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Adapting Constant-volume Manometry for Studying Gas Exchange by Bulky Plant Organs

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Abstract. **Constant-pressure manometry, previously designed to study O_2 and CO_2 gas exchange in small pieces of tissue, cells, and organelles, was adapted to study bulky organs. According to this new procedure, a near-zero-volume Devaux chamber connects a manometer to the internal atmosphere volume (V_G) of a plant organ covered by a layer of epoxy, submerged in unstirred water, kept at constant temperature, and kept at the same V_G pressure. Equations, based on CO_2 and O_2 solubility at equilibrium with V_G, were used to follow O_2 consumption as a function of reduced internal O_2 pressure over time [for organs with V_G < 0.1 (v/v) and respiratory quotient (RQ) of 0.7 to 1.3] to observe the transition between aerobiosis and anaerobiosis and to measure CO_2 evolution during the anaerobic phase. For those measurements, bulky-organ manometry performed consistently in tomato [V_G = 6.41% (v/v)], sweetpotato [V_G = 8.57% (v/v)], and potato [V_G = 0.34% (v/v)]. The results indicate that constant-volume manometry is sufficiently precise to detect differences in respiratory metabolism as a function of intercellular O_2 concentration in intact plant organs.**

Constant-volume manometry has been used to study the consumption and production of gases such as CO_2, N_2, and O_2 during fermentation, photosynthesis, nitrate reduction, and protein decomposition and to study some specific respiratory enzymes (Burk and Milner, 1932). The equipment used for constant-volume manometry is constructed of a manometer with a rigid removable jar placed inside a temperature-controlled bath with shaking, to allow rapid equilibrium between the gas and liquid phases. According to this method, under constant temperature and volume, a change in the number of gas molecules can be followed by measuring pressure (Umbreit et al., 1972). Perkins (1943) described three main types of manometry equipment. The first was a constant-volume Warburg-Bancroft respirometer. The second was a differential respirometer in which the side of the manometer, which is usually open to the air, is connected to another closed jar to eliminate small errors caused by temperature and barometric changes during the assay. The third, used with the Warburg-Barcroft equipment, allows direct and differential manometric measurements.

Several methods were developed to extract and evaluate the intercellular concentrations of different gaseous components such as CO_2, ethylene, O_2, and N_2 from bulky organs. The main principles used for the extraction techniques are the concentration equilibrium between the gaseous components of an internal chamber (Wardlaw and Leonard, 1939) or an external chamber (Devaux, 1891) and the plant internal atmosphere. In addition, there are several vacuum-extraction techniques (Burton and Spragg, 1950; Calbo and Sommer, 1987; Magness, 1922) that also have been used to measure porosity or V_G (Burton and Spragg, 1950; Calbo and Sommer, 1987). In the case of porosity measurements, other principles such as pycnometry (Jensen et al., 1969) and inert gases (Cameron and Yang, 1982) have also been used. For concentration measurements of very small gaseous samples with CO_2, O_2, and N_2, the method of Bonnier and Mangin (Thoday, 1913), or one of its adaptations such as the one made by Scholander (1947), is reliable and inexpensive.

The main objective of this work was to present an extension of the Warburg-Bancroft technique to apply constant-volume manometry to bulky organs using a new approach to achieve equilibrium between the liquid and the gas phases in plant organs sealed in a vessel maintained at a constant temperature and volume. Bulky-organ manometry was evaluated as a tool to study respiration as a function of intercellular O_2 partial pressure and anaerobic CO_2 evolution.

Theory

Assumptions. The basic assumptions of this work are that 1) the volume of an externally sealed organ remains constant during the measurement; 2) the equilibrium between the gas and the liquid phase inside the organ is very rapid and, consequently, changes in the liquid phase can be followed in the gas phase; 3) changes of the internal gradients of O_2 and CO_2 at the intercellular level are not a limiting factor to the method; and 4) the half-time in seconds ($t_{1/2}$) for equilibrium between the pressure-measuring device with near-zero volume and the internal atmosphere is small and can be disregarded.

Aerobic phase. The total number of gas molecules (n_T) inside an externally sealed bulky organ at constant temperature can be represented as the sum of its components:

$$n_T = n_{O_2} + n_{CO_2} + n_{N_2} + n_{Ar} + ... \quad [1]$$

where n is the number of moles of each specified gas molecule.

Assuming that for each molecule of O_2 consumed one molecule of CO_2 is released (or the converse) and that N_2, Ar, H_2O, and all minor components of the air can be disregarded for calculations of CO_2 and O_2 exchange, then n_T could be represented by

$$n_T = (n_{O_2} + n_{CO_2})_L + (n_{O_2} + n_{CO_2})_G \quad [2]$$

where the subscripts L and G are the liquid and gas phases, respectively.

Substituting the number of molecules of each species by the product of the concentration (mol·liter^{-1}) and the volume of the

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system (V) in liters, Eq. [3] is obtained:

$$n_T = V_L ([O_2] + [CO_2])_L + V_G ([O_2] + [CO_2])_G \quad [3]$$

The ideal gases could be rewritten for CO_2 and O_2 as follows:

$$PV = nRT \quad [4]$$

$$[O_2]_G = p_{O_2}/RT \quad [5]$$

$$[CO_2]_G = p_{CO_2}/RT \quad [6]$$

where P is the partial pressure (kPa), R is the gas constant (8.3143 $J \cdot K^{-1} \cdot mol^{-1}$), and T is the temperature.

