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TECHNICAL REPORT

A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb

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Abstract. A simple stem hygrometer for attachment to a bared section of sapwood or a cross-sectional cut end of a shoot is described. Two welded chromel-constantan thermocouples inside the chamber, one touching the sample and the other in the chamber air, allowed measurement of and correction for the temperature gradient between the sample and the dewpoint measuring junction. The instrument was attached to the cut end of an apical shoot of *Thuja occidentalis* L. protuding from a Scholander-Hammel pressure bomb. Cut-end water potential (ψ_{hyg}), measured using the stem hygrometer, was compared to xylem pressure potential (ψ_{xp}) while the latter was manipulated in the pressure bomb. After an initial equilibration time of 3–4 h, hygrometer equilibrium values were achieved within 1.5–4.0 min of changing ψ_{xp} in the pressure bomb. The half-time ($t_{1/2}$) for vapour pressure equilibration was 15–40 s. Stable temperature gradients between the sample and dewpoint measuring junction of 0.01–0.1 °C were measured. Correcting ψ_{hyg} for the temperature gradient resulted in excellent agreement with ψ_{xp}.

Key-words: stem hygrometer; equilibration time; temperature gradients; pressure bomb.

Introduction

Although a relatively new technique in the field of water relations research, the thermocouple hygrometer (Spanner,1951) has enjoyed a wide audience. Recently, the attraction of continuous non-destructive monitoring of plant tissue water potential has received attention and variations of this highly adaptive technique have appeared in the literature (Neumann & Thurtle, 1972; Michel, 1977; McBurney & Costigan, 1982). Most *in situ* hygrometers have been designed for attachment to leaves, but there are advantages in using stem hygrometers. Less significant energy balance disruptions and ease of attachment of the stem hygrometer favour its use over that of the leaf hygrometer.

This paper describes a simple stem hygrometer and its performance relative to the Scholander-Hammel pressure bomb (Scholander *et al.*, 1965). Unlike its use in similar studies in the past (e.g. McBurney & Costigan, 1982), the stem hygrometer is attached to the end of a shoot protruding from the pressure bomb. This reduces problems concerning water potential gradients between the sites of hygrometer and pressure bomb measurements and facilitates a more precise comparison.

The thermocouple hygrometer relies, for its success, on the accurate determination of very small differential temperatures (0.01–0.5 °C), and also on the assumption that the initial measuring junction and sample temperatures are identical. It has long been realized that failure to achieve the latter is a major source of error in hygrometry.

Michel (1979) addressed the problem of temperature gradients in stem hygrometers but stopped short of actually measuring the error (i.e. the error-inducing gradient is that between the sample and the measuring junction, not the measuring junction and the reference or instrument temperature). He relied on predicting the former by measuring the latter. In our experience, however, the variability between instruments, as well as installations, precluded reliable predictions. Calissendorf & Gardner (1972) described a leaf psychrometer which did measure the temperature gradient between the sample and the region of the measuring junction. However, the results they reported when using their instrument on corn leaves were unrealistically low compared to results from similar studies (e.g. Neumann & Thurtle, 1972).

This paper describes an instrument similar, in principle, to that described by Calissendorf & Gardner (1972) in that it allows measurement of error-inducing temperature gradients within the chamber, but for use on stems rather than leaves. Furthermore, the technique described allows concurrent and directly related measurements of water potential via an independent method (pressure bomb).

Materials and methods

A thermocouple hygrometer chamber (Fig. 1) was machined from the head of a 25 mm stainless-steel

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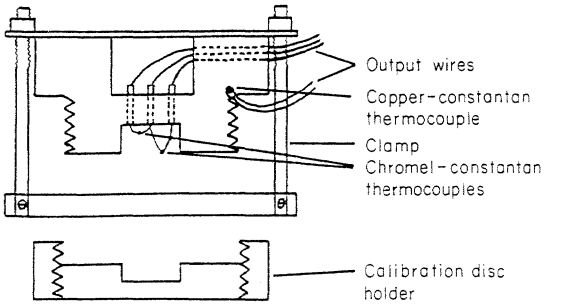

Figure 1. Diagram (not to scale) of stainless-steel stem hygrometer and calibration disc holder. The clamp can be used to attach the instrument to the side of stems.

bolt (AISI 304). It was fitted with two welded chromel-constantan measuring junctions (wire available from Goodfellow Metals, Milton Rd, Cambridge, U.K.). One of the junctions was in contact with the sample surface while the other was in the chamber air. A Wescor HR-33T microvoltmeter (Wescor, Inc., Logan, Utah, U.S.A.) was used to measure the temperature difference between the two junctions as well as to provide the Peltier cooling current for the junction in the chamber air. A soldered copper-constantan thermocouple was embedded in the chamber body to measure instrument temperature. The instrument was designed to allow attachment to either the cut end of a shoot or the side of a bared section of sapwood.

