Communication

Pressure Probe Technique to Study Transpiration in *Phycomyces* Sporangiophores[1]

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ABSTRACT

The growth equation for the rate of water uptake is augmented with a transpiration term. The obtained augmented growth equations are used to develop methodology which employs the pressure probe to measure transpiration rates from single plant cells. Experiments are conducted on the sporangiophores of *Phycomyces blakesleeanus* to demonstrate this technique.

Pressure probe techniques have been used to determine the biophysical and biomechanical properties of higher plant cells which control water transport and plant cell growth (2–4, 8, 14). These pressure probe techniques have also been used to study the water relations of the sporangiophores of *Phycomyces blakesleeanus* (5). In general, pressure probe techniques require that there is a known relationship between the turgor pressure and other parameters which represent the biophysical and biomechanical properties of the plant cell. Equations which establish such a relationship for many plant cells have been termed the growth equations (13). Recent pressure probe studies have demonstrated that the growth behavior of the sporangiophores of *P. blakesleeanus* correlates well with that predicted by the growth equations and augmented growth equation (11).

The growth equations were first derived and published by Lockhart (9). Two equations were derived based on the premise that the rate of irreversible plant cell enlargement is determined predominately by the rate of two interrelated and simultaneous physical processes: the rate of water uptake and the rate of irreversible cell wall extension. The derivation assumes that the turgor pressure is constant and that the plant cell is not transpiring. The first growth equation relates the relative rate of change in volume of the cell contents $\dot{v}$, to the rate of water uptake:

$$\dot{v} = (dV/dt)/V = L\,(\sigma\Delta\pi - P), \qquad (1)$$

where V is the volume of the cell contents, t is the time, L is the relative hydraulic conductance, σ is the solute reflection coefficient, $\Delta\pi$ is the difference in osmotic pressure between the inside of the cell and the external medium, and P is the turgor pressure (the pressure difference between the cell interior and the external medium).

The second growth equation relates the relative rate of change in volume of the cell wall chamber, $\dot{v}$, to the irreversible extension

of the cell wall:

$$\dot{v} = (dV/dt)/V = \phi\,(P - P_c), \qquad (2)$$

where V is the volume of the cell wall chamber, ϕ is the irreversible extensibility of the cell wall, and P_c is the critical turgor pressure or yield threshold (the magnitude of which must be exceeded by the turgor pressure before irreversible wall extension occurs). It is apparent that the volume occupied by the cell contents and the volume of the cell wall chamber must be the same, and the rate of change of the respective volumes must also be equal. Equations 1 and 2 have been termed the growth equations (13). Both the utility and success of these growth equations are demonstrated in recent reviews by Taiz (13), Boyer (2), and Cosgrove (4).

In general, the utility of the growth equations for pressure probe studies is limited by the assumption that the turgor pressure must be constant. Ortega (10) augmented the growth equation for the rate of irreversible cell wall extension with an elastic (reversible) extension term so as to include dynamic growth, *i.e.* when the turgor pressure is changing. This augmented growth equation is:

$$\dot{v} = (dV/dt)/V = \phi\,(P - P_c) + (dP/dt)/\varepsilon, \qquad (3)$$

where ε is the volumetric elastic modulus. Some of the extended utility of this augmented growth equation is discussed and demonstrated by Ortega (10), and Cosgrove (3, 4).

Another limitation of the growth equations is that they are only valid for plant cells that do not transpire. In this paper the growth equation describing the rate of water uptake is augmented with a 'transpiration term,' thus extending the applicability of the growth equations to single plant cells which transpire. Experimental methodology is developed which uses the pressure probe, together with the augmented growth equations, to determine the rate of transpiration in single transpiring plant cells. The results of experiments, which used this pressure probe technique to determine the transpiration rate of the sporangiophores of *P. blakesleeanus*, are presented and the transpiration rates are compared to those measured by other methods (1, 7).

