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How Do Stomata Read Abscisic Acid Signals?[1]

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When abscisic acid (ABA) was fed to isolated epidermis of *Commelina communis* L., stomata showed marked sensitivity to concentrations of ABA lower than those commonly found in the xylem sap of well-watered plants. Stomata were also sensitive to the flux of hormone molecules across the epidermal strip. Stomata in intact leaves of *Phaseolus acutifolius* were much less sensitive to ABA delivered through the petiole than were stomata in isolated epidermis, suggesting that mesophyll tissue and/or xylem must substantially reduce the dose or activity of ABA received by guard cells. Delivery of the hormone to the leaf was varied by changing transpiration flux and/or concentration. Varying delivery by up to 7-fold by changing transpiration rate had little effect on conductance. At a given delivery rate, variation in concentration by 1 order of magnitude significantly affected conductance at all but the highest concentration fed. The results are discussed in terms of the control of stomatal behavior in the field, where the delivery of ABA to the leaf will vary greatly as a function of both the concentration of hormone in the xylem and the transpiration rate of the plant.

During the last 15 years, evidence has accumulated to support the view that in the early stages of soil drying ABA produced in the roots and transported in the transpiration stream can function as a physiological signal in the regulation of gas exchange (Davies and Zhang, 1991). Laboratory experiments have shown a clear correlation between xylem ABA concentration and the degree of stomatal opening (Zhang and Davies, 1989, 1990; Khalil and Grace, 1993). Field results have supported this conclusion and have also shown a tight and even more sensitive correlation between xylem ABA concentration and stomatal conductance (Wartinger et al., 1990; Tardieu et al., 1992). This is perhaps surprising, since during its passage through the plant xylem sap is exposed to several factors that can alter the amount of ABA arriving at the sites of ABA action on the guard cells (Hartung and Slovik, 1991). Much ABA will move out of the xylem into alkaline compartments, and Trejo et al. (1993) have shown that the mesophyll and its capacity to metabolize ABA can play an important role in determining the amount of the growth regulator arriving in the epidermis and the final extent of stomatal closure. In a recent paper, Gowing et al. (1993) described an experiment designed to address the question of whether the guard cells perceive the concentration of ABA in the xylem or respond to the flux of the growth regulator into the leaf. The results led the authors to conclude that both variables are important in the determination of a local ABA concentration (around the guard cells) to which the guard cells react but that this concentration is not necessarily similar to the xylem ABA concentration.

In the experiments performed by Gowing et al. (1993), the amount of ABA arriving at the guard cells was varied by feeding short pulses of the hormone, preceded and followed by hormone-free artificial xylem sap. In nature, increases in VPD will lead to increased transpiration flux, and if xylem ABA concentration does not decrease dramatically, such an increase in transpiration flux must result in an increase in hormone flux. Tardieu and Davies (1992) have shown that xylem ABA concentrations are relatively constant throughout the day, and therefore as the day progresses increased VPDs must result in increased flux of ABA molecules to the guard cells. We would like to understand how stomata of plants in the field might "read" ABA signals arriving in the transpiration stream. To this end, we have tried in this experiment to mimic the hormone signals received by leaves as transpiration flux changes throughout the day. To evaluate the effects on stomatal behavior of increased fluxes and concentrations of ABA on leaf conductance of whole leaves, we have used a transpiration bioassay. The assay was performed at different temperatures with the aim of varying transpiration rate and therefore the flux of ABA into the lamina. In another experiment, epidermal strips received a continuous flux of different ABA concentrations. Flux of the hormone across the strips was also varied. Results of the two experiments were compared to assess the role of the mesophyll in regulating the amount of ABA reaching the guard cells.

MATERIALS AND METHODS

Experiment with Whole Leaves

Growth Conditions

Seeds of *Phaseolus acutifolius* Gray. were germinated in several layers of wet laboratory towel, and after the radicle appeared (after approximately 2 d) the seeds were sown into 90- × 90-mm pots containing vermiculite. Plants were grown in a controlled-environmental cabinet with a temperature of 25/14°C (day/night) and a photoperiod of 14 h with a PPFD of 320 μmol m^{-2} s^{-1}. The pots were watered

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Abbreviations: g_w, leaf conductance; VPD, vapor pressure deficit.

every 2 d with a full-strength modified Hoagland nutrient solution (Epstein, 1972).

Transpiration Bioassay

When the primary leaves reached their maximum length (approximately 10 d after emergence), the stem was cut under degassed, distilled water to avoid embolism and immediately placed into glass vials of 25 cm^3 volume, filled with distilled and degassed water. The vial tops were covered with aluminum foil to reduce evapotranspiration. After the excised shoots were placed in the vials, they were left in darkness for 12 h. After the dark period, the excised shoots were transferred to similar vials containing ABA solutions made up in distilled and degassed water. The vials were transferred to a growth cabinet and weighed every 30 min to determine transpiration rate. Measurements were carried out for 4.0 h and then the leaf area was determined in a planimeter and the transpiration rates were expressed per unit leaf area. g_w was calculated using the expression $g_w = E/\Delta W$, where E is the transpiration rate and ΔW (estimated from measurement of leaf and air temperature) is the driving force for evaporation expressed as a mole fraction. Under the conditions in the growth chamber (see below), boundary layer conductance was estimated from a filter paper replica and was found to be approximately 3.0 mol m^{-2} s^{-1}, which is large enough to be ignored in the calculation of g_w.

