG lowered leaf Ca content in unsprayed trees but had no effect when trees were sprayed with K (Table 3).

Sprayed trees (treatments 2 and 4) contained higher ($P < 5\%$) K in the roots than unsprayed trees (treatments 1 and 3). Root

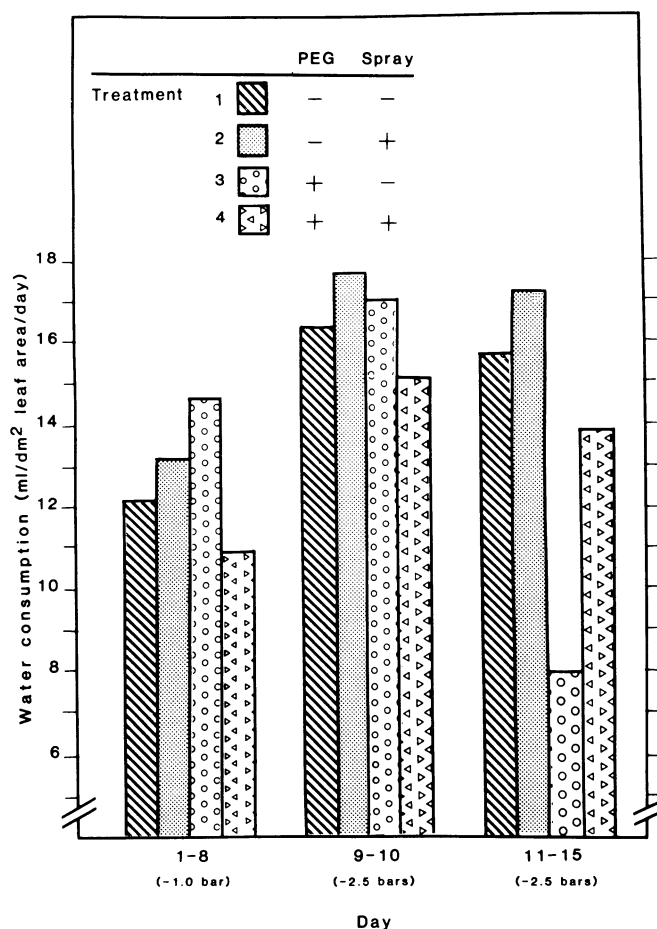


Fig. 1. 'York Imperial' apple seedling water consumption as affected by PEG and/or K_2SO_4 sprays.

K concentrations of sprayed and unsprayed plants were 1.16 and 1.00% K on a dry weight basis, respectively.

Photosynthesis determinations. Photosynthesis, which was determined in a separate growth chamber, changed daily and displayed different patterns from all treatments (Fig. 2). There was a significant PEG \times spray \times day interaction (Table 4), which implies that responses among treatments varied over time (Fig. 2). Photosynthesis at the end of the experiment was elevated for the PEG-treated trees (treatment 3) and reduced in those trees which were stressed and sprayed (treatment 4, Fig. 2).

Table 1. Analysis of variance for 'York Imperial' apple seedling water consumption.

Source of variation	Day 1-8 (PEG at -1.0 bar)			Day 9-10 (PEG at -2.5 bars)			Day 11-15 (PEG at -2.5 bars)		
	df	MS	Significance	df	MS	Significance	df	MS	Significance
PEG	1	1.2	NS	1	10	NS	1	483	*
Spray	1	20	NS	1	0.0	NS	1	224	NS
PEG \times spray	1	113	*	1	10	NS	1	81	NS
Day	7	---	---	1	---	---	4	---	---
PEG \times day	7	0.7	NS	1	1.2	NS	4	6.5	NS
Spray \times day	7	0.9	NS	1	1.7	NS	4	6.2	NS
PEG \times spray \times day	7	0.7	NS	1	5.2	NS	4	1.4	NS

NS, *Not significant (NS) or significant at 5% level (*).

Table 2. The effect of PEG and K₂SO₄ sprays on root:shoot ratio of 'York Imperial' apple seedlings.

PEG	Root:Shoot ratio (dry wt/dry wt)	
	K ₂ SO ₄ spray	
	-	+
-	0.48	0.48
+	0.38	0.59

ANOVA			
Source of variation	df	MS	Significance
PEG	1	0.03	NS
Spray	1	0.37	NS
PEG × spray	1	0.53	*

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

Table 3. The effect of PEG and K sprays on Ca concentration in 'York Imperial' apple leaves.