THEORY

An expanded form of Eq. 1, which includes a transpiration term, may be derived from a statement of the conservation of water mass in a plant cell. Consider a single plant cell of arbitrary shape. The rate of change of the water mass in the cell must be equal to the net flow rate of water into the volume enclosed by the surface of the cell. Mathematically this conservation law is stated in integral form as follows:

$$d/dt \iiint \rho \, dV = -\iint \rho \bar{u} \cdot \bar{n} \, dS, \qquad (4)$$

where the unit normal of the surface, $\bar{n}$, is pointing out of the

cell and therefore negative. For plant cell growth, water may be considered incompressible, *i.e.* ρ = constant, and then the conservation equation becomes:

$$dV/dt = -\iint \bar{u} \cdot \bar{n} \, dS. \tag{5}$$

Eq. 5 simply states that the rate of increase of the water volume in the plant cell, dV/dt, is equal to the net rate at which water is flowing into the cell. This equation is equivalent to that used by Silk and Wagner (12). Now it is assumed, as it was by Lockhart (9), that plant cell enlargement is due primarily to net water uptake. Then dV/dt of the plant cell contents may be substituted for dV/dt of the water in the plant cell.

The net flow rate of water into the plant cell may be expressed simply as the flow rate into the cell minus the flow rate out of the cell. For simplicity, consider that there are two distinct areas of the cell surface by which water flows into and out of the cell, A_i and A_o respectively, then Eq. 5 may be rewritten as:

$$dV/dt = J_i A_i - J_o A_o, \tag{6}$$

where the nomenclature is changed to conform to that used in the literature, that is, J_i and J_o represent the average velocity of water flow into and out of the cell, respectively (J is sometimes referred to as the flux of water in the literature). Now, from the thermodynamics of irreversible processes, Dainty (6) obtained an expression for the water flow into the cell, J_i:

$$J_i = L_p(\sigma \Delta \pi - P). \tag{7}$$

Substituting Eq. 7 into Eq. 6, Eq. 8 is obtained:

Now dividing by the volume of the cell, V, the following relationship is obtained:

$$\dot{v} = (dV/dt)/V = (L_p A_i/V)(\sigma \Delta \pi - P) - J_o A_o/V. \tag{9}$$

Furthermore, by letting $L = L_p A_i/V$ and $\dot{T} = J_o A_o/V$, Eq. 9 may be simplified to obtain:

$$\dot{v} = (dV/dt)/V = L(\sigma \Delta \pi - P) - \dot{T}. \tag{10}$$

The term $\dot{T}$ represents the relative volumetric flow rate of water out of the plant cell which evaporates and is lost to the atmosphere, *i.e.* the relative transpiration rate. It is apparent that $\dot{v}$ is positive (cell volume is increasing) when the rate of water uptake is greater than the rate of water loss due to transpiration, *i.e.* $L(\sigma \Delta \pi - P) > \dot{T}$. Of course, $\dot{v}$ is negative (cell volume is decreasing) when $\dot{T}$ is greater than $L(\sigma \Delta \pi - P)$. This condition may occur when the water uptake is reduced (perhaps the water supply is diminished or eliminated) and transpiration continues at near normal rates. The cell volume remains constant ($\dot{v} = 0$) when $\dot{T} = L(\sigma \Delta \pi - P)$. This condition may be obtained when the water source is eliminated (then the rate term for water uptake must equal zero; $L(\sigma \Delta \pi - P) = 0$) and transpiration is eliminated ($\dot{T} = 0$), perhaps by providing an environment of 100% RH.

The augmented growth equations (Eqs. 3 and 10) which describe the expansion rate (growth rate) of the plant cell, may be simplified when special conditions exist. Consider a plant cell in a nongrowing stage of development, which is suddenly removed from its water source. When the plant cell is in a nongrowing state, the irreversible wall extensibility is zero ($\phi = 0$) and only reversible extension of the cell wall can occur. Furthermore since there is no water uptake, the rate term for water uptake, $L(\sigma \Delta \pi - P)$, is zero. Applying these conditions to Eqs. 3 and 10, and combining by eliminating $\dot{v}$, we obtain the following equation:

$$-dP/dt = \varepsilon \dot{T}. \tag{11}$$

This equation establishes a relationship between the turgor pressure and the relative transpiration rate. The differential equation

is easily intergrated to solve for P when ε and $\dot{T}$ can be assumed to be constant. Using the initial condition that $P = P_i$ at $t = 0$, the following solution is obtained for P:

$$P(t) = P_i - \varepsilon \dot{T} t. \tag{12}$$

Eq. 12 describes a linear decay of turgor pressure in time from some initial value P_i. Thus, if the decay rate ($-dP/dt$) and ε are determined experimentally, then $\dot{T}$ may be calculated from Eq. 11.