Temperature Experiment

The transpiration bioassay was used as explained above at five different air temperatures: 15.9, 19.9, 24.0, 29.4, and 36.1°C. Air temperature was measured with a shielded thermocouple held at leaf level a few centimeters from the leaves. The temperature of leaves from each treatment was determined with a fine (0.08 mm) thermocouple held against the lower leaf surface. PPFD and wind speed were maintained at constant levels during the assay (wind speed, 5 m s^{-1}; PPFD, 500 μmol m^{-2} s^{-1}). The RH was not controlled and the VPD varied depending on the temperature: 0.503, 1.316, 1.913, 2.617, and 3.346 kPa, respectively. At each temperature the effects of four concentrations of ABA were assessed: 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} mol m^{-3}. The concentration of ABA measured in the xylem sap of well-watered plants was used as a control (6×10^{-5} mol m^{-3}).

At the end of the experiment and after the area of the leaves was measured, the leaves were immediately frozen in liquid nitrogen and freeze dried. Bulk leaf ABA concentration was determined using a radioimmunoassay. The monoclonal antibody (AFR MAC 62) used, which is specific for (+)-ABA, was kindly provided by Dr. S.A. Quarrie (Institute of Plant Science Research, Norwich, UK). Samples were extracted using different ratios of extraction (leaf dry weight:solvent) depending of the concentration of ABA fed (1:65–1:150), and further dilutions were made if it was found to be necessary.

Experiment with Epidermal Strips

Growth Conditions

Seeds of *Commelina communis* L. were sown in John Innes No. 2 compost. After emergence, seedlings were transplanted into 90- × 90-mm pots and grown in a controlled-environment cabinet with environmental conditions as described above. The pots were watered daily to the drip point, and once a week they were watered with a full-strength modified Hoagland nutrient solution. When the plants were 4 or 5 weeks old, either the fourth or the fifth leaf, which was the youngest fully expanded leaves at that time, was used as a source of experimental material.

Mounting Epidermal Peels

Two 10- × 20-mm segments of leaf parallel to the veins were obtained from each side of the main central vein and floated in a Petri dish containing 10 mol m^{-3} Mes buffer (Sigma) and 50 mol m^{-3} KCl (Sigma) adjusted to pH 6.15 with KOH. After a few minutes, a leaf segment was blotted dry with a tissue paper and the epidermis was carefully peeled from the abaxial surface. Afterward, the epidermis was mounted with the cuticle side down on a microscope slide covering a 5-mm-diameter hole in the middle of the slide. The epidermis was flattened gently against the glass and stuck to it by means of silicone grease (RS Components, Northants, UK), which had been previously spread evenly around the hole. Thereafter a 7-mm-diameter plastic rubber O-ring was placed over the hole of the microscope slide on top of the epidermis, creating a chamber above the epidermis with a volume of 80 μL (Fig. 1).

Stomatal Measurements and Experimental Conditions

After the epidermis was mounted, the microscope slide was placed on the stage of an inverted microscope (IMT-2, Olympus). In most experiments, the epidermis was perfused continuously by gravity with a CO_2-free solution at a rate of 0.06 mL s^{-1} (full flux). In other experiments, fluxes were reduced to one-tenth, one-quarter, and one-half of full flux. In a further experiment, one-tenth of the full flux was applied and this was then increased to full flux after the stomatal aperture had reached a steady state. Solution was

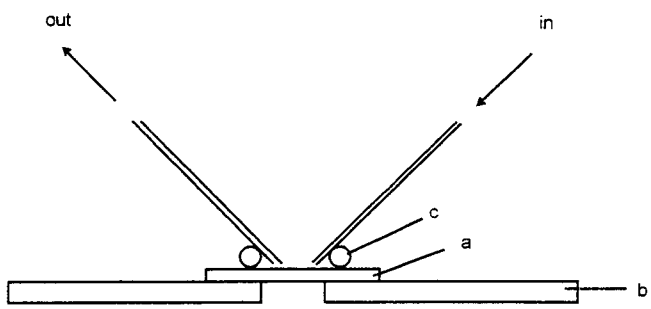

Figure 1. An epidermal strip of *C. communis* L. (a) mounted with the cuticle side down on a microscope slide (b) and perfused with different solutions in a chamber of 80 μL volume created by means of an O-ring mounted on top of the epidermis (c).

supplied along an insulated pipe from a reservoir aerated with CO_2-free air and maintained at 25°C in a temperature-controlled bath. In general, closed stomata were observed under the microscope after the epidermis was mounted. The epidermis was perfused with Mes buffer (10 mol m^{-3}) and KCl (50 mol m^{-3}) and illuminated continuously with a PPFD of 400 μm^{-2} s^{-1} from a Schott (Cologne, Germany) KL1500 halogen cold-light source to promote stomatal opening. Stomata with apertures between 16 and 18 μm were obtained after 2 h of this treatment. After the stomata were opened, the microscope was fixed to a particular field with the ×40 objective and the same population of stomata (between 10 and 15 μm) was measured with a calibrated graticule fixed to the eyepiece and continuously observed for several hours. Mes buffer with KCl was used as a control solution in which stomata attained "maximum apertures," which did not vary during several hours of continuous observation. In other cases after the above stomatal apertures were obtained, the Mes buffer solution was replaced by ABA solutions made up in Mes buffer at the following concentrations: 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} mol m^{-3}. The synthetic (±)-ABA (Sigma) was supplied in the racemic form, but the concentration given in each treatment was calculated from the active (+)-enantiomer. In all of the treatments stomatal measurements were taken every 30 min for several hours.

