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SIMULTANEOUS MEASUREMENT OF LEAF HYDRAULIC AND STOMATAL CONDUCTANCES BY PRESSURE BOMB, GAS EXCHANGE, AND β-GAUGE TECHNIQUES

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Abstract. A combination of pressure bomb, gas exchange, and β-gauge techniques was used to simultaneously measure stomatal diffusional conductance and leaf hydraulic conductance between the petiole and the intercellular spaces of infiltrating bean (*Phaseolus vulgaris*) leaves. Measured leaf hydraulic and stomatal conductances were very variable, but their stationary values correlated with each other. A striking coordination between the initial values of gaseous and liquid phase conductances existed independently of experimental conditions: leaf hydraulic and stomatal conductances were lower in the dark than in the light and lower in unwatered than in well-watered plants. The initial values of leaf hydraulic conductance did not correlate with the applied hydraulic pressure.

The new method fits to investigate coordination between hydraulic conductance and stomatal conductance in shoots of different plant species.

Key words: *Phaseolus vulgaris* L.; combination of β-gauge, gas exchange and pressure chamber techniques; leaf hydraulic conductance; stomata.

INTRODUCTION

Desiccation can sometimes be a primary cause of the abscission of plant leaves. A catastrophic water loss from the leaves is possible when evaporation is not correctly balanced with water uptake. The plant parameters determining the water uptake and evaporation under constant environmental conditions are hydraulic and stomatal conductances, respectively. There are several papers that describe changes in the hydraulic conductance of the xylem, especially in trees (Sperry & Tyree, 1988; Cochard et al., 1992; Tyree et al., 1994; Zotz et al., 1994). Little is known about the interactions between the hydraulic and stomatal conductances, although it is evident that both these conductances determine the water status of plant leaves and branches. A rapid stomatal closure should restore the leaf water potential that was reduced by a decrease in the hydraulic conductance (caused for example by xylem embolism or by low root temperature). For sugarcane plants it was shown that vapour phase and liquid phase conductances changed in coordination (Meinzer & Granz, 1991). It is not known how widely that coordination occurs in different

plant species, but it is possible that it exists also at leaf level. There is some evidence that leaf hydraulic conductance in herbaceous species depends on transpiration, being higher at higher transpiration (Hailey et al., 1973; Boyer, 1974; Black, 1979a, 1979b; Boyer, 1985).

This work sought proof for the existence of coordination between hydraulic and stomatal conductances in bean leaves.

MATERIALS AND METHODS

The used method involves the application of pressure to leaf petioles in water in a pressure chamber with a simultaneous measurement of the infiltration rate with β-gauge techniques and measurement of the transpiration rate and leaf temperature to estimate stomatal conductance. The method used to determine the leaf hydraulic conductance assumes that (1) the water potential in intercellular spaces equals zero in the infiltrating leaves and (2) the main resistance to liquid water flow in its path from the xylem to the intercellular spaces is located in the xylem and cell walls, not in the bulk water of intercellulars.

It is possible that the hydraulic conductance measured in this way is higher than that without positive pressure in the xylem, because some of the air-filled cavities may be filled with water immediately after pressure application. Despite this disadvantage, the used method allows of the establishment of coordination between stomatal and hydraulic conductances, if this coordination exists.

Plant material. Dwarf bean (*Phaseolus vulgaris* L. cv. Oregon) plants were used. Plants were grown in a growth chamber in separate 1000 cm^3 stainless steel pots containing a mixture of soil and peat (3 : 1 by volume). Moisture was held at 60% of the mixture's full water capacity. The light/dark period was 14/10 h, and the PAR density was 410 $\mu E\ m^{-2}s^{-1}$ (mercury fluorescent lamp). There was a normal ambient CO_2 concentration, 25/25 °C day/night temperature, and 50—60% relative humidity in the growth chamber. Experiments were performed when plants had three to four trifoliolate leaves (14 to 18 days from emergence).

Experimental procedures. A trifoliolate leaf of the plant was cut near the stem. The cut end of the petiole was driven through the rubber seal of a pressure bomb. Thereafter, the cut end of the stem was immersed in water in the bomb and recut under water. The bomb was closed, and one leaf from three was fitted into a leaf chamber. The leaf chamber has been designed for simultaneous measurements of relative changes in water content, transpiration rate, and the temperature of the leaf under controlled humidity and CO_2 concentrations. The leaf water content was monitored by the β-gauge technique: β-particles from a radioactive source ($Ba^{14}CO_3$, with the activity of 1 mC on the window for leaf illumination) that passed through the leaf were counted with a Geiger-Müller counter (window of 1 cm diameter and 4—5 μm thickness).