In general, ε is a function of P for most plant cells. Thus, Eq. 11 will assume a more complex form, which depends on the relationship between ε and P (typically nonlinear). In many cases a linear relationship between ε and P provides a good approximation over a limited range of P. Then for that range of P the following equation may be used to describe the relationship between ε and P:

$$\varepsilon(P) = \varepsilon_o + mP, \tag{13}$$

where m is the slope of the line and ε_o is the intercept on the ε axis. Substituting Eq. 13 into Eq. 11 yields:

$$dP/dt + m\dot{T}P = -\varepsilon_o \dot{T}. \tag{14}$$

Again using the initial condition that $P = P_i$ at $t = 0$, the solution to Eq. 14 is obtained:

$$P(t) = (P_i + \varepsilon_o/m) \exp(-m\dot{T}t) - \varepsilon_o/m. \tag{15}$$

Eq. 15 describes an exponential decay of P from an initial value, P_i, to another value, $-\varepsilon_o/m$. The time constant, t_c, for the exponential decay is $1/m\dot{T}$. In general, Eqs. 14 and 15 will be valid for only part of turgor pressure decay, over that range of turgor pressure where Eq. 13 is a good approximation. Within this range, $\dot{T}$ may be calculated from the following expression which is obtained from Eq. 15:

$$\dot{T} = [1/(m\Delta t)] \ln [(P_1 + \varepsilon_o/m)/(P_2 + \varepsilon_o/m)], \tag{16}$$

where P_1 and P_2 are values along the turgor pressure decay curve, and Δt is the time interval required for the decay of the turgor pressure from a value of P_1 to P_2. The transpiration rate, dV/dt, can be calculated from the expression: $dV/dt = V_o \dot{T}$, where V_o is the volume of the cell.

MATERIALS AND METHODS

Vegetative spores of the wild type strain of *P. blakesleeanus* NRRL 1555 (−) were inoculated in glass shell vials on sterile growth medium consisting of 4% (w/v) potato dextrose agar, 0.1% (v/v) Wesson oil, and 0.006% (w/v) thiamine. After inoculation the vials were incubated under continuous light from an incandescent light bulb (40 W) at high humidity and constant temperature (20 ± 1°C). Normally, sporangiophores appeared by the end of the third day. The sporangiophores were plucked daily so that a new crop was available the following day. Large nongrowing stage III sporangiophores, 1.5 to 3.0 cm in length, were selected for experiments from the third to seventh crop.

The turgor pressure of the sporangiophore was continuously measured with a manual version of the pressure probe which is almost identical in design to the pressure probe used in the water relations study of *Phycomyces* sporangiophores (5). This version of the pressure probe differs from that reported by Husken *et al.* (8) in that a manually adjustable control rod (plunger) is used to adjust the pressure in the pressure probe chamber and thus control the location of the cell sap-silicon oil interface in the microcapillary tip which impales the sporangiophore. The pressure transducer used in the pressure probe was obtained from Kulite Semiconductor Products Inc., Ridgefield, NY (model XT-190-300G) and was calibrated inside the pressure probe with a Heise Bourdon Tube Pressure Gauge; Dresser Ind., Newton,

CT (model CMM, 0-200 PSIG Range). The transducer's output (0.307 mV/psi) was determined to be linear over the range of turgor pressures measured in the experiments.

The following procedure was used for the experiments conducted. Initially, a large stage III sporangiophore was carefully removed from the mycelium with tweezers (plucked), and transferred to a small chamber. Plucked stage IV sporangiophores continue to grow at almost normal rates after their base is immersed in water (1). The chamber (1 × 1.5 × 4 cm) was constructed from transparent plexiglass. The base of the chamber was connected to a syringe by a flexible plastic tube so that water could be added to, or removed from, the bottom of the chamber. The sporangiophore was attached to two thin horizontal supports inside the chamber. A thin layer of vaseline was applied to the edge of the supports so that the sporangiophore would stick to the supports. The bottom of the chamber was filled with water so that the base of the sporangiophore was immersed in water. The plucking and transfer procedure was completed in 5 to 10 s.