RESULTS

Temperature Experiment

The effects of VPD or leaf temperature on leaf conductance and transpiration of *P. acutifolius* leaves are shown in Figure 2. VPD and leaf temperature in the range 1.316 to 3.346 kPa and 19.9 to 36.1°C, respectively, had no significant effect on leaf conductance, whereas the rate of leaf transpiration increased linearly as the two variables increased. ABA fed at different concentrations to leaves held at different temperatures inhibited the leaf conductance, with inhibition proportional to the negative logarithm of ABA concentration (Fig. 3). It can be seen that with increasing leaf temperature, the shape of the relationship between stomatal conductance and ABA concentration did not change. Each of the points represents a steady-state condition that was reached approximately 1 h after ABA was fed.

Figure 4 shows the rates of ABA delivery to the leaf at four of the temperatures tested and at the four ABA concentrations fed. ABA delivery showed an approximately linear relationship with temperature, VPD, and concentration of ABA fed. However, despite the increase in ABA delivery as flux increased at a particular ABA concentration fed, the restriction in stomatal conductance was not affected by increased ABA flux. No further reductions in leaf conductance were registered until a new and higher ABA concentration was detected (Fig. 5). Bulk leaf ABA content was measured at the end of the experiment, and it can be seen in Table I that the leaf ABA content increased as the transpiration increased and as the applied ABA concentration increased at each of the different temperatures.

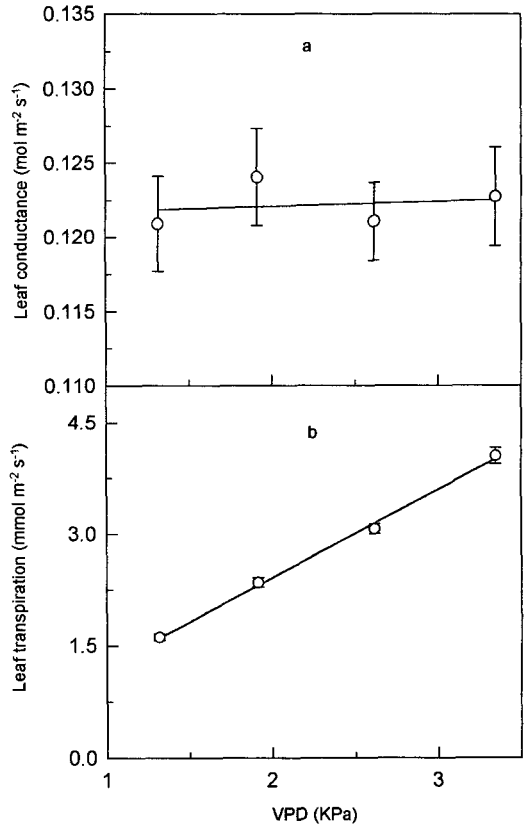

Figure 2. Effect of VPD on leaf conductance (a) and leaf transpiration (b) of detached leaves of *P. acutifolius*. Values are the averages ±SE of five different leaves.

Although the flux of a given concentration of ABA to the leaf did not affect the extent of the stomatal response to the hormone, higher hormone fluxes did result in more rapid stomatal responses to the hormonal treatment (Fig. 6).

When the transpiration bioassay was run at 15.9°C, g_w of leaves fed with the control solution was reduced compared to g_w of control leaves at the other four temperatures. For this reason, data collected at 15.9°C were not included in the main analysis. These data were useful, however, since we were able to test the effect on relative conductance (compared to controls) of comparable fluxes of ABA solutions of different concentrations. The combination of treatments described in Table II allowed us to compare the effects of concentration and flux of ABA on stomata over three separate ranges of flux (7–8, 45–50, and 270–350 pmol m^{-2} s^{-1}). At each flux there was a clear effect of concentration of the hormone, whereas at three of the four concentrations fed there was no effect of flux. Only at the highest concentration fed (10^{-1} mol m^{-3}) was there a small effect of flux.

Epidermal Strip Experiment

Mounting epidermis in the manner described above apparently did not affect the functionality of the stomata. This was confirmed by exposing epidermal peels to pulses of ABA of various durations (Fig. 7). At the end of each pulse

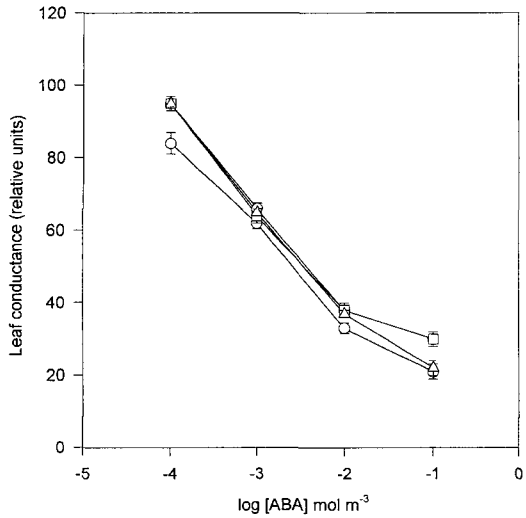

Figure 3. Steady-state leaf conductance of detached leaves of *P. acutifolius* (as a percentage of values for control plants fed artificial xylem sap containing 6×10^{-5} mol m^{-3} ABA) that were fed with different concentrations of ABA at different temperatures: 19.9°C (□), 24°C (○), 29.4°C (△), and 36.1°C (◇). Values are the averages ±SE of five different leaves.

the epidermis was perfused with Mes buffer and KCl, but the stomata continued to close for some time, after which the effect caused by the ABA pulse was gradually overcome. Stomatal apertures similar to those recorded before ABA treatment were obtained after approximately 3 h.