Substituting Eqs. [4] and [6] into Eq. [3] generates

$$n_T = V_L([O_2]_L + [CO_2]_L) + V_G[(p_{O_2}/RT) + (p_{CO_2}/RT)] \quad [7]$$

The concentrations of O_2 and CO_2 in the liquid phase can be calculated knowing that α_{O_2} and α_{CO_2} are the solubilities of O_2 and CO_2 in water at normal pressure ($mol \cdot liter^{-1}$) (Armstrong, 1979; Umbreit et al., 1972).

$$[O_2]_L = \alpha_{O_2} p_{O_2} \quad [8]$$

$$[CO_2]_L = \alpha_{CO_2} p_{CO_2} \quad [9]$$

An expression relating pressure variation and O_2 consumption can then be obtained by substituting Eqs. [8] and [9] into Eq. [7]:

$$n_T = p_{CO_2}(\alpha_{CO_2} V_L + V_G/RT) + p_{O_2}(\alpha_{O_2} V_L + V_G/RT) \quad [10]$$

Separating p_{CO_2} from Eq. [10] yields

$$p_{CO_2} = [n_T - p_{O_2}(\alpha_{O_2} V_L + V_G/RT)]/(\alpha_{CO_2} + V_G/RT) \quad [11]$$

The sum of partial pressures of O_2 and CO_2 (H) could be expressed as

$$H = p_{O_2} + p_{CO_2} \quad [12]$$

Substituting p_{CO_2} from Eq. [11] into Eq. [12] gives

$$p_{CO_2} = [H(\alpha_{CO_2} V_L + V_G/RT) - n_{T]}]/[V_L(\alpha_{O_2} - \alpha_{CO_2})] \quad [13]$$

Differentiating Eq. [13] with relation to H gives

$$dp_{O_2}/dH = (\alpha_{CO_2} + V_G/V_L RT)/(\alpha_{O_2} - \alpha_{CO_2}) \quad [14]$$

Equation [14] represents the change in P_{O_2} as a function of the pressure change measured in the sealed organ.

Anaerobic phase. In the anaerobic phase, n_T is no longer constant and its variation is a function of CO_2 production. In this particular case, the pressure variation is a measurement of dn_{CO_2}:

$$dn_{CO_2} = V_G d[CO_2]_G + V_L d[CO_2]_L \quad [15]$$

Under anaerobiosis, $dp_{O_2} = 0$ (Eq. [12]) and dp_{CO_2} is dH. Substituting Eqs. [6] and [9] into [15] gives

$$dn_{CO_2} = (\alpha_{CO_2} V_L + V_G/RT) dp_{CO_2} \quad [16]$$

Assumption 1. The constancy of the sealed bulky-organ volume (V_o) is a requirement for using the constant-volume manometry method. Assume then the behavior of a sealed organ enclosed within an incompressible chamber filled with water at constant temperature (Fig. 1a) so that

$$\Delta V_t = \Delta V_o + \Delta V_w \quad [17]$$

$$\Delta V_o = \Delta V_G + \Delta V_{SL} \quad [18]$$

$$\Delta V_w = \Delta V_{SL} \quad [19]$$

Since the organ is sealed and the expected variations of solids and liquids (V_{SL}) are small because there is no net influx of water (V_w), then V_{SL} could be ignored. Accordingly, the variation of V_G will be equal to the total system volume variation (V_t), which is zero due to chamber incompressibility. Consequently, a pressure equilibrium among the measured pressure (H), organ pressure (p_o), and liquid pressure (p_L) should be sustained (Eq. [20]):

$$H = p_o = p_L \quad [20]$$

Equation [20] describes the sufficient condition that can be used to set up alternative systems even without using undeformable recipients (Fig. 1b).

Assumption 2. The O_2 and CO_2 equilibrium rate between the liquid and gas phase inside the organ is fundamental in this method, as it is in the Warburg-Bancroft respirometer. Perkins (1943) stressed the necessity of a good shaker to accelerate this equilibrium. In the present work, diffusion plays a larger role than shaking to accelerate the equilibrium, since no mechanical shaker can be used inside the organ.

The mentioned reasons indicate that it is important to know the time required by a front of gas molecules, located at a volume element between two cell walls, to reach the protoplast. Figure 2A shows a section through the plant tissue with a gas volume between two cell walls. Considering that initially (t = 0) all O_2 molecules are in this volume element, then Eq. [21] derived from Ficks's second law can be used to estimate the time intervals t_1 and t_2 expended for a fraction 1/e of the molecules to travel a distance equal to or larger than x_1 and x_2 within the cell.

$$\bar{x}^2 = 4Di \cdot t \quad [21]$$

where $\bar{x}$ is the average distance traveled by the molecules, Di is the diffusion coefficient, and t is the time expended by the molecular front.

Using Eq. [21] requires that all O_2 molecules be lined in the orthogonal plane that contains X_0; this is not the situation in Fig. 2. However, since the Di for an O_2 molecule in air (0.207 $cm^2 \cdot s^{-1}$) is $\approx 10^4$ times larger than in water (2.38×10^{-5} $cm^2 \cdot s^{-1}$) at 25C (Armstrong, 1979), the V_G could be considered an extremely thin layer.

An estimation of the time to reach equilibrium between V_G and the protoplast of the cells can be obtained by applying Eq. [21] with two initial conditions.

a) It is assumed that cyclosis stirs the cytoplasm efficiently and the only resistance to diffusion is located at the cell wall water layer (Fig. 2A). If the average thickness of the cell wall is ≈10 µm (Nobel, 1970), then the average time for 1/e O_2 molecules to cross the cell wall according to Eq. [21] is 1.0×10^{-4} sec.

b) It is assumed that the cytoplasm of those cells remains inert (Fig. 2b). Under this condition, the time for diffusion of 1/e of the O_2 molecules from the V_G at the initial position $x_0 = 0$ to a point located at $x_1 = 100$ µm apart is 1.0×10^{-2} sec.

These estimates suggest that all cells may almost be in equilibrium with the V_G in their vicinity.