The sporangiophore was then allowed to equilibrate for the next 10 to 20 min, during which time the length was measured periodically to ensure that no growth occurred. Then the sporangiophore was impaled with the microcapillary tip of the pressure probe. The turgor pressure was continuously measured and recorded on a Houston Omniscribe Stripchart Recorder (model D5217-2) for the remainder of the experiment. After the turgor pressure stabilized, the chamber was emptied to isolate the plucked sporangiophore from the water source. Typically, when the sporangiophore was exposed to room air, the turgor pressure began to decay immediately afterwards. Sometimes it was necessary to use the corner of a tissue to absorb small drops of water adhering to the base of the sporangiophore, and to the supports. After the turgor pressure decayed to a small value, a series of small step-ups in turgor pressure were produced by injecting inert silicon oil (Dow Corning Corp., fluid 200, 1 cs viscosity) into the vacuole. A horizontally mounted microscope (Gaertner, 7011 K eyepiece and 32 m/m EFL objective) was used to measure the change in length, Δl, resulting from the step-up in turgor pressure, ΔP, (there was no detectable change in diameter). The volumetric elastic modulus, ε, was calculated from the expression: $\varepsilon = l(\Delta P/\Delta l)$.

For these experiments the sporangiophore was exposed to the room air. The room air temperature was maintained at 22 ± 1°C and the relative humidity was measured for each experiment with the wet bulb-dry bulb technique. A fiber-optic illuminator (Flexilux 90; HLU Light Source 90/W with two armed swan neck light guide), which filters out infrared light, was used to illuminate and view the sporangiophore through a horizontally mounted EZM-2TR Trinocular Zoom Stereomicroscope.

RESULTS AND ANALYSIS

The results of typical experiments are presented in Figures 1 and 2. Figure 1A (lower graph) is the turgor pressure trace from the strip chart recorder, which demonstrates a turgor pressure decay after the water is removed. The large black arrow on the time scale indicates when the water source was removed. The room air temperature was 22°C and the RH of the room air was 40%.

After the turgor pressure decayed to approximately 0.015 MPa (not shown), a series of small step-ups in turgor pressure were produced with the pressure probe. The resulting length changes were measured, and ε was calculated for each step-up. A plot of ε versus P is presented in Figure 1B (inset). The relationship between ε and P (dashed line) is generally nonlinear and very similar to those measured for other higher plant cells (8, 14). The ε-P relationship can be approximated by the equation:

$$\varepsilon = \varepsilon_{max} \left(1 - (\varepsilon_{max} - \varepsilon_o) \exp(-kP)/\varepsilon_{max}\right), \quad (17)$$
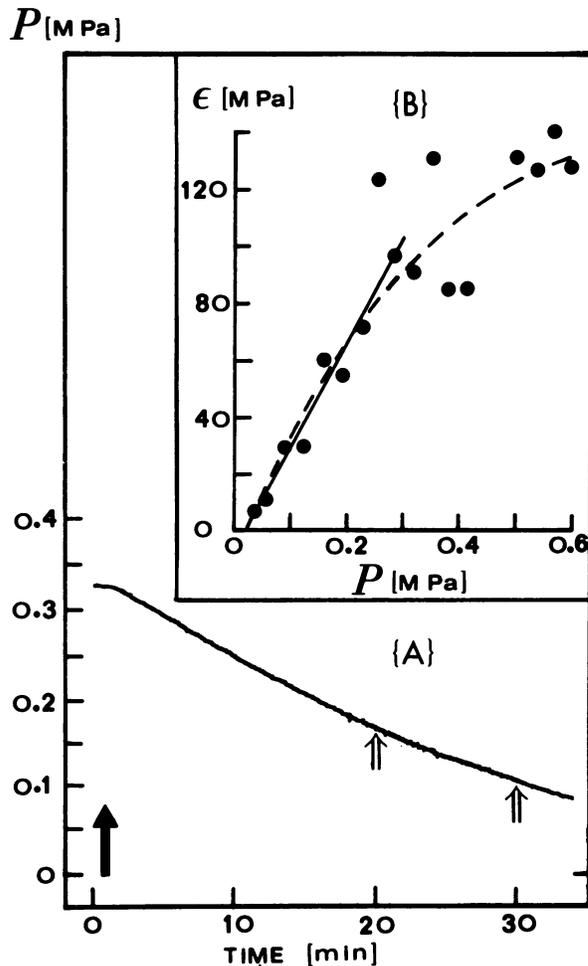