The effect of perfusing epidermal peels with different concentrations of ABA is shown in Figure 8. All concentrations of ABA affected stomatal aperture within 30 min of the initiation of the treatment. A concentration as low as 10^{-7} mol m^{-3} restricted stomatal aperture by approximately 15%, whereas 10^{-6} and 10^{-5} mol m^{-3} ABA restricted stomatal aperture by more than 50%. ABA at a concentration of 10^{-4} mol m^{-3} completely closed stomata within 60 min.

Varying the flux of a given concentration of ABA across the epidermis was shown to influence stomatal aperture (Fig. 9), although only a reduction of flux to one-tenth of the full flux resulted in a stomatal aperture that was significantly different from that resulting from the application of the same concentration of hormone at full flux. A 5-fold change in flux had no effect on stomata.

In an experiment in which stomatal response to a low concentration of ABA had reached a steady state at the low flux, flux was increased and an immediate stomatal response was seen (Fig. 10). Stomata responded even further when, after this treatment, ABA delivery was increased by 2 orders of magnitude by an increase in concentration at the same solution flux.

DISCUSSION

We have shown that by perfusing epidermal strips with a constant input of ABA the response of the stomata is much more sensitive than that of stomata in intact leaves (cf. Figs. 3 and 8). It may be that under the conditions of the experiment described here the stomata in isolated epidermis show something close to their maximum sensitivity. A concentration of ABA (10^{-6} mol m^{-3}) even lower than that measured in the xylem sap of well-watered plants of *C. communis* has a very significant effect on the final stomatal aperture after 3 h (50–60% reduction of the initial aperture). These results raise the question of what would happen if the concentration of ABA carried in the transpiration stream arrived unmodified at the stomatal complex. The results in Figure 8 suggest that stomata would be almost permanently closed even when the soil is well watered. However, stomata of well-watered plants commonly do open under most conditions, and we commonly observe clear relationships between stomatal aperture and [ABA] in the xylem, albeit with a much-reduced sensitivity (Zhang and Davies, 1989; Tardieu et al., 1992).

Many experiments reported in the literature show how stomata react to different ABA concentrations at which the fluxes of solutions into the leaves are tightly controlled and not varied. In natural conditions, the transpiration flux will determine the amount of ABA delivered to the leaf

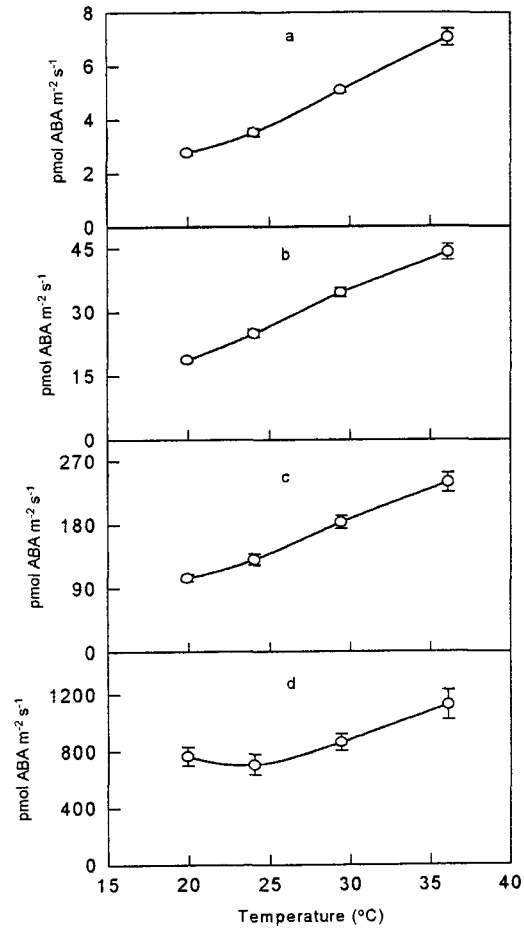

Figure 4. Effect of different temperatures on the fluxes of ABA arriving in detached leaves of *P. acutifolius* that were fed with different concentrations of ABA: a, 10^{-4} mol m^{-3}; b, 10^{-3} mol m^{-3}; c, 10^{-2} mol m^{-3}; and d, 10^{-1} mol m^{-3}. Values are the averages ±SE of five different leaves.

