

The effects of abscisic acid and vapor pressure deficit on leaf resistance of *Paphiopedilum leeanum*

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Evidence is presented to show that $1 \times 10^{-5} M$ abscisic acid reduces transpiration of *Paphiopedilum leeanum* by 42% and increases leaf resistance by 79%. Increasing vapor pressure deficit from 0.5 kPa to ca. 3.0 kPa increases leaf resistance from ca. $6 S cm^{-1}$ to ca. $10 S cm^{-1}$. These results are discussed in view of the lack of guard cell chlorophyll and the lack of K^+ involvement in stomatal movement in this species.

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Des données montrent que l'acide abscisique $1 \times 10^{-5} M$ diminue de 42% la transpiration de *Paphiopedilum leeanum* et augmente la résistance foliaire de 79%. L'augmentation du déficit de pression de vapeur de 0.5 kPa à environ 3.0 kPa augmente la résistance foliaire d'environ $6 S cm^{-1}$ à environ $10 S cm^{-1}$. Ces résultats sont discutés en rapport avec l'absence de chlorophylle dans les cellules stomatiques et avec le fait que l'ion K^+ n'est pas impliqué dans le mouvement stomatique chez cette espèce.

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Introduction

Potassium ion is considered to be the primary osmotic agent causing stomatal opening in many species (Raschke 1976). K^+ flux has been shown to accompany stomatal closure after abscisic acid (ABA) application (Jones and Mansfield 1970, 1972; Horton 1971). The effect of relative humidity or vapor pressure deficit (VPD) upon stomata is of considerable interest (Hall et al. 1976). Sheriff (1977) reported that the stomata of 10 out of 26 species studied were sensitive to relative humidity, closing at higher VPD's. Löscher and Schenk (1978) found the stomatal aperture change of *Valerianella locusta* (L.), in response to a change in VPD, preceded K^+ movement, suggesting that K^+ transport may be a secondary process.

Nelson and Mayo (1975) found that *Paphiopedilum* spp. guard cells lack chlorophyll but respond to light and CO_2 similarly to other species. They also found that K^+ is not involved as the major osmoticum due to its near absence in the epidermis (Nelson and Mayo 1977). The objectives of this research were to determine (i) the effect of relative humidity, and (ii) the effect of ABA upon leaf resistance of the lady slipper orchid *Paphiopedilum leeanum*.

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Materials and methods

Fully expanded lady slipper orchid leaves obtained from greenhouse grown plants were used in both experiments. The experiments were carried out with cut leaves rather than intact plants. Cut leaves were sealed in 25-mL beakers containing water or $1 \times 10^{-5} M$ ABA as the experiment dictated. The beakers plus leaves were placed in desiccators over saturated salt solutions to precisely control VPD. The desiccators were placed in a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH) where the photosynthetically active radiation (PhAR) was at least $200 \mu E m^{-2} s^{-1}$ at the leaf surface. This is sufficient to cause full stomatal opening (Nelson and Mayo 1975). Fans inside the desiccators stirred the air to reduce the boundary layer at the leaf surface. Transpiration was determined by weighing the beaker plus leaf at approximately 4-h intervals on a Mettler H10 analytical balance. The balance was placed inside the growth chamber, which was maintained at 70% relative humidity and 22°C, to minimize disturbance during the weighing process.

Leaf resistance (R_L) was calculated according to Slavik (1974):

$$[1] R_L = [(C_{sat} - C_a)/q] - R_a$$

where R_L = leaf resistance in $S cm^{-1}$; C_{sat} = saturation absolute humidity at leaf temperature; C_a = absolute humidity at air temperature, controlled by the saturated salt solution; q = transpiration rate; and R_a = leaf boundary layer resistance estimated according to Nobel (1974):

$$[2] R_a = \alpha^a / D_1$$

where D_1 = the diffusivity of water vapor; α^a = boundary layer thickness estimated from Eq. 3:

$$[3] \alpha^a = 0.4 (\ell_{leaf} / V_{wind})^{1/2}$$

where l = the leaf dimension in the down wind direction; and V = wind velocity.

Leaf and air temperatures were measured with 0.0076-cm diameter copper-constantan thermocouples and a Fluke digital thermometer (model 2100A). Wind speed within the desiccators was measured with a Hastings air meter (Model AB-27).

Vapor pressure deficits in the desiccators were controlled using the saturated salt solutions given in Table 1. The effect of ABA was studied in the $\text{NH}_4\text{Cl-KNO}_3$ desiccator. The VPD effects upon leaf resistance were studied in the KOH and KCl desiccators. Since KOH was one of the salts used, CO_2 concentration would be lowered, favoring open stomata. Accordingly, a small dish with two or three KOH pellets was placed in each desiccator. Thus, in all experiments, stomatal opening was favored by PhAR and CO_2 concentration.