FIG. 1. Decay in turgor pressure, P, as a function of time after the water source is removed (indicated by the arrow) from a plucked stage III sporangiophore (A); B (inset), measured behavior of the volumetric elastic modulus, ε, as a function of the turgor pressure, P, for the same sporangiophore.

(dashed line). This relationship has the properties that at low pressures it has a constant slope ($\sim k\varepsilon_{max}$), and at higher pressures the slope is zero and ε becomes a constant ($\varepsilon = \varepsilon_{max}$). The following values were used to generate the curve represented by the dashed line: $\varepsilon_{max} = 160$ MPa, $\varepsilon_o = -10$ MPa, and $k = 3.0$ MPa.

Visual inspection of the data points also suggests that the ε-P relationship may be approximated by a linear relationship at low turgor pressures (less than 0.3 MPa). In fact, a good fit may be obtained with a linear least squares fit of the data points between 0.0 and 0.3 MPa (solid line). For this range, Eq. 13 may be used to describe $\varepsilon(P)$, where $m = 363$ and $\varepsilon_o = -6.3$ MPa (solid line). Then Eq. 15 describes $P(t)$, and Eq. 16 may be used to calculate T using values of P between 0.0 and 0.3 MPa.

The results presented in Figure 1A demonstrate that the turgor pressure decays from a value of 0.162 MPa at 20 min to 0.106 MPa at 30 min (indicated by vertical arrows on the turgor pressure trace). Then, T is calculated from Eq. 16 to be 0.000135/min. Multiplying T by the volume of the sporangiophore, $8.3 \times 10^8 \ \mu m^3$ ($dV/dt = V_o T$), the transpiration rate is calculated to be $1.1 \times 10^5 \ \mu m^3$/min or 0.11 nl/ min.

Interestingly, if the linear relationship is obtained from Eq. 17 (at low pressures), then $m \cong k\varepsilon_{max} = 480$, and $\varepsilon_o = -10$ MPa. Now, T is calculated from Eq. 16 to be 0.000105, and the transpiration rate is calculated to be 0.09 nl/min.
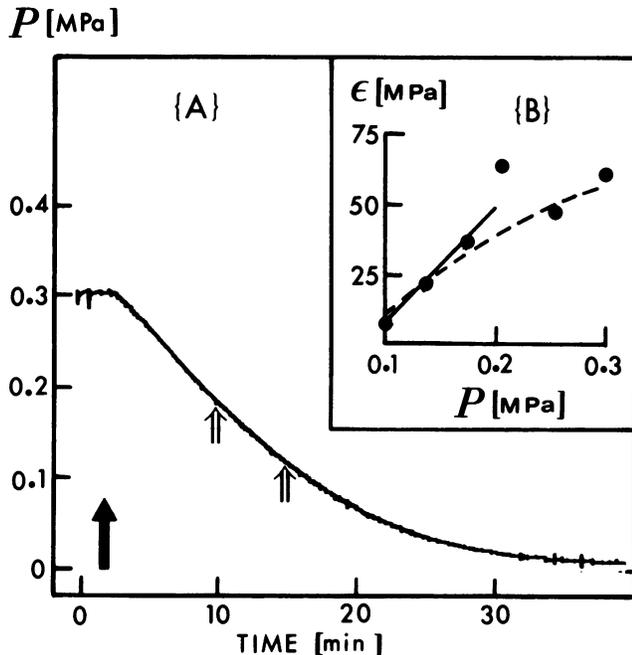
FIG. 2. Decay in turgor pressure, P, as a function of time after the water source is removed (indicated by the arrow) from a plucked stage III sporangiophore (A); B (inset), measured behavior of the volumetric elastic modulus, ε, as a function of the turgor pressure, P, for the same sporangiophore (the origin is not shown).