Assumption 3. Changes of the internal gradients of O_2 and CO_2 at the intercellular level should not be a limiting factor because the Devaux chamber (Fig. 3) closely approximates the average com-

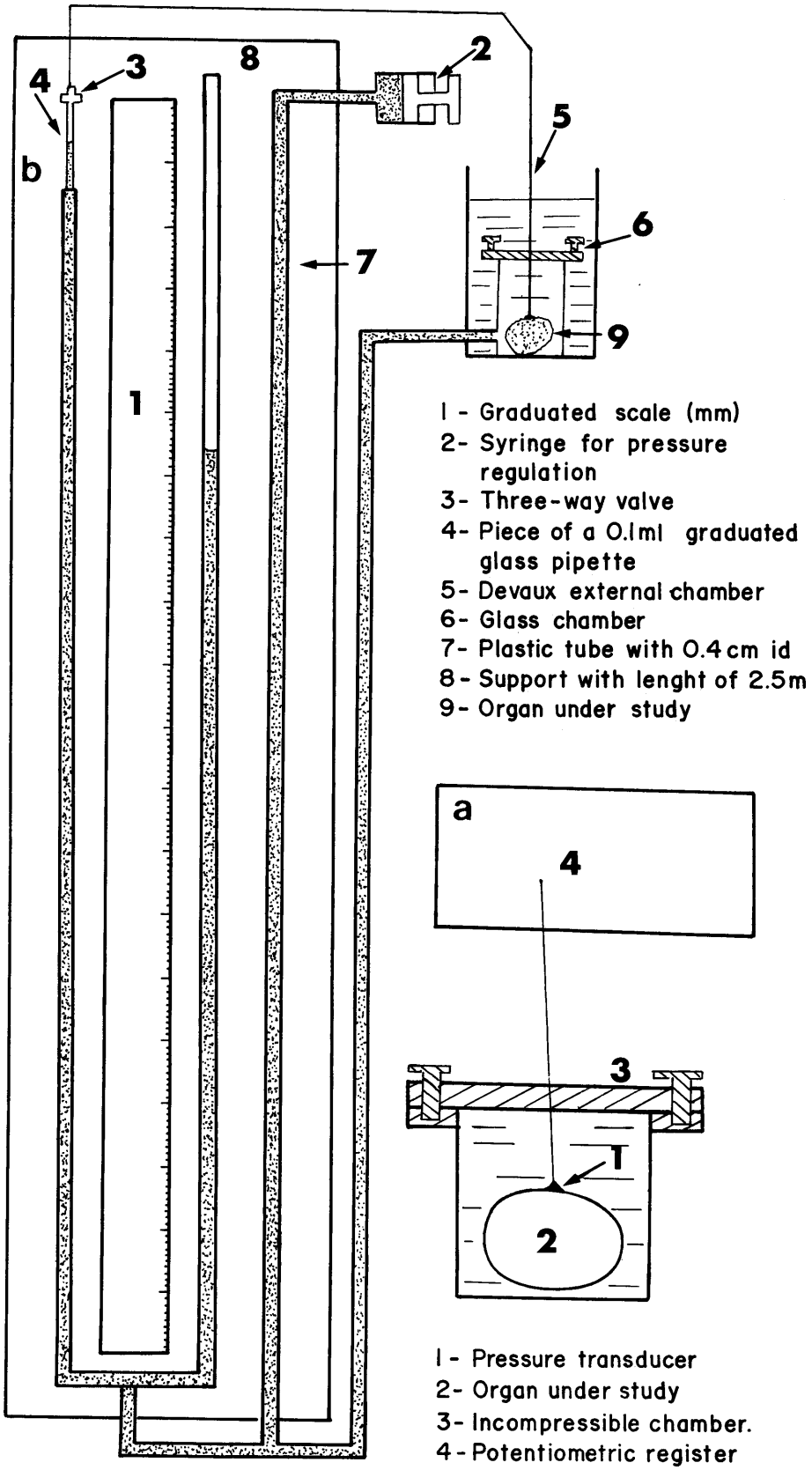

Fig. 1. Equipment to measure aerobic O_2 and anaerobic CO_2 evolution of a sealed bulky organ (2, 9) kept inside a chamber filled with water (3, 6) using constant-volume manometry. (**a**) Alternative electronic system that could be also used to apply the constant-volume manometry technique, with the bulky organ kept inside an incompressible chamber. In this automated model, the Devaux chamber is replaced by a pressure transducer (1) sealed against the organ (2) and connected to an X-Y potentiometer (4) that should register the pressure changes in time with an accuracy of at least 50 Pa. (**b**) The intercellular air pressure is followed in a millimetric 2.5-m U-shaped manometer (1) filled with water connected to the organ (9) by a Devaux chamber with near-zero dead volume (5). The manometer column height is slowly changed in time with a syringe (2) to keep the air volume constant, as shown by the water level (4).