Different aspects of the method used were discussed in our previous publications (Sõber & Moldau, 1977; Sõber, 1992). After recording the intensity of β-radiation corresponding to the initial value of the leaf water content, the pressure in the bomb was increased until infiltration started. The appearance of infiltration was denoted as point O on the monitored time courses of the leaf water content (see Fig. 1). Infiltration started at a pressure of about 100 (not zero) kPa in the dark and at a pressure of about 500 kPa at the highest rates of transpiration. This indicates that the water flow out of the cell walls was restricted even under very low transpiration.

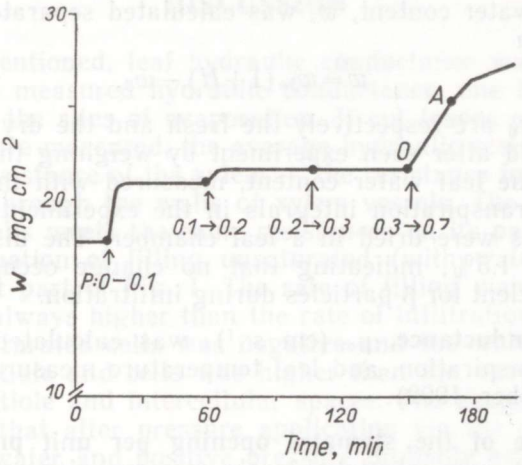

Fig. 1. Typical time course of the leaf water content, w, after a stepwise pressure increase at the moments indicated by arrows. Numbers at arrows designate pressure in MPa before and after its rise. Maximal leaf hydraulic conductance was calculated using the rate of the increase of the leaf water content in its linear region between the points O (initiation of infiltration) and A.

To get lower values of stomatal conductance some plants were not watered for two days prior to the experiment and some experiments were performed after having kept the leaf in a darkened leaf chamber for an hour.

The leaf hydraulic conductance, μ_w, was calculated from the formula

$$\mu_w = FL/\Delta P, \tag{1}$$

where FL is the flow rate of liquid water through a petiole and the leaf to intercellular spaces per leaf area unit (g cm^{-2} s^{-1}), and ΔP is pressure difference at the path of water. The flow rate of liquid water, FL, was calculated as the sum of the infiltrating water flow rate, v_i, measured by β-gauge technicques, and the transpiration rate, E:

$$FL = v_i + E. \tag{2}$$

The infiltration rate was got from the formula

$$v_i = \Delta w/\Delta t, \tag{3}$$

where Δw is the change in the absolute water content of the infiltrating leaf and Δt is the time interval used.

The absolute values of the leaf water content w (g cm^{-2}) cannot be calibrated against β-radiation intensity for all leaves because leaf absorption coefficients for β-radiation are different in leaves with different dry matter content and structure. At the same time the relative difference in the water content, H, can be easily measured:

$$H = (\ln I_e - \ln I)\,/(\ln I' - \ln I_e), \tag{4}$$

where I and I' are the intensities of β-radiation with and without the leaf in the leaf chamber respectively, and I_e is the β-radiation intensity at the end of experiment.

The current water content, w, was calculated separately for each leaf from the formula

$$w = w_{fe}(1+H) - w_d, \quad (5)$$

where w_{fe} and w_d are respectively the fresh and the dry weight per unit leaf area obtained after each experiment by weighing the leaf segments.

Changes in the leaf water content, measured with the β-gauge, were compared with transpiration integrals in the experiments where infiltrated leaf segments were dried in a leaf chamber. The discrepancies were not higher than 1.5%, indicating that no change occurred in the leaf absorption coefficient for β-particles during infiltration.

The stomatal conductance, μ_{st} (cm s^{-1}), was calculated as usual from air humidity, transpiration, and leaf temperature measurements (Sõber & Moldau, 1977; Sõber, 1992).