The results of another typical experiment are presented in Figure 2. Figure 2A is the turgor pressure trace from the strip chart recorder, which demonstrates a turgor pressure decay after the water is removed. The large black arrow on the time scale indicates when the water source was removed. The room air temperature was 22°C and the relative humidity was 34%.

After the turgor pressure decayed to approximately 0.01 MPa, a series of small step-ups in turgor pressure were produced with the pressure probe to generate the ε-P curve presented in Figure 2B (inset). As before the relationship between ε and P is nonlinear and very similar to those measured for other higher plant cells (8, 14). Again, Eq. 17 may be used to approximate this ε-P relationship (dashed line) where ε_{max} = 80 MPa, ε_o = -40 MPa, and k = 5.3 MPa. However, the ε-P relationship may be approximated by a simpler linear relationship over a limited range. A linear relationship seems to provide a good fit for the turgor pressure range between 0.1 and 0.2 MPa. For this range, Eq. 13 may be used to describe $\varepsilon(P)$, where m = 408 and ε_o = -34 MPa (solid line). Then, as before, Eq. 15 describes $P(t)$, and Eq. 16 may be used to calculate $\dot{T}$ using values of P between 0.1 and 0.2 MPa.

The results demonstrate that the turgor pressure decays from a value of 0.186 to 0.119 MPa in 5 min (as indicated by the vertical arrows on the turgor pressure trace in Fig. 2A). Then, $\dot{T}$ is calculated from Eq. 16 to be 0.00053/min. Multiplying $\dot{T}$ by the volume of the sporangiophore, 1.19 × 10⁹ μm³, the transpiration rate is calculated to be 6.3 × 10⁵ μm³/min or 0.63 nl/min.

If the linear relationship is obtained from Eq. 17 (at low pressures), then $m \cong k\varepsilon_{max}$ = 424, and ε_o = -40 MPa. The transpiration rate is calculated to be 0.73 nl/min, and $\dot{T}$ is calculated to be 0.00061/min. In general, the calculated transpiration rates from other similar experiments ranged from 0.09 nl/min to 0.8

nl/min for stage III sporangiophores, 2.0 to 3.3 cm in length and with RH between 25 and 40%. The average transpiration rate, and standard deviation, was 0.41 ± 0.39 nl/min (n = 9).

Control experiments were conducted to ensure that the observed decay rates in P, after the water was removed, were due to transpiration. In these experiments the sporangiophore was placed in a sealed chamber lined with wet tissue paper to achieve an environment of nearly 100% RH. The results demonstrate that the turgor pressure remained constant after the water was removed, i.e. $\dot{T}$ = 0.

DISCUSSION

Bergman *et al.* (1) measured the water uptake of plucked stage IV sporangiophores with their base sealed into a small manometer system. The experiments were conducted at 75% RH. The water uptake values ranged from 1.2 nl/min for young stage IV sporangiophores to 0.1 nl/min for older ones. The measured uptake rates ranged from seven times the volume growth rate at the maximum growth rate to a little more than one time the volume growth rate for old sporangiophores. Bergman *et al.* (1) also measured the uptake of tritiated water into the sporangiophore and obtained rates that varied between 0.28 to 0.62 nl/min.

More recently Geer *et al.* (7) measured the water loss from single stage IV sporangiophores by measuring the weight loss as a function of time with an ultrasensitive electrobalance. The mean rate of water loss for plucked sporangiophores was 0.52 ± 0.05 μg/min in dry air (RH < 10%) and 0.16 ± 0.06 μg/min in humid air (RH > 90%).

The transpiration rates for stage III sporangiophores reported in the present paper appear to be the first. In general, the range of transpiration rates determined by the pressure probe technique for stage III sporangiophores are nearly the same as those previously reported for stage IV sporangiophores (1, 7).

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