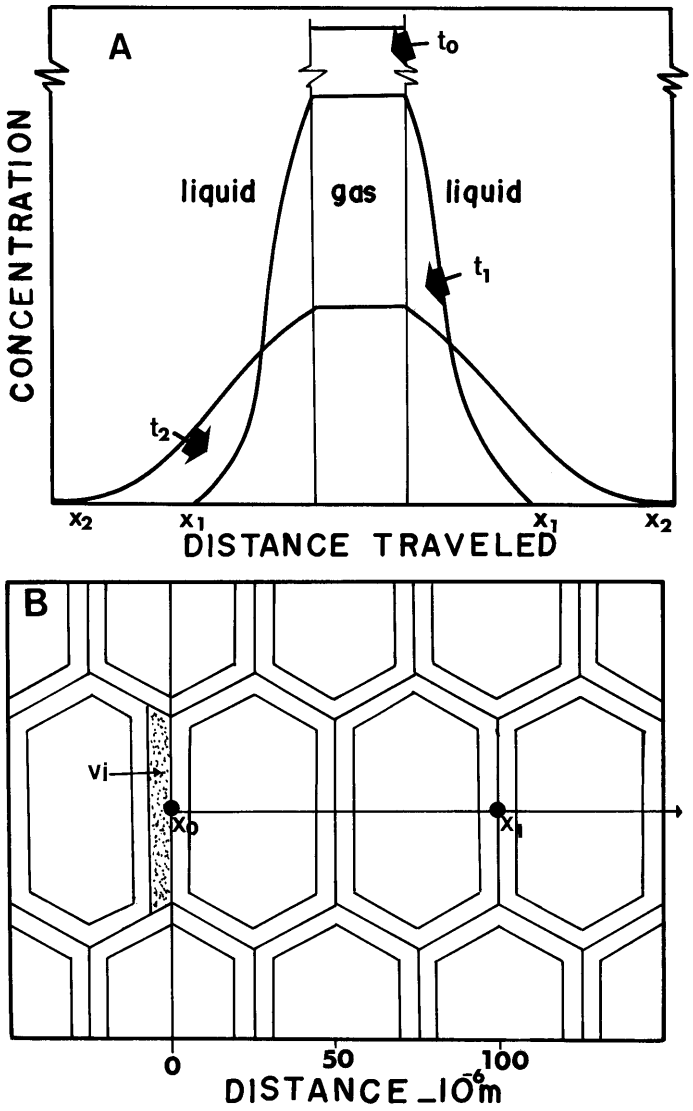

Fig. 2. Unidirectional O_2 diffusion models. (**A**) Oxygen random distribution from an intercellular air volume (V_i) located between two cell walls. X_1 and X_2 are the distances traveled by the molecular front after the time intervals t_1 and t_2 ($t_2 = 10t_1$) and the ordinate is the local concentration. (**B**) Diagram of a transversal section showing V_i and a 100-μm O_2 path between X_0 and X_1 within the plant tissue.

position for those organs (Cameron, 1982). Furthermore, it can be hypothesized that, for the sealed organ under study, the internal gradients will vanish during the measurement. From those considerations, it is assumed that, even for organs without a clear superficial barrier to diffusion, the internal gradients of concentration should not limit the use of this manometric technique.

Assumption 4. For constant-volume manometry of bulky organs, the gaseous composition of the near-zero dead-volume Devaux chamber that connects the organ and the pressure-measuring device should be in equilibrium with V_G (Fig. 3a).

Assuming that a Devaux chamber with a volume V_C is loosing O_2 to an organ at rate J (mol·s^{-1}), with t the time in seconds and n_C the number of O_2 molecules in the chamber, the changes of n_C in time can be expressed by Eq. [22]:

$$Jdt = dn_C \quad [22]$$

Since J is proportional to the concentration gradient between the internal atmosphere (c_i) and the Devaux chamber (c_C) (mol·liter^{-1}) and is inversely related to the diffusion resistance (ϕ) of the region of the organ in contact with the Devaux chamber, Eq. [22] can be rewritten as

$$(c_i - c_C)dt/\phi = V_c dc_c \quad [23]$$

or in its integral form as

$$-t/\phi V_c = Ln(c_i - c_c) \quad [24]$$

From Eq. [24], it can be shown that

$$t_{1/2} = -V_c \phi Ln_{1/2} \quad [25]$$

where $t_{1/2}$ is the time it takes for the Devaux chamber to reach half of the internal organ concentration.

The unit of ϕ_o is liters·s^{-1}, which is convenient since the average difference in O_2 concentration between the internal atmosphere and the air divided by the rate of consumption, an average resistance or transforming factor, is available in the literature for many organs and can be expressed as liters·kg^{-1}·s^{-1}. As a consequence, this transforming factor divided by the specific organ mass estimates the organ diffusion resistance (ϕ).

Fick's first law of proportionality between the cross-section area and the flux can be used to show that

$$\phi = \phi_o A_o / A_c \quad [26]$$

where ϕ is the whole-organ diffusion resistance, A_o is the organ external area (cm^2), and A_c is the Devaux chamber area.

According to Eqs. [25] and [26], $t_{1/2}$ will become smaller as the Devaux chamber area A_c grows bigger and V_c gets smaller.

Materials and Methods

Plant material. Tuberous roots of 'Coquinho' sweetpotato [*Ipomea batatas* (L.) Lam], 'Bintje' potato tubers (*Solanum tuberosum* L.), and 'Kada' tomato (*Lycopersicon esculentum* Mill.) grown at Centro Nacional de Pesquisa de Hortaliças, Brasília, Brazil, were stored at 13 ± 2C for ≈40 days, 9 ± 2C for ≈20 days, and 20 ± 3C for 1 day, respectively. Organs were transferred to the exact assay temperature 1 day before each measurement to achieve a steady-state temperature.

Devaux chamber. A chamber was placed at the surface of the organ that had a smaller resistance to diffusion in an area abraded 24 h before with a sharp razor blade to work as an extension of the V_G in dynamic equilibrium conditions (Devaux, 1891) (Fig. 3). The Devaux chamber was reduced to a slightly curved copper plate with a 1- to 3-cm^2 (potatoes and sweetpotatoes) and a circular copper plate for tomatoes with a 0.18-mm-i.d. plastic capillary inserted in its center for gas sampling with a hypodermic syringe. The surface of the chamber had a coarse texture to avoid air blockage by the contact between the chamber and the organ. This chamber was fixed to the organ with araldite.

Manometer. The Devaux chamber (Fig. 1b, 5), with a capillary in its center, was connected to the organ (Fig. 1b, 9) and the manometer through the three-way valve (Fig. 1b, 3). To reduce the system's air volume (Fig. 1b, 4), a small piece of a 0.1-ml glass pipette was set on the tip of the plastic manometer tube. The three-way valve (Fig. 1b, 3) is used to connect the organ to the air and the manometer and to align it with the scale. During operation, the system pressure is changed with a syringe (Fig. 1b, 2) by adding or removing water in the manometer when the water meniscus departs from its origin in this pipette.