The relative rate of the stomatal opening per unit pressure increase, v_0 (% s^{-1} MPa^{-1}), was calculated by the maximal slope of the time course of stomatal conductance after an infiltration-inducing pressure rise:

$$v_0 = \Delta\mu_{st}/\Delta t \Delta P \mu_{st}. \quad (6)$$

RESULTS

Leaf hydraulic conductance, calculated by Eqs. (1)—(5), varied greatly among bean leaves. Typical graphs of leaf hydraulic conductance after pressure application are presented in Fig. 2*a*. Leaf hydraulic conductance was at its maximum immediately after a pressure rise, decreasing afterwards to a new stationary level. The infiltration rates were 2 to 15 times higher than the transpiration rates, i.e. the correction of transpiration to the measured infiltration rate was not large. The transpiration rate increased during infiltration when the leaf was in the light, but the increase in transpiration was lower than the decrease in the infiltration rate, i.e. μ_w, calculated by Eqs. (1)—(5), decreased in time. The graphs show that leaf hydraulic conductance was constant only when the infiltration rate was low (e.g. in the case of a darkened leaf chamber, see Fig. 2*a*, curve *3*). Typical changes in the hydraulic conductance, μ_w, after the second increase in pressure are presented in Fig. 2*b*. Leaf hydraulic conductance remained at a constant level for 5—6 min again, then it began to decrease. This means that during the first 5—6 min μ_w was approximately the same as before the infiltration-enchancing pressure increase.

The initial values of the leaf hydraulic conductance, μ_{w0}, varied greatly (values between 3 and 80 μg cm^{-2} s^{-1} MPa^{-1}) and did not depend on the pressure applied in the pressure chamber (data not shown). The initial leaf hydraulic conductance, μ_{w0}, correlates with the initial stomatal conductance, μ_{st0}, estimated before the first pressure rise (Fig. 3*a*). All values of μ_{w0} measured in the dark or on the leaves of unwatered plants were quite low as compared with the values measured in the light on the leaves of watered plants.

The relative rate of stomatal opening per unit pressure increase, v_0, calculated by Eq. (6), was also in positive correlation with the initial leaf hydraulic conductance when the latter was low, but v_0 saturated at high values of hydraulic conductance. The relative rate of stomatal opening of water-deficient leaves was higher than that of control leaves and did not saturate (see different curves in Fig. 3*b*).

DISCUSSION

As already mentioned, leaf hydraulic conductance was very variable. To interprete the measured hydraulic conductance, one has to know the path of water to the sites of evaporation. If cut leaves or branches with petiole in water are measured, the average hydraulic resistance cannot be lower than the resistance of the xylem + the resistance for water flow out from the xylem through the walls of xylem vessels. The water flow that enters the leaf cells meets the same resistances on its path.

A typical situation of filling unsaturated (with water) leaf cells is shown in the left part of Fig. 1. The rate of filling water-deficient cells with water was always higher than the rate of infiltration, because water potential of unsaturated cells was negative and the water potential gradient between petiole and cells was higher than the water potential gradient between petiole and intercellular spaces. The shape of the curve in Fig. 1 suggests that after pressure application via the petiole leaf cells are filling with water and positive pressure probably exists in cell walls

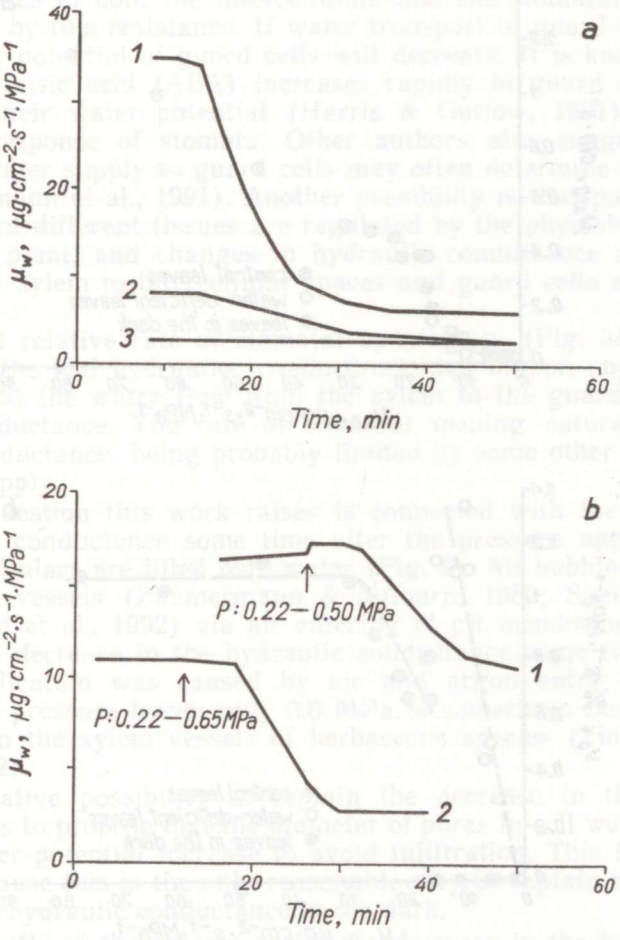

Fig. 2. Typical time courses of leaf hydraulic conductance, μ_w, after infiltration-inducing pressure rise in the pressure chamber (*a*) and during infiltration after the second pressure rise (*b*). Curves *1* and *2*, different leaves in the light; curve *3*, leaf in the dark. Arrows indicate the moment of pressure rise, numbers at arrows show pressure before and after its rise in MPa.