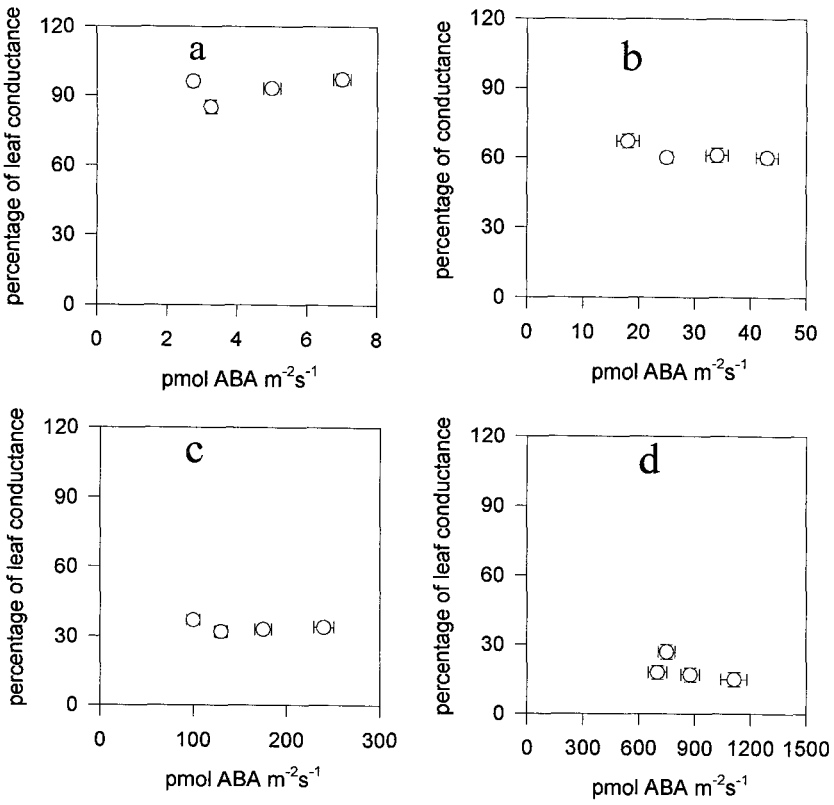

Figure 5. Effect of different fluxes of ABA on leaf conductance (as a percentage of values for leaves fed with artificial xylem sap) of detached leaves of *P. acutifolius* that were fed with different concentrations of ABA: a, 10^{-4} mol m^{-3}; b, 10^{-3} mol m^{-3}; c, 10^{-2} mol m^{-3}; and d, 10^{-1} mol m^{-3}. Values are the averages ± SE of five different leaves.

(Jackson, 1993), and this observation raises the important question of whether transpiration fluxes can provide enough ABA molecules to change the local concentration and influence stomata. Indeed, Jackson has argued strongly that we can properly interpret stomatal responses to ABA signals only if we are able to quantify hormone fluxes. In a recent model in which the control of stomatal behavior was described by chemical and hydraulic signaling (Tardieu and Davies, 1993), the relationship between [ABA] and stomatal conductance was a central component of the control system but it was necessary to include a sensitivity component to complete the control loop (Tardieu, 1993; Trejo et al., 1993). It was recognized that high apparent sensitivity at high transpiration rates could reflect the increased flux of ABA molecules under these conditions.

The results reported in the present paper show that stomata in isolated epidermis are sensitive to variation in the number of ABA molecules arriving at the epidermis when this variation is generated as a result of variation in "transpiration rate" as well as by variation in concentration in the xylem stream. The data reported in Figure 10 show that an increase from a low to a higher flux of ABA molecules at a given concentration will cause a significant restriction in the aperture of stomata within 3 h. The same population of stomata retains sensitivity to an additional increase in delivery of ABA molecules in the form of an increase in concentration. Nevertheless, it is important to note that we can detect stomatal sensitivity to flux only when we change the supply rate by 1 order of magnitude, whether this is brought about by an increase in delivery rate or by a step increase in concentration.

Table I. *Bulk leaf ABA content (nmol g^{-1} dry weight) of detached leaves of P. acutifolius fed with different concentrations of synthetic ABA for 4.0 h at different temperatures*

Values are the averages ± SE of five individual leaves.

[ABA] Fed	Temperature (°C)			
	19.9	24.0	29.4	36.1
mol m^{-3}				
Control	1.93 ± 0.11	2.48 ± 0.2	4.21 ± 0.04	6.86 ± 0.76
10^{-4}	2.14 ± 0.21	2.90 ± 0.30	3.77 ± 0.21	6.80 ± 1.47
10^{-3}	6.10 ± 0.90	9.91 ± 0.86	12.3 ± 0.50	17.5 ± 0.55
10^{-2}	22.5 ± 0.81	32.5 ± 0.99	44.9 ± 1.70	50.9 ± 3.73
10^{-1}	87.1 ± 5.35	142.4 ± 5.0	160 ± 15.40	190.9 ± 12.0