Alternative CO_2 and O_2 measurements. After allowing time for equilibrium between the gaseous components of the internal atmosphere and the Devaux chamber attached to a nonsealed organ (Eq. [25]), 0.1-ml gas samples were withdrawn from the chamber

using a 1-ml syringe with near-zero dead volume. The concentrations of CO_2 and O_2 were measured with the Bonnier-Mangin microtechnique (Scholander, 1947; Thoday, 1913).

Operation. The organ, with the Devaux chamber attached, was completely covered with araldite and enclosed in the chamber (Fig. 1b, 6). The chronometer is started when the three-way valve position is changed to connect the manometer and the organ internal atmosphere through the Devaux chamber. With small movements of the syringe (Fig. 1b, 2) the meniscus was adjusted a millimeter below (for aerobic O_2 consumption) or above (for anaerobic CO_2 evolution) the zero at the pipette in the left arm of the manometer. The height in the right arm was then recorded. At the exact moment that the meniscus went back to zero, the corresponding time was also recorded. The height increments used in this feedback process should not be >2.0 cm due to the time required for equilibrium between the Devaux chamber pressure and the organ internal atmosphere pressure.

The presented curves relating the rate of O_2 consumption or CO_2 evolution vs. time and O_2 evolution vs. internal O_2 partial pressure represent data from at least five consecutive experiments.

A reliable electronic feedback mechanism could be used with a pressure transducer (Fig. 1a) to automate this process.

Intercellular gas determinations. V_G was estimated with a semipycnometric method (Calbo and Nery, 1994).

The internal O_2 partial pressure was estimated backwards, knowing that at the point of transition between aerobiosis and anaerobiosis the intercellular O_2 partial pressure is near zero (Beevers, 1961; Yocum and Hanckett, 1957). The initial internal concentration could either be measured in samples taken from the Devaux external chamber or directly estimated from the manometric reading (Table 1).

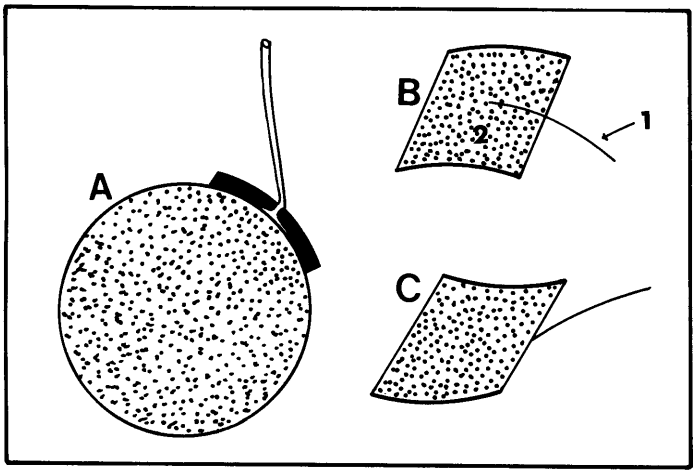

Fig. 3. Devaux external chamber with near-zero dead volume connected to the organ surface by a sealant to work as an extension of the internal organ atmosphere. This chamber was used to take internal atmosphere gas samples and connect the plant organ to the manometer for constant-volume manometry. A = Devaux external chamber connected to the organ. B = top view. C = underneath view, 1 = copper capillary, 2 = slightly curved copper plate.

The O_2 consumption rate ($mol \cdot kg^{-1} \cdot h^{-1}$, R_{O_2}) was obtained with Eq. [27]:

$$R_{O_2} = (\alpha_{O_2} V_L + V_G/RT) dp_{O_2} M_o dt \quad [27]$$

and the rate of CO_2 evolution ($mol \cdot kg \cdot h^{-1}$, R_{CO_2}) was obtained with Eq. [28]:

$$R_{CO_2} = (\alpha_{CO_2} V_L + V_G/RT) dp_{CO_2} M_o dt \quad [28]$$

where V_L was estimated as the volume of the organ minus its gaseous volume and Mo is the organ mass (kg).

Results and Discussion

Carbon dioxide evolution, V_G/V_L, and RQ effects. Figure 4 shows the maximum theoretical CO_2 pressure developed when all O_2 molecules contained inside an organ having an initial internal O_2 pressure of 20 kPa are consumed in respiration, with an RQ of 1 and an initial CO_2 pressure of 0.0 kPa as a function of the V_G/V_L (Eq. [29]).

$$p_{CO_2} = RTn_T/(RT\alpha_{CO_2} V_L + V_G) \quad [29]$$

The maximum CO_2 pressure developed during aerobic respiration increases as a function of V_G/V_L and becomes asymptotic to 20 kPa, as V_G/V_L tends to be infinite (Fig. 4). If V_G/V_L is very large, p_{CO_2} becomes equal to the initial p_{O_2} and the total pressure variation becomes null. Consequently, this method could no longer be applied. Thus, organs having V_G/V_L <0.5 and usually <0.1 are suitable for use with this manometric technique. Accordingly, for bulky organs having V_G/V_L <0.1 (v/v), dH should estimate dp_{O_2} with an error <2.5%.

Equation [29] can also be used to estimate the errors caused by RQ values different from 1. For this purpose, the right side of Eq. [29] has to be multiplied by RQ. Using this procedure, it can be proved that, for 0.7 < RQ < 1.3, the errors in the estimated O_2 consumption rate are negligible for V_G/V_L < 0.1.

Respiration and intercellular O_2. Constant-volume manometry of bulky organs can be used efficiently to measure respiration as a function of the internal O_2 partial pressure in organs, such as roots, tubers, and fruit, with variable V_G and firmness (Fig. 5). These respiration rates were similar to standard chromatographic estimates of CO_2 evolution in organs for which RQ is presumably close to the unit (Table 1).