PEG	Ca concn (% dry wt)	
	K ₂ SO ₄ spray	
	-	+
-	1.36	1.22
+	1.13	1.22

ANOVA			
Source of variation	df	MS	Significance
PEG	1	0.06	*
Spray	1	0.00	NS
PEG × spray	1	0.06	*

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

Stomatal conductance displayed a pattern similar to that of P_n (Fig. 3). Photosynthesis was significantly correlated to stomatal conductance over the range of treatments applied (Fig. 4).

Discussion

Apple seedlings responded to PEG-induced water stress depending upon whether or not the trees were sprayed with K₂SO₄. The - 1.0 bar water stress (considered a mild water stress) applied alone had a stimulative effect on water consumption, P_n, and stomatal conductance (Fig. 1, 2, and 3). These responses can be explained in various ways. The slight (- 1.0 bar) water stress could decrease turgor pressure in epidermal cells and consequently decrease the pressure exerted by the epidermis on guard cells. This in turn could cause wider stomatal opening (17). However, application of a PEG stress of - 1.0 bar to K-sprayed trees lowered their water consumption (Fig. 1), P_n rate and C_s (Fig. 2 and 3). Thus, K sprays altered the response of plants exposed to - 1.0 bar water stress.

In our previous work (18), we reported that lowering nutrient osmotic potential to - 1.0 bar by addition of PEG induced apple seedlings responses typical of water-stressed plants. However, in the present study, the same level of osmotic potential (via PEG) was insufficient to induce such responses in unsprayed plants. This discrepancy is probably due to the high light (800 μEm⁻²s⁻¹) preconditioning of the plants used in the present study

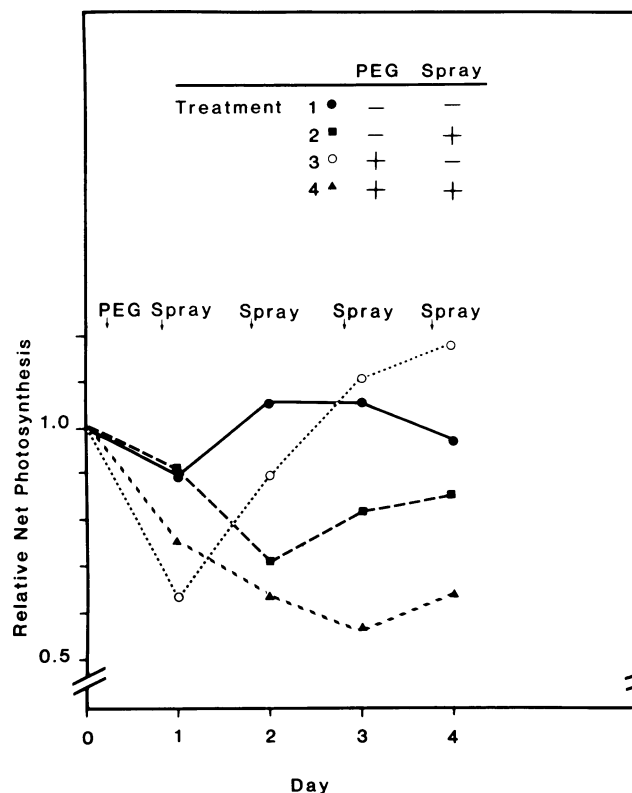


Fig. 2. Relative net photosynthesis of the 'York Imperial' apple seedlings from the PEG and/or K₂SO₄ spray treatments.

for 3 weeks prior to treatment applications. To the contrary, the plants reported in the previous paper (18) were grown in poor light conditions in the greenhouse prior to treatment applications. As shown by Davis and Lakso (2), apple trees preconditioned under high light were more capable to withstand subsequent drought than low-light preconditioned plants, as measured by stomatal conductance and P_n rate. Moreover, the plants preconditioned with high light had lower root resistance (3).

Reduced water consumption and stomatal closure occurred with the - 1.0 bar water stress and K sprays. These responses probably improved plant water balance. Earlier closure of stomata during development of water stress is believed to improve plant water balance and to prevent rapid water depletion at high level of stress (16). Nagarajach and Ratnasurja (12) also reported that earlier closure of stomata during development of water stress delays commencement of plant wilting. Indeed, when the water

Table 4. Analysis of variance for relative P_n rate and relative stomatal conductance (C_s) in 'York Imperial' apple leaves.