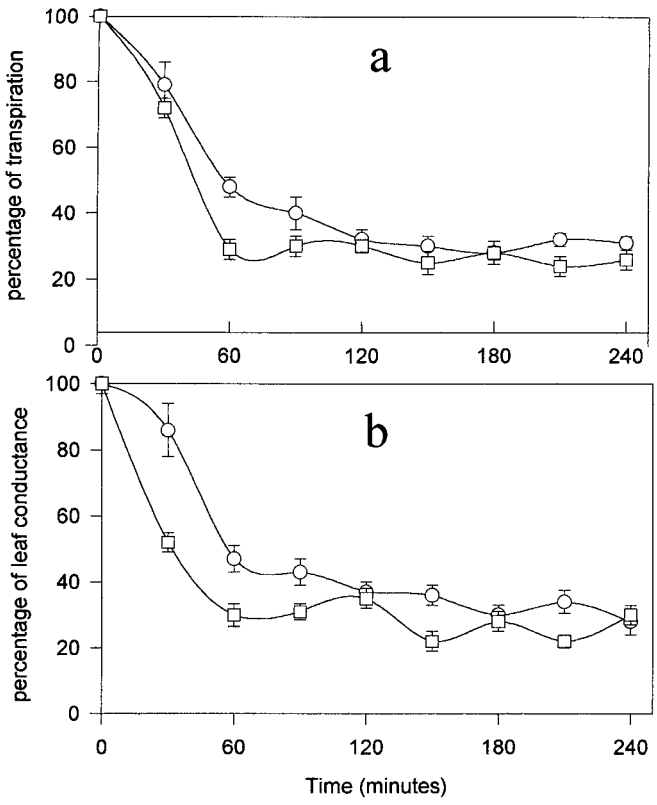

Figure 6. Effect of feeding 10^{-2} mol m^{-3} ABA on transpiration (a) and leaf conductance (b) of detached leaves of *P. acutifolius* (as a percentage of transpiration and conductance before ABA was fed) at 15.9°C (○) or 36.1°C (□). Values are the averages ± SE of five replicates.

It is important to know whether stomata in intact leaves show the same degree of sensitivity to ABA signals entering the leaf from the transpiration stream. A solution of ABA fed through the transpiration stream into an intact *P. acutifolius* leaf causes a rapid reduction in diffusive conductance, although as we noted above the stomata of intact leaves are not as sensitive to ABA signals as stomata in isolated epidermis. ABA-induced reductions in leaf conductance seem to be a function of the ABA concentration supplied, with higher concentrations eliciting greater reductions in conductance. These effects of ABA were not mediated by VPD or temperature, since these two variables had no significant effect on leaf conductance in the range analyzed in Figures 2 to 5. It is important to note that transpiration increased linearly as these two variables increased; therefore, the increase in amount of ABA delivered between 19.9 and 36.1°C was 2.5-fold when feeding 10^{-4} mol m^{-3}, 2.3-fold when feeding 10^{-3} mol m^{-3}, 2.3-fold when feeding 10^{-2} mol m^{-3}, and 1.5-fold when feeding 10^{-1} mol m^{-3} ABA. Despite these increases in delivery at high temperature or VPD, the maximum reduction of leaf conductance was achieved by each concentration at 19.9°C or 1.316 kPa (VPD) and was not increased at higher temperatures or VPD (Fig. 5). These results show that over the range of delivery rates achieved at each concentration we cannot detect an effect of hormone flux on conductance. Furthermore, when comparable fluxes of ABA were generated with solutions of different concentrations (Table II), the solution of the higher concentration always promoted a larger stomatal response. At three concentrations, an increase in flux of up to 7-fold had no detectable effect on conductance. Only at the highest concentration fed did an increase in flux (4-fold) limit stomatal opening. This concentration (10^{-1} mol m^{-3}) is higher than concentrations found even in severely stressed plants.

These results suggest strongly that by one means or another the plant is able to prevent a buildup of the hormone in the vicinity of the binding sites on the guard cells. It seems clear that ABA metabolism and conjugation (Grantz et al., 1985; Zeevaart and Creelman, 1988) and compartmentation (Hartung and Slovik, 1991) play an important role in determining local concentration of ABA and therefore may significantly modify stomatal response to ABA signals. We need more information concerning the regulation of these important processes. When delivery was increased by 1 order of magnitude by an increase in hormone concentration, then a new equilibrium conductance was reached, presumably because more ABA molecules gained access to binding sites on guard cells.

An alternative explanation for our whole-leaf flux data is suggested by the results of MacRobbie (1990), namely that when stomata have received an initial dose of the hormone they are insensitive to subsequent doses provided within at least the next 28 min. At the start of our experiment, stomata were closed and therefore initial differences in the flux of molecules into the leaf at different temperatures must have been relatively small. As stomata opened in response to PPFD, differences in flux would have been generated, but the stomatal response may already have been fixed as a function of the concentration of the first molecules arriving at the sites of action on the guard cells. Our experiments with isolated epidermis suggest that this explanation is unlikely. Stomata seem to be sensitive to changes in the number of ABA molecules delivered, re-

Table II. *Effect of g_w on four different concentrations of ABA applied to detached leaves of P. acutifolius at two different temperatures to generate different fluxes of the hormone into the leaf*

Parameter	ABA Concentration (mol m^{-3})							
	10^{-4}		10^{-3}		10^{-2}		10^{-1}	
	15.9°C	36.5°C	15.9°C	36.5°C	15.9°C	36.5°C	15.9°C	36.5°C
Flux of ABA (pmol m^{-2} s^{-1})	1.1 ± 0.04	7.7 ± 1.1	7.5 ± 0.9	50.4 ± 3.6	45.3 ± 3.7	271.4 ± 17.8	347.1 ± 33.3	1360.9 ± 80.5
Percent of original g_w	95.5 ± 4.5	106 ± 11.8	62 ± 2.6	60 ± 5.8	33.1 ± 1.8	31.1 ± 2.9	19.7 ± 0.9	12.3 ± 1.1