Since vapor pressure deficit can cause stomatal closure in two ways: (1) directly reducing guard cell turgor or (2) indirectly via bulk leaf water status, the relative water content (RWC) of leaves in the VPD experiment was determined according to Slavik (1974):

$$[4] \text{ RWC} = [(\text{initial weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})] \times 100$$

Results and discussion

The effects of $1 \times 10^{-5} M$ ABA fed through the transpiration stream upon R_L and transpiration are given in Table 2. The experiment was carried out over two light periods and the results analyzed according to Steel and Torrie (1960) for unpaired observations and unequal variances. The minimum leaf resistances measured by this technique are within the range reported by Nelson and Mayo (1975) using a diffusive resistance porometer on intact plants at 70% relative humidity, indicating that the cut-leaf-desiccator technique is comparable. It is obvious that ABA increases R_L and reduces transpiration. Davies (1978) reported that ABA reduced transpiration by one half in *Vicia faba*, Raschke (1974) reported that $1 \times 10^{-5} M$ ABA reduced transpiration of *Xanthium strumarium* by one half, and Jones and Mansfield (1972) reported a reduction of approximately 30% by $1 \times 10^{-4} M$ ABA in *Hordeum vulgare*. Thus, the 42% reduction in transpiration of *Paphiopedilum* is similar to the effect reported for other species. This is interesting since K^+ is not believed to be involved in

TABLE 1. Relative humidities and vapor pressure deficits (VPD) of unsaturated salt solutions used to control transpiration at 25°C (Weast 1968; Winston and Bates 1960)^a

Saturated salt	Relative humidity, %	VPD, kPa
KOH	8.0	3.014
KCl	85.0	0.475
NH_4Cl and KNO_3	71.2	0.9

^aRelative humidities and VPDs were corrected to the actual temperature within the desiccators.

TABLE 2. The effects of $1 \times 10^{-5} M$ abscisic acid upon transpiration and leaf resistance

Transpiration, $\text{mg dm}^{-2} \text{h}^{-1}$		R_L , S cm^{-1}		Comments
H_2O	+ABA	H_2O	+ABA	
576.00	298.80	4.47	9.36	First light period leaves, 7 h in solution
579.60	252.00	4.44	11.24	
381.60	270.00	7.15	11.24	
428.40	262.80	6.28	10.75	
417.60	309.60	6.47	9.00	
\bar{X} 476.64	278.4 ^a	5.76	10.32 ^b	
698.40	331.20	3.76	8.82	Same plants 24 h later, rates during the 2nd light period
691.20	277.20	3.80	10.69	
428.40	270.00	6.64	11.00	
478.80	352.80	5.85	8.23	
471.60	374.40	5.96	7.71	
\bar{X} 553.68	321.12 ^c	5.20	9.29 ^d	

^aThe reduction in transpiration rate is 41.56% and the difference is highly significant.

^bThe increase in R_L is 79% and the difference is highly significant.

^cThe reduction in transpiration rate is 42% and the difference is highly significant.

^dThe increase in R_L is 78.65% and the difference is highly significant.

stomatal opening in *Paphiopedilum* (Nelson and Mayo 1977), and the effect of ABA is presumed to be upon K^+ transport. Pitman and Wellfare (1978) found that ABA inhibits the transport of Na^+ , Mg^{2+} , Ca^{2+} , and phosphate into the xylem of barley roots; thus the effect could conceivably be upon the transport of another cation. Raschke (1976) indicates that ABA may block H^+ transport out of cells. Thus the effect could be more indirect by preventing the elevation of guard cell pH, long associated with stomatal opening (Scarath 1932).

The effects of VPD upon transpiration rate, leaf resistance, and RWC are given in Table 3. Leaf resistance increased with increasing VPD but so did the transpiration rate. RWC decreased with increased VPD suggesting that the increased R_L is due to change in bulk leaf water status. This along with the large increase in transpiration rate would seem to preclude any direct effect of VPD upon the stomata (Farquhar 1978).

Nelson and Mayo (1977) found that K^+ is not involved in *Paphiopedilum* stomatal function. Lösch and Schenk (1978) have shown that K^+ transport between the guard cells and subsidiary cells occurs after the stomatal response to a changed VPD rather than concomitantly. Recently Rogers et al. (1979) have shown that K^+ decreases in the guard cells of open stomata even though the aperture remains the same. They suggest that K^+ is necessary for stomatal opening but other solutes such as sugar, organic acids, or proline obtained

TABLE 3. The effect of vapor pressure deficit (VPD) upon transpiration rate, leaf resistance (R_L), and relative water content (RWC)

Leaf No.	VPD = 0.475 kPa			VPD = 3.014 kPa		
	Transpiration, $\text{mg dm}^{-2} \text{h}^{-1}$	R_L , S cm^{-1}	RWC, %	Transpiration, $\text{mg dm}^{-2} \text{h}^{-1}$	R_L , S cm^{-1}	RWC, %
1	211.7	6.3	81.7	1311.1	6.0	67.2
2	273.4	4.7	84.0	668.9	11.8	80.0
3	258.4	5.0	79.4	522.4	15.2	72.2
4	342.4	3.6	84.1	1364.4	5.8	71.1
5	228.7	5.8	83.2	974.2	8.1	80.8
6	152.3	9.1	81.5	650.5	12.1	77.4
\bar{X}	244.5 ^a	5.8 ^b	82.3 ^c	915.2	9.8	74.8

^aThe increase in transpiration rate is 274% and the difference is highly significant.

^bThe increase in R_L is 69% and the difference is significant.

^cThe decrease in RWC is 7.5% and is significant.

from starch may be necessary for long-term maintenance of the aperture.

The results presented here provide further evidence of the similarity between *Paphiopedilum* stomata and many other species; namely low R_L in the light, high R_L in the dark, CO_2 sensitivity, and sensitivity to ABA. It suggests, in conjunction with the work cited above, that the effect of ABA is not directly upon the ion transport mechanism.

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