Source of variation	df	P _n		C _s	
		MS	Significance	MS	Significance
PEG	1	0.06	NS	0.11	NS
Spray	1	0.41	NS	0.37	NS
PEG × spray	1	0.04	NS	0.04	NS
Day	3	---	---	---	---
PEG × day	3	0.02	NS	0.01	NS
Spray × day	3	0.08	NS	0.08	NS
PEG × spray × day	3	0.04	*	0.05	NS

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

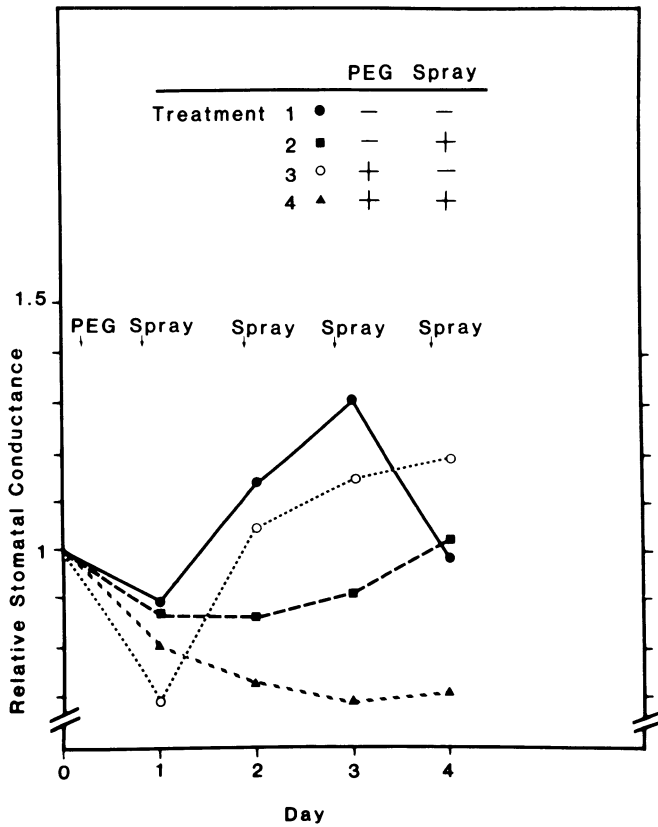


Fig. 3. Relative stomatal conductance of the 'York Imperial' apple seedlings from the PEG and/or K_2SO_4 spray treatments.

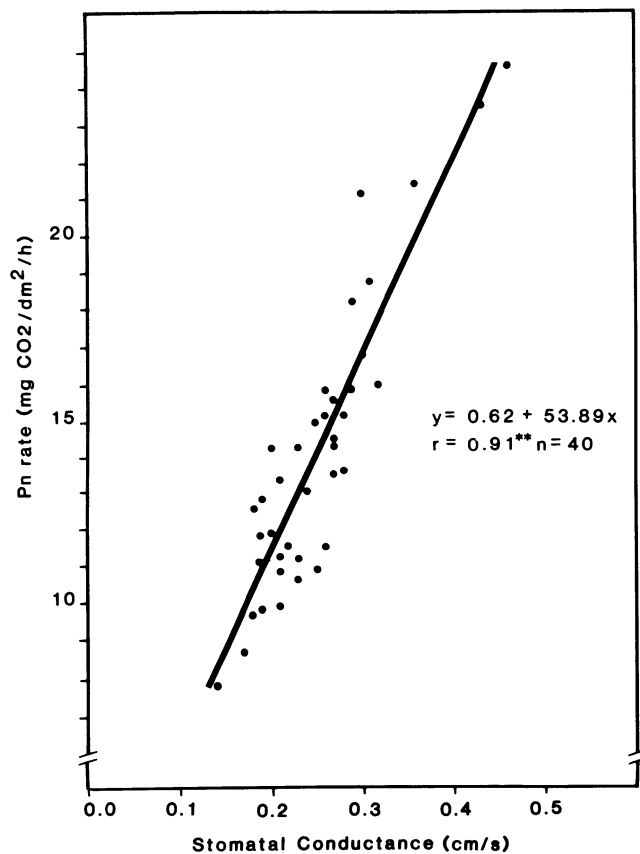


Fig. 4. Plot of the regression equation between stomatal conductance and photosynthesis rate (P_n) from apple seedlings treated with PEG and/or K_2SO_4 sprays.

potential of the nutrient solution was further decreased from -1.0 to -2.5 bars, the stressed but unsprayed plants (treatment 3) showed wilting symptoms within 2 days, whereas those simultaneously stressed and sprayed (treatment 4) did not. Thus, these results support previous reports (12, 16) that earlier closure of stomata during water stress development prevents rapid depletion of water and appearance of wilting symptoms. Although stressed and unsprayed trees (treatment 3) showed a drastically lowered water consumption during days 11–15 (Fig. 1), this effect was due mainly to a reduction in the transpiring leaf area caused by wilting and burning of the leaf edges.