The manometric method can even be used to estimate the cytochrome oxidize apparent Km for organs with very low respiration rate and a nearly null CO_2 evolution rate in the anaerobic

Table 1. Estimates[z] of intercellular O_2 pressure reduction obtained by manometry and using the Devaux external chamber method, intercellular air volume (V_G), O_2 consumption rate by manometry, and CO_2 evolution rate by gas chromatography. These assays were performed at a barometric pressure of 91.2 kPa at 11C for potatoes and sweetpotatoes and at 23C for tomatoes.

	Pressure reduction	O_2 partial pressure (kPa)		V_G	O_2[y]	CO_2
Organ	(kPa) at O_2 = 0	Manometry	Devaux	[% (v/v)]	($mmol \cdot kg^{-1} \cdot h^{-1}$)	
Potato	17.02 ± 0.40	17.60 ± 0.41	16.75 ± 0.41	0.34 ± 0.23	0.117 ± 0.005	0.132 ± 0.006
Sweetpotato	16.84 ± 0.61	18.71 ± 0.69	17.04 ± 0.59	8.57 ± 0.44	0.388 ± 0.044	0.413 ± 0.040
Tomato	12.07 ± 0.86	13.62 ± 1.1	15.04 ± 0.32	6.41 ± 0.8	0.551 ± 0.089	0.544 ± 0.031

[z]Each value represents mean of five replicates ±SD.
[y]Oxygen consumption rates at an O_2 partial pressure of 9.61 kPa.

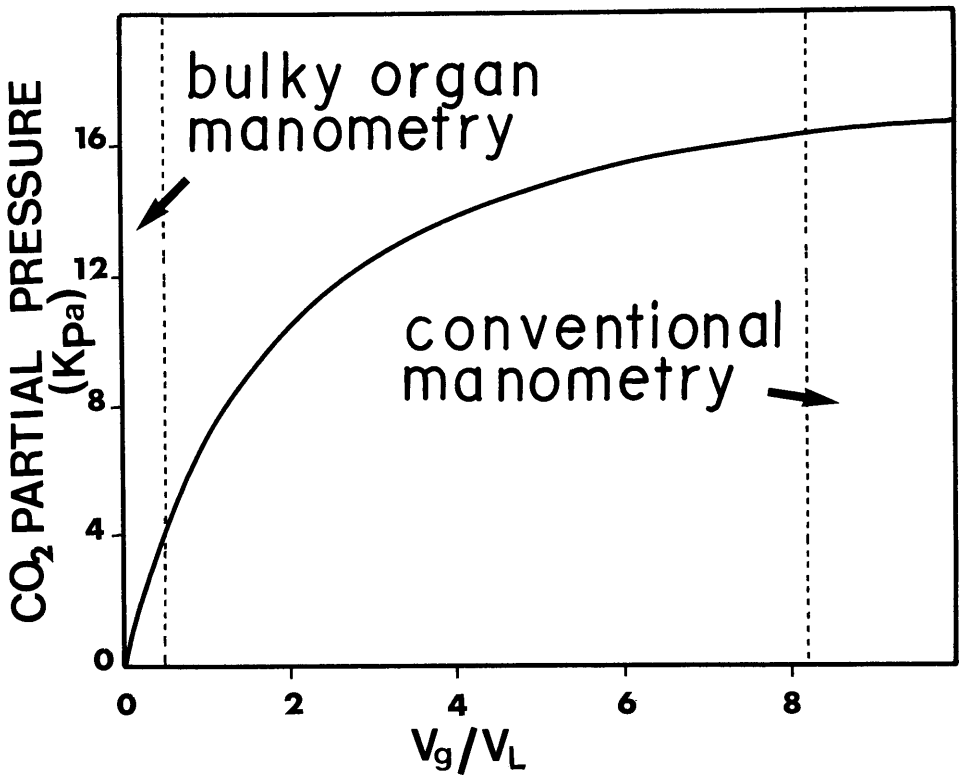

Fig. 4. Theoretical relationship between the CO_2 partial pressure generated by CO_2 evolution during the complete respiratory consumption of O_2 as a function of the ratio between internal gas (V_G) and volume (V_L) of an organ according to Eq. [29].