when the cells are already in full turgor, but the leaf is not yet infiltrated. In a certain moment O, when the water potential in the cell walls is high enough, the transpiring air-filled hydrophobic pores are filled with water and the liquid water flows through formed channels to intercellular spaces. The value of the pressure that initiates infiltration in different leaves is lower at lower transpiration, but it is not zero at very low transpiration in the dark. This result is in accordance with data of other authors (Passioura, 1984), and shows that cell walls that are close to intercellulars are hydrophobic, as proposed by Scott (1950). As there is no increase in hydraulic conductance after the second pressure increase (Fig. 2*b*) and there is no positive correlation between the initial hydraulic conductance and the applied pressure (data not shown), it is probable that most channels to intercellular spaces are filled with water at about the same pressure and almost simultaneously.

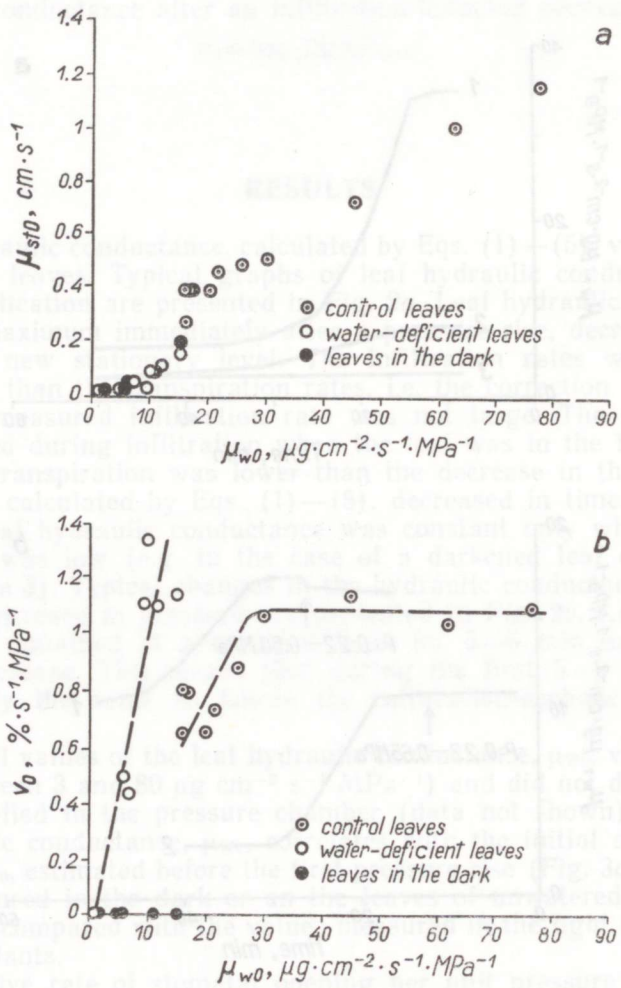

Fig. 3. Correlations of values of initial leaf hydraulic conductance, μ_{w0}, with the initial stomatal conductance, μ_{st0} (*a*) and the initial relative rate of stomatal opening, v_0 (*b*), estimated after a pressure rise in the pressure chamber.

The leaf hydraulic conductance from the petiole to intercellular spaces was in the same order or lower compared to the hydraulic conductance of individual cells. In case the maximal leaf hydraulic conductance per intercellular area was calculated taking into account that the area of the intercellular spaces in bean leaves is about ten times higher than the external projected leaf area, the maximal values of the initial leaf hydraulic conductance per intercellular area were 8×10^{-6} cm s^{-1} MPa^{-1}. These values were similar to those reported for most cells of higher plants (Steudle et al., 1983). Other measured values of hydraulic conductance were up to 30 times lower, but never higher than the hydraulic conductance of cells. Consequently the hydraulic resistance should be located in cells, not in infiltrating intercellular spaces.