It is noteworthy that K_2SO_4 sprays alone did not significantly affect water consumption (Fig. 1, Table 1), P_n or stomatal conductance (Fig. 2 and 3, Table 4).

Lack of wilting symptoms on sprayed trees at the -2.5 bar osmotic stress also could be attributed to higher root:shoot ratio (Table 2) and higher root K concentration. The importance of increased root weight and root:shoot ratio for plant tolerance to drought conditions has been reported (20, 21). In the present study, PEG-induced water stress decreased root:shoot ratio, which is consistent with the findings of Haber (7). However, K-sprayed trees exposed to PEG-induced water stress increased root:shoot ratio (Table 2). Potassium is known to elavate plant water uptake and decrease root resistance (1, 6, 16). Thus increased K content in the roots of sprayed plants could enhance water flow through the root. Since root resistance to water flow was not measured, the above supposition needs confirmation in future research.

The results obtained in the photosynthetic portion of this study support previous data (10, 18) that photosynthesis rate in apple leaves is strongly correlated to stomatal conductance.

The results of the present experiments show that under low-level water stress, K_2SO_4 sprays should not be applied since they decrease stomatal conductance and consequently photosynthesis. However, when water stress is expected to progress, earlier stomatal closure and reduced transpiration are of high importance. Stomatal closure just before the stress prevents depletion of water and delays commencement of plant wilting. Under field conditions, however, water stresses that cause wilting of apple trees are very rarely observed. Stomatal closure and reduced transpiration under moderate water stress can be of interest with reference to Ca level in fruits. As shown by some researchers (15, 19), midday, summer water stress induces water movement along with Ca from fruit to leaves. Stomatal closure in these circumstances may improve plant water balance and allow the plant to avoid the stress.

Under conditions in which these experiments were conducted, K sprays had no influence on growth and photosynthesis of unstressed apple seedlings.

Literature Cited

1. Baker, D. A. and P. E. Weatherley. 1969. Water and solute transport by exuding root systems of *Ricinus communis*. J. Expt. Bot. 20:485–496.
2. Davies, F. S. and A. N. Lakso. 1979. Water stress responses of apple trees. I. Effects of light and soil preconditioning treatments on tree physiology. J. Amer. Soc. Hort. Sci. 104:392–395.
3. Davies, F. S. and A. N. Lakso. 1979. Water stress responses of apple trees. II. Resistance and capacitance as affected by greenhouse and field conditions. J. Amer. Soc. Hort. Sci. 104:395–397.
4. Fisher, R. A. 1968. Stomatal opening: Role of potassium uptake by guard cells. Science 160:784–785.
5. Gergely, I., R. F. Korcak, and M. Faust. 1980. Polyethylene glycol induced water stress effect on apple seedlings. I. Meth-