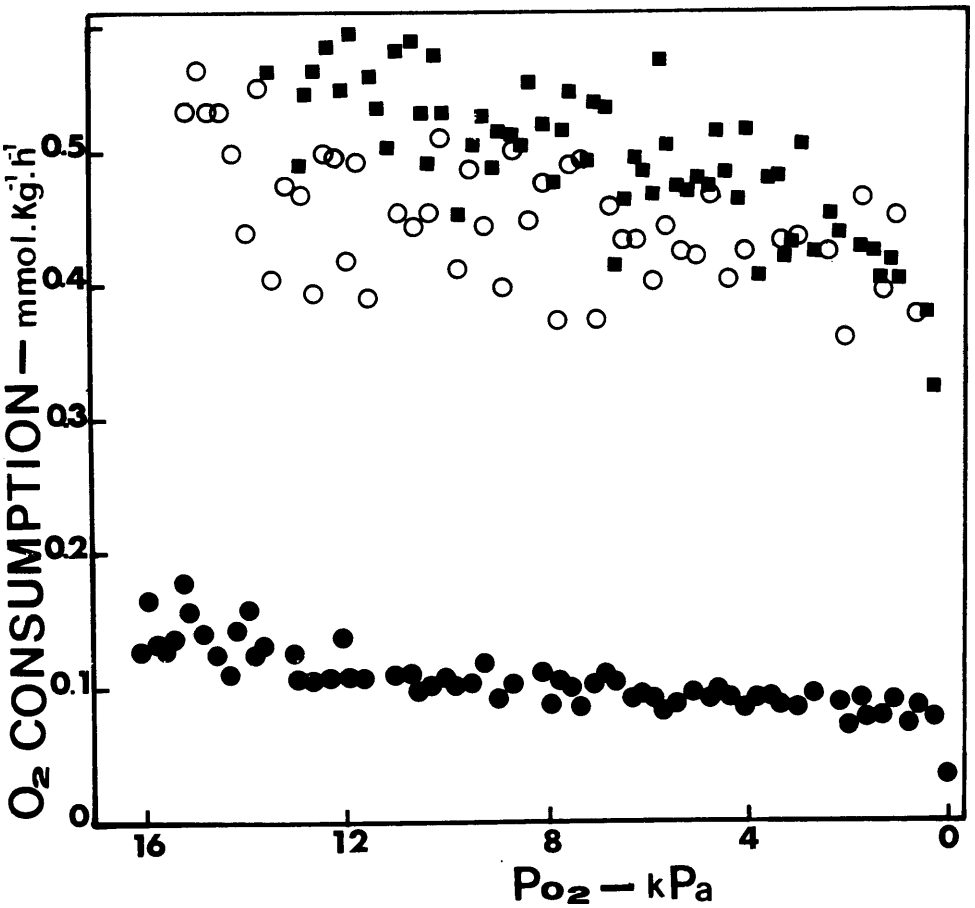

Fig. 5. Typical O_2 consumption rates as a function of the internal O_2 partial pressure (P_{O_2}) reduction in potatoes (●) and sweetpotatoes (❍) at 11C and tomatoes (■) at 23C under a local barometric pressure of 91.2 kPa.

phase. Potato, an organ that produces lactate instead of ethanol and CO_2 in the absence of O_2 (Beevers, 1961), had a CO_2 evolution rate too low to be followed accurately in the anaerobic phase. A Michaelis-Menten least square fit for estimated O_2 levels between 0% and 5% in Fig. 6a give a Km estimate of $0.0447 \pm 0.0100\%$ O_2 (n = 5) or 7.29 μM of dissolved O_2, a result that nearly agrees with that of Yocum and Hanckett (1957).

The presence of a dermal diffusion barrier can affect the shape of the curves relating internal O_2 partial pressure and respiration, while the water manometer registers the initial 40 cm of pressure reduction. This fact was observed in potatoes (Fig. 6a), for which a rapid apparent respiratory reduction occurred in the first 20 cm of pressure reduction. In sweetpotatoes, the surface abrasion before the Devaux chamber attachment removed this initial error. For potato tubers, however, abrasion was not sufficient. In tomatoes, this error was not observed. If this anomaly occurs for any given organ, then the respiration estimates during the first 40 cm should be ignored.

The observed dead volume for the Devaux chamber, measured according to the required volume of petroleum jelly required to fill the space between the organ and the internal chamber surface, was 0.04 ml; n = 5 was roughly similar to the potato V_G, from which O_2 diffusion toward the organ could cause this initially rapid pressure reduction, a departure from the desired equilibrium. For organs with low intercellular air volume, this dead volume should be added to V_G, otherwise the respiration rate, according to Eqs. [14] and [27], will be underestimated.

There were near-linear relationships between time and the intercellular pressure inside a sweetpotato and a tomato (Figs. 6b, 7). Two factors may have caused a rapid transition from the near-linear O_2 consumption to the anaerobic CO_2 evolution. First, the low Km value of the cytochrome oxidize was estimated to be