A strikingly high positive correlation existed between the initial leaf hydraulic conductance and the stomatal conductance before the pressure rise (Fig. 3*a*). It is not clear whether there exists a causal relationship between hydraulic and stomatal conductances or not. One way to explain this correlation is to assume that a relatively high hydraulic resistance occurs in the bundle sheath and (or) mesophyll tissue. In this case the water flow rates to both the intercellulars and the stomatal guard cells are regulated by this resistance. If water transport to guard cells is limited, the water potential of guard cells will decrease. It is known that the content of abscisic acid (ABA) increases rapidly in guard cells after a decrease in their water potential (Harris & Outlow, 1991), causing a hydroactive response of stomata. Other authors also suggest that the shortage of water supply to guard cells may often determine the stomatal response (Nonami et al., 1991). Another possibility is that pore diameters in cell walls of different tissues are regulated by the physiological status of the whole plant, and changes in hydraulic conductance at the water path from the xylem to intercellular spaces and guard cells are changing in parallel.

The initial relative rate of stomatal opening, v_0 (Fig. 3*b*), was also higher when the leaf hydraulic conductance was higher, supporting our conclusion that the water flow from the xylem to the guard cells limits stomatal conductance. The rate of stomatal opening saturates at high hydraulic conductance, being probably limited by some other process, e.g. by energy sypply.

Another question this work raises is connected with the decrease in the hydraulic conductance some time after the pressure application and before intercellulars are filled with water (Fig. 2). Air bubbles can appear in the xylem vessels (Zimmermann & Milburn, 1982; Sperry & Tyree, 1988; Cochard et al., 1992) via air entering at pit membranes. It is possible that the decrease in the hydraulic conductance some time after the pressure application was caused by air and argon entry via the leaf petiole in the pressure bomb: only 0.6 MPa is sometimes needed to form air bubbles in the xylem vessels of herbaceous species (Zimmermann & Milburn, 1982).

An alternative possibility to explain the decrease in the hydraulic conductivity is to propose that the diameter of pores in cell walls decreases after the water potential increase to avoid infiltration. This is even more probable, because this is the only reasonable way to explain the measured low values of hydraulic conductance in the dark.

Independently of the process causing a decrease in the hydraulic conductance, the initial values of the measured leaf hydraulic conductance can be used to describe the leaf hydraulic conductance before the pressure application. The used method fits to investigate gaseous and liquid phase water conductances in different plant leaves, if measurements of hydraulic conductance are made during 5—6 min after the appearance of infiltration.

The main conclusion drawn from this work is that stomatal conductance can be regulated by cell wall properties. As cell wall properties are known to depend on metabolism of the cells, there exists a possibility to regulate stomatal conductance through mesophyll cell metabolism.

To verify this conclusion, the relations between stomatal conductance and leaf hydraulic conductance require a closer study. The problem deserves study also because coordination between the stomatal and hydraulic conductances may be different in different species and it would modify plant tolerance to water deficit.

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LEHE HÜDRAULILISE JUHTIVUSE JA ÕHULÕHEDE JUHTIVUSE ÜHEAEGNE MÄÄRAMINE, KASUTADES KOMBINEERITULT SURVEKAMBRIT NING GAASIVAHETUSE JA β-OSAKESTE NEELDUMISE MÕÕTMIST

Anu SÕBER

On välja töötatud metoodika, mis võimaldab üheaegselt määrata lehtede hüdraulilist juhtivust ja õhulõhede juhtivust. On leitud, et aedoa lehtede hüdraulilise juhtivuse ja õhulõhede juhtivuse muutlikud väärtused on omavahel väga heas korrelatsioonis: vee vedel- ja gaasifaasi juhtivused olid võrreldes kontrolltaimede lehtede juhtivusega väiksemad nii pimedas kui ka vee defitsiidi korral.

ОДНОВРЕМЕННОЕ ОПРЕДЕЛЕНИЕ ГИДРАВЛИЧЕСКОЙ И УСТЬИЧНОЙ ПРОВОДИМОСТИ У ЛИСТЬЕВ С ИСПОЛЬЗОВАНИЕМ КОМБИНАЦИИ МЕТОДОВ КАМЕРЫ ДАВЛЕНИЯ ГАЗООБМЕНА И ПОГЛОЩЕНИЯ β-ЧАСТИЦ

Ану СЫБЕР

Выработана методика для одновременного измерения гидравлической и устьичной проводимости листьев. Установлена корреляция между значениями этих проводимостей у листьев фасоли. Можно утверждать, что обе проводимости были меньше по сравнению с таковыми у контрольных растений в темноте и при водном дефиците.