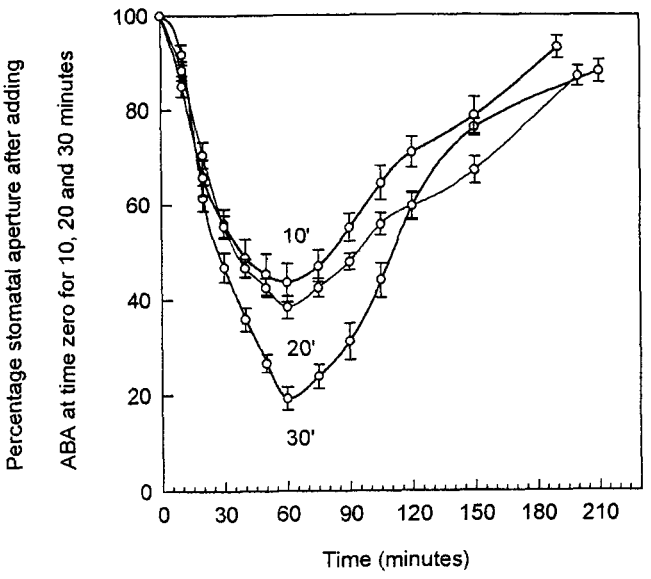

Figure 7. Stomatal apertures in epidermal strips of *C. communis* after exposure to a pulse of ABA at 10^{-2} mol m^{-3}. Pulses were of 10, 20, and 30 min duration. After each pulse, the epidermis was continuously perfused with Mes buffer for 3 h. Values are the averages ± SE of at least five different stomata.

gardless of how the delivery is varied, if the change in delivery is large enough. One can conclude only that the insensitivity of stomata in whole leaves to changes in the flux of hormone into the leaf reflects the fact that local hormone concentration at the epidermis will be dominated by the concentration of hormone arriving in the transpiration stream, i.e. unless the transpiration flux changes by 1 order of magnitude or more. This means that in investigations of ABA signaling between roots and leaves we should

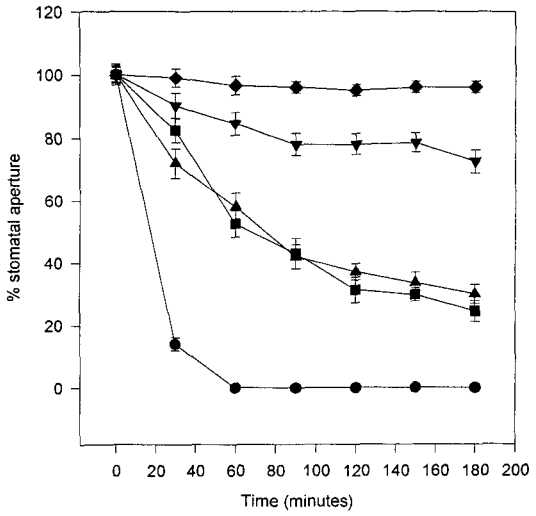

Figure 8. Effect of perfusing epidermal peels of *C. communis* with different concentrations of ABA: 10^{-7} mol m^{-3} (▼), 10^{-6} mol m^{-3} (▲), 10^{-5} mol m^{-3} (■), and 10^{-4} mol m^{-3} (●). Mes buffer was used as a control (◆). Values are the averages ± SE of at least three different fields from different epidermes.

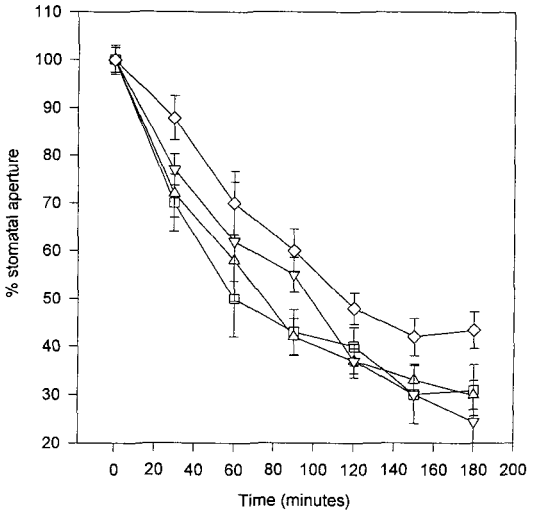

Figure 9. Effect of different fluxes of ABA solution supplied to isolated epidermis at 10^{-6} mol m^{-3}. Fluxes are 0.016 pmol s^{-1} (△), 0.008 pmol s^{-1} (□), 0.004 pmol s^{-1} (▽), and 0.0016 pmol s^{-1} (◇). Values are averages ± SE of replicates of three experiments in which at least 15 stomata were recorded.

be able to quantify physiologically meaningful changes in the amount of ABA entering the leaves of whole plants and interpret stomatal responses to soil drying by making reliable estimates of concentrations of stomata-active compounds in the xylem.