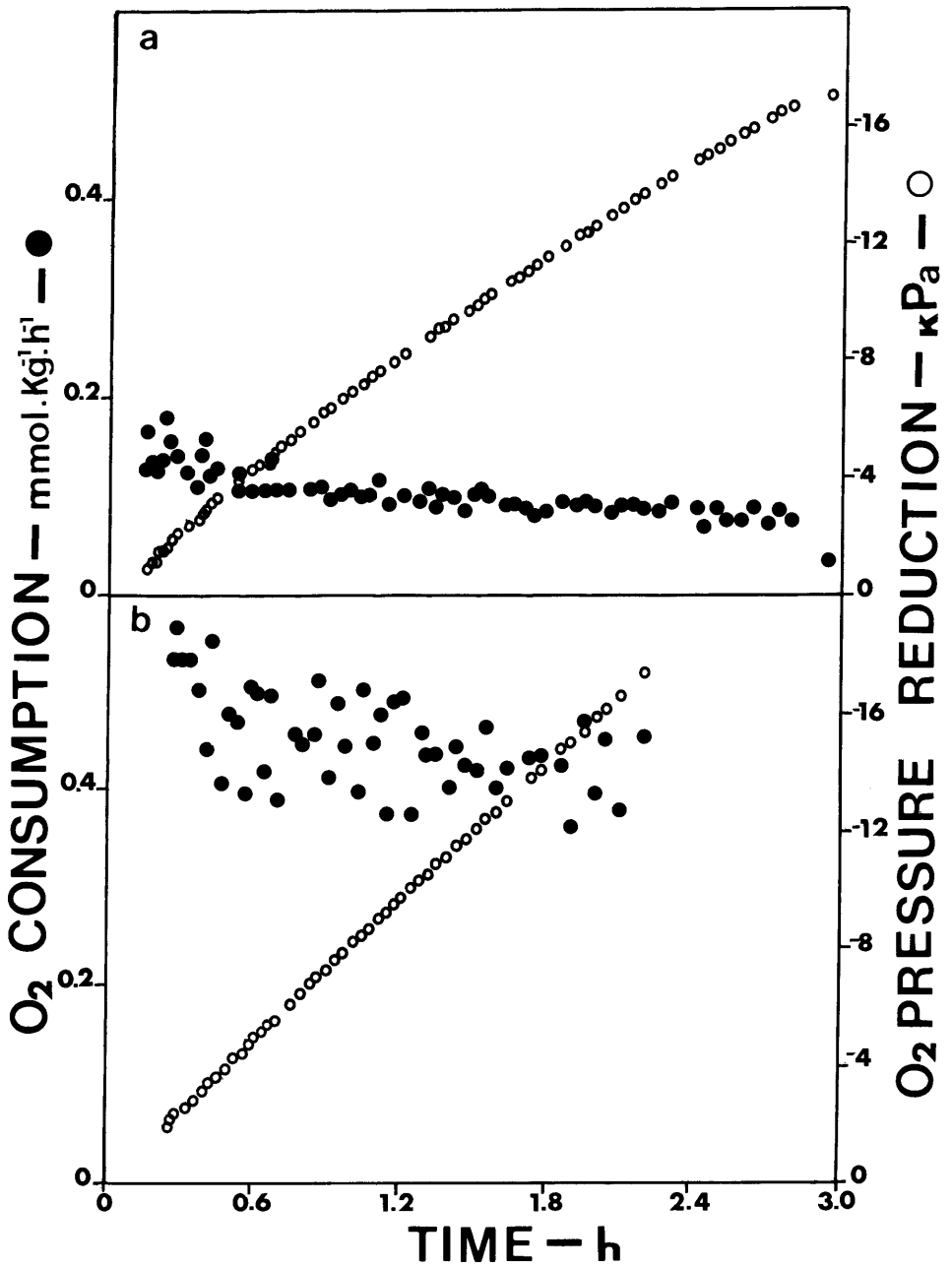

Fig. 6. Data obtained from bulk-organ constant-volume manometry applied to 'Bintje' potato tubers (**a**) and 'Coquinho' sweetpotato roots (**b**) at a barometric pressure of 91.2 kPa at 11C. Typical time-courses of the intercellular O_2 pressure gradient (○); O_2 consumption as a function of time (●).

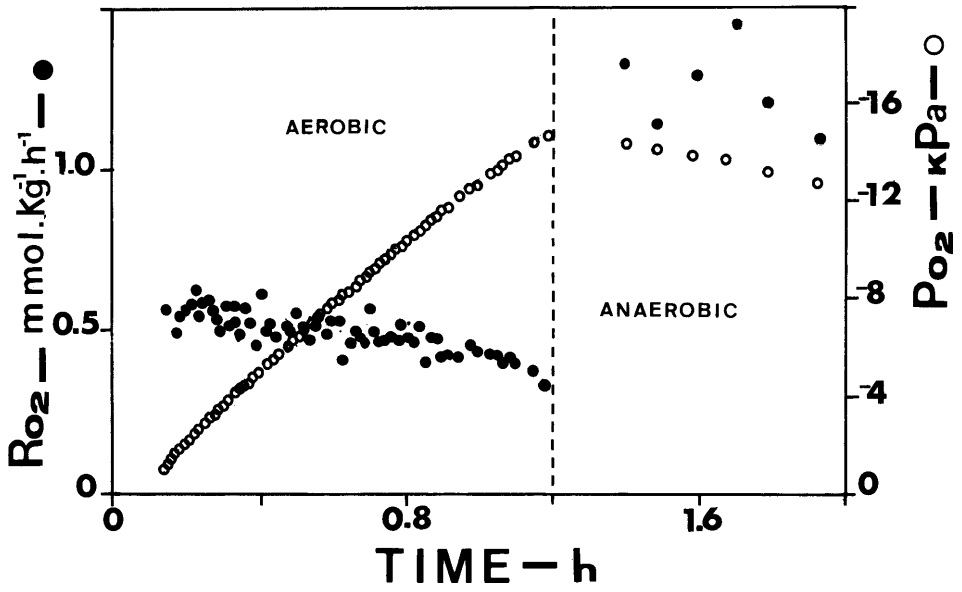

Fig. 7. Typical aerobic O_2 consumption rate or anaerobic CO_2 evolution rate (●) and internal pressure (○) during manometric assay of tomatoes at 23C under a local barometric pressure of 91.2 kPa.

<0.091 kPa O_2. Second, the plant tissues may require a near constant ATP supply and demand, possibly related to a rapid anaerobic respiration triggered as soon as the O_2 was depleted.

The time spent to consume all of the O_2 (Figs. 6 a and b, 7) was a function of the product's respiratory rate, temperature, and V_G. These periods were ≈1.5 h for sweetpotato and 3.5 h for potato, both at 11C, and 0.5 h for tomato at 23C (n = 5). These long periods were considered experimentally desirable, since they allowed the required time for equilibrium between the liquid and gas phase.

Table 1 shows agreement between the estimates of the internal O_2 pressure reduction by this constant-volume manometric technique and those obtained from gas samples taken from the Devaux (1891) external chamber. Small experimental differences between these methods, however, were conceivable since, besides the random experimental errors, at least the following factors were involved.

a) The plant intercellular air pressure usually is not in perfect equilibrium with the external atmosphere (Dancey, 1987). Gradients of 200 to 500 Pa were observed for sweetpotato roots at 11C and 90% relative humidity. For these observations, the manometer was connected to the organ using a Devaux chamber (Fig. 3).

b) The time before the beginning of the experiment, while the organ was being sealed, caused the largest differences in the tomatoes, since the connection of the Devaux chamber to the peduncle insertion nearly blocks fruit gas exchange.

c) There was some error in assessing the transition between aerobiosis and anaerobiosis (50 Pa).

Constant-pressure manometry using the intercellular gas volume of bulky organs with very different V_G, anatomy, and morphology performed surprisingly well. The proposed method contrasts with Warburg-Bancroft constant-volume manometry, which uses an analogous set of equations derived for a chamber with a gas volume many times larger than the sample volume, and was specific for cells, tissue sections, or organs of very small dimensions (Perkins, 1943; Umbreit et al., 1972) with negligible diffusion resistance.

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