- odology, water consumption and dry matter production. *J. Amer. Soc. Hort. Sci.* 105:854–857.
6. Graham, R. D. and A. Ulrich. 1972. Potassium deficiency-induced changes in stomatal behavior, leaf water potential and root system permeability in *Beta vulgaris* L. *Plant Physiol.* 49:105–109.
 7. Haber, M. F. 1980. The effects of simulated soil water deficit on calcium uptake and root growth of peach seedlings. MS Thesis, Rutgers, The State University of New Jersey, New Brunswick.
 8. Hsiao, T. C. 1973. Plant water responses to water stress. *Annu. Rev. Plant Physiol.* 24:519–570.
 9. Jackson, M. L. 1970. Phosphorus determinations for soils. Vanado-molybdo-phosphoric yellow color method, in nitric acid system, p. 151–154. In: M. L. Jackson (ed.), *Soil chemical analysis*. Prentice-Hall, Englewood Cliffs, N.Y.
 10. Lakso, A. N. and E. J. Seeley. 1978. Environmentally induced responses of apple tree photosynthesis. *HortScience* 13:646–650.
 11. Nagarajah, S. 1979. The effect of potassium deficiency on stomatal and cuticular resistance in tea (*Camellia sinensis*). *Physiol. Plant* 47:91–94.
 12. Nagarajah, S. and G. B. Ratnasurija. 1978. The effect of phosphorus and potassium deficiencies on transpiration in tea (*Camellia sinensis*). *Physiol. Plant* 42:103–108.
 13. Pearson, C. J. 1975. Fluxes of potassium and changes in malate within epidermis of *Cammellina cyanea* and their relationships with stomatal aperture. *Austral. J. Plant Physiol.* 2:85–89.
 14. Peaslee, D. E. and D. N. Moss. 1968. Stomatal conductivities in K-deficient leaves of maize (*Zea mays* L.). *Crop Sci.* 8:427–430.
 15. Perring, M. A. 1979. The effects of environment and cultural practices on calcium concentration in the apple fruit. *Commun. Soil Sci. Plant Anal.* 10:279–293.
 16. Radin, J. W. and L. L. Parker. 1979. Water relation of cotton plants under nitrogen deficiency. II. Environmental interactions on stomata. *Plant Physiol.* 64:499–501.
 17. Raschke, K. 1975. Stomatal action. *Annu. Rev. Plant Physiol.* 26:309–340.
 18. Swietlik, D., M. Faust, and R. F. Korcak. 1982. Effect of Mineral nutrient sprays on photosynthesis and stomatal opening of water stressed and unstressed apple seedlings. I. Complete nutrient sprays. *J. Amer. Soc. Hort. Sci.* 107:XXX–XXX.
 19. Terblanche, J. H., K. H. Gurgun, and I. Hesebeck. 1980. An integrated approach to orchard nutrition and bitter pit control. p. 71–82. In: D. Atkinson, J. E. Jackson, R. O. Sharples, and W. M. Waller (eds.), *Mineral nutrition of fruit trees*. Butterworth, London.
 20. Turner, N. C. 1979. Drought resistance and adaptation to water deficits in crop plants. p. 343–372. In: H. Mussell and R. C. Staples (eds.), *Wiley*, New York.
 21. Turner, N. C. and J. E. Begg. 1981. Plant-water relations and adaptation to stress. *Plant & Soil* 58:97–131.

J. Amer. Soc. Hort. Sci. 107(4):572–581. 1982.

Freezing Resistance of Ornamental Trees and Shrubs

A. Sakai

The Institute of Low Temperature Science, Hokkaido University, Sapporo, 060 Japan

Additional index words. cold hardiness

Abstract. Freezing resistance of winter twigs from about 300 tree species and cultivars native to different climates in the world was assessed. Dormant 1-year-old twigs collected from mature trees were artificially hardened. Almost all of the trees, which ranged from tropical to subtropical in both Asia and America, sustained freezing injury to some of their tissues at temperatures below -5°C . Evergreen and deciduous broad-leaved trees native to warm-temperate climates survived freezing at -7° to -20° . In general, flower buds were far less resistant than vegetative buds. Winter minimum temperature is among the important factors governing the northern limits of cultivated plants.

Little reliable information is available on the hardiness levels in midwinter of important ornamental trees and shrubs, when assessed by the same method. Although low temperature is only one of many environmental stresses, and resistance to midwinter minimum temperature is only one of several types of winter hardiness, the close relationship between the two suggested that freezing resistance has played a significant role in plant distribution (13, 14). Freezing resistance in midwinter also proved useful in plant introduction programs and contributed to our understanding of plant adaptations to cold climates (12). Freezing resistance of Ericoideae (11) and of the genus *Camellia* (9) have been published. The purpose of this study is to present systematic data on winter-freezing resistance of cultivated trees

and shrubs including both the Northern and Southern Hemispheres.

Materials and Methods

Dormant 1-year-old twigs were collected by the author from about 300 species of flowering and ornamental trees which were mainly planted in Sapporo ($43^{\circ}03'N$), Tokyo ($35^{\circ}41'N$), Irozaki, and Hamamatsu (Shizuoka Prefecture) in Japan. Sample collection sites are shown in each table. Five uniform twig sections, 10 cm long, cut from each twig sample were enclosed in sealed polyethylene bags. Because freezing resistance of winter twigs can change in response to previous environmental temperatures including those encountered during transport, twigs were subjected to an artificial hardening regime of -2° to -3°C for 20 days and -5° for 3 days for hardy samples, or of 0° to -1° for 20 days for less-hardy samples. This procedure has been shown to promote maximum freezing resistance of the samples (5, 7, 13, 14). After hardening, most of the less-hardy twigs were cooled in 2° or 3° increments at 4-hr intervals to succes-

¹Received for publication Dec. 7, 1981. Contribution No. 2399 from the Institute of Low Temperature Science.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.