We must ask why there is no apparent stomatal response to an accumulation of ABA after the ABA in solution is delivered and the water carrying it is lost by transpiration (Table I). Bulk leaf ABA contents were more than doubled as delivery rate was increased by increasing temperature for each concentration fed, and it seems reasonable to expect that such an accumulation would keep the stomata largely closed at higher temperatures. The fact that this was not the case suggests that compartmentalization and catabolism of ABA were sufficient to prevent any accumulation of ABA in the vicinity of the guard cells. In such a situation, only the "new" ABA molecules arriving in the transpiration stream would exert an effect on the stomata, and aperture would then be determined by the ABA content of the transpiration stream. It is important to point out that in many cases the bulk leaf [ABA] cannot reflect the magnitude or rapidity with which the stomata are affected by soil drying (Tardieu et al., 1992). Kefu et al. (1991) calculated that the amount of ABA carried in the transpiration stream of cotton plants each day is 9 times in excess of the amount of ABA found in leaves. The authors assumed that a large proportion of ABA entering a leaf is metabolized or exported back to the roots. Trejo et al. (1993) showed that by inhibiting the catabolism of ABA in the mesophyll the amount of ABA reaching the epidermis was increased, and therefore xylem ABA exerted an increased effect on stomatal aperture. The results of the present paper confirm the view that the leaf has an important influence on how the stomata respond to whatever moves from the roots in the transpiration stream.

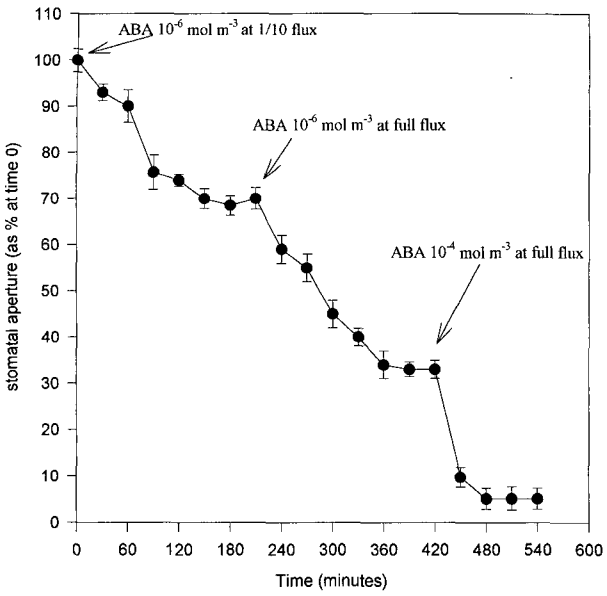

Figure 10. Effect of changing flux or concentration of ABA reaching epidermal peels of *C. communis* after a steady-state stomatal aperture had been reached. Values are the averages ±SE of three different fields from different epidermes.

During the last 30 years, a role for ABA in the control of stomatal functioning has become a well-established part of our thinking about plant water relations. Our data suggest that it may be necessary to think rather differently about the chemical control of stomatal behavior. With many others, we have previously argued that closure of stomata is promoted by an increase in the supply of ABA to leaves, which is usually generated by soil drying. We do not wish to modify this view, but it must also be noted that the data reported here suggest that xylem sap of even well-watered plants will close stomata unless it is modified by the influence of the leaf.

The results described in this paper suggest that the access that ABA molecules have to guard cells can be tightly regulated. We argue that under most conditions the mesophyll will sequester and metabolize enough ABA to allow the stomata to open, but it is important to note that closure of stomata could be caused either by the synthesis of more ABA than the leaf can cope with in its most active state (a turgid leaf?) or by a reduction in the capacity of the mesophyll to cope with even a low concentration of ABA arriving at the leaf, allowing more ABA access to the guard cells. This state might result from a change in leaf water status (Tardieu and Davies, 1992; Trejo and Davies, 1994) or a change in temperature (Rodriguez and Davies, 1982; Cornic and Ghashghaie, 1991), conditions that have been shown to vary the apparent sensitivity of guard cells to ABA, perhaps via an effect on compartmentalization or metabolism. Such a mechanism could provide a clear link between the influence of edaphic and climatic variables on leaf gas exchange and might explain why there is commonly an excellent correlation between mesophyll activity (often quantified as photosynthesis rate) and stomatal behavior (Ball et al., 1987). This observation has given rise to the suggestion that stomatal behavior is controlled by mesophyll activity (Wong et al., 1979). Although this can be shown to be the case under many circumstances, under other conditions (perhaps when the influence of root activity is dominant) this correlation can be broken (Jarvis and Morison, 1981; Zeiger, 1983). The results reported in this paper provide some mechanistic understanding of stomatal behavior of this kind.

In a recent paper, Tardieu (1993) suggested that the steady-state responses of stomata to VPD could be explained by an interaction between ABA and the water relations of the leaf, without any explicit account taken of a humidity response mechanism for the stomata. Others (Raschke, 1979; Blackman and Davies, 1983) have suggested that hormones may play a part in determining the CO_2 responses of stomata. If we are to argue that this is the case, attention must be given to the dynamics of the responses of guard cells to ABA signals and to changes in other environmental variables. We have seen here that the transpiration flux will influence the rapidity with which stomata will respond to a concentration signal arriving from the roots (Fig. 6). The relatively sluggish stomatal response to the removal of the ABA signal (Fig. 7) suggests that, if we are to argue that ABA is involved in the more dynamic stomatal responses to abrupt variation in climatic factors, then we will have to argue for variation in sensitivity of the stomatal response (Davies et al., 1994). To make further progress in this area, we will also need to be able to quantify the supply of hormones to guard cells under different environmental conditions. Until we can do this in vivo, the microscopic technique described in this paper (see also McAinsh et al., 1990) provides a method by which precise control can be exerted on isolated stomata.

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