The hygrometer was calibrated using filter-paper discs soaked with standard sodium chloride solutions of molality 0.1–0.5 mol kg^{-1} in increments of 0.1 mol kg^{-1}. The water potential of each standard solution at 25 °C was obtained from tables (Lang, 1967).

An apical shoot of *Thuja occidentalis*, 0.6–0.8 m long and 10–12 mm butt diameter, was enclosed in a Scholander-Hammel pressure bomb (Scholander *et al.*, 1965) with 30–60 mm of the butt-end protuding. The stem hygrometer was attached to the cut end of the shoot (Fig. 2). Grease was applied at the junction of wood and metal surfaces to ensure a vapour seal. The assembly at the top of the bomb was then enclosed by a temperature-controlled chamber (Fig. 2). This consisted of a metal cylinder (105 mm diam. × 175 mm high), completely lined with 5-mm-diameter copper tubing and covered with 30-mm-thick Styrofoam (Styrofoam SM). The ends of the cylinder were sealed with cotton wool and Styrofoam. Air was circulated within the chamber by a 6 V fan (Micronel Ltd, Zurich, Switzerland). Fluid from a constant-temperature bath (Exacal, Model EX100, Neslab Instruments, Portsmouth, New Hampshire, U.S.A.) was pumped through the copper tube and maintained at 25 ± 0.01 °C. Fluctuations in ambient air temperature were minimized (± 1.0 °C) using an air conditioner.

The sample shoots were air-dehydrated overnight (10–12 h) before they were placed in the pressure

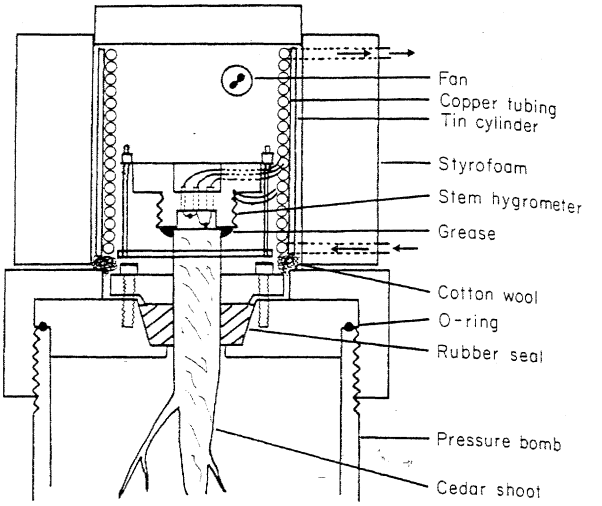

Figure 2. Diagram (not to scale) of a cedar shoot clamped in a pressure bomb with the stem hygrometer attached to the cut end and enclosed in a temperature-controlled chamber.

bomb. Xylem pressure potential (ψ_{xp}), as determined at the start of each experiment, was typically −1.0 to −2.0 MPa. Following ψ_{xp} determination the bomb pressure was lowered 0.1 MPa and held. The hygrometer and temperature control system were then installed and allowed to achieve vapour pressure and temperature equilibrium (usually 3–4 h). The bomb pressure was then lowered in 0.2–0.4 MPa steps at a rate of 3.0 kPa s^{-1} and held for at least 15 min at each step. Hygrometer readings of water potential (ψ_{hyg}) at the cut end were taken every 5 min and compared to computed ψ_{xp} (present bomb pressure minus initial balance pressure). Pressure changes were reversed upon reaching the lowest ψ_{xp} (i.e. bomb pressure = 0).

Vapour pressure equilibration

Analysis of vapour pressure equilibration time was achieved by operating the HR-33T in the Dew Point Mode (Wescor Instruction Manual, 1982) and continuously recording the output on a strip chart recorder while manipulating ψ_{xp} in the pressure bomb. The bomb pressure was raised or lowered at a rate of about 40.0 kPa s^{-1} to effect a total change of 0.8 MPa in each case. In the case of these rapid pressure changes, temperature changes inside the pressure bomb were inevitably caused (Puritch & Turner, 1973) and the extent to which this affected the temperature at the cut end of the shoot was assessed as outlined in the following section.

Temperature gradients

Stable temperature gradients within the hygrometer chamber between the sample surface and the dewpoint measuring junction were achieved using the temperature-controlled chamber. The value of the

gradient was determined from the differential output of the two welded thermocouples. In the temperature range of interest, the temperature coefficient of chromel-constantan thermocouples is 0.061 mV/°C (Omega Engineering, Inc., *Temperature Measuring Handbook*, 1980, p. A-11).

Transient temperature gradients within the chamber, which were apparent only during rapid bomb pressure changes, were also measured in the above manner and continuously recorded during rapid pressure changes.

A correction to ψ_{hyg} resulting from the temperature gradient measured at the time of each reading was applied according to the formula:

$$\psi_c = \psi_e + \frac{kRT_s}{V_w}\Delta T,$$

where

ψ_c = corrected water potential (Pa),
ψ_e = erroneous water potential (Pa),
R = gas constant (J mol^{-1} K^{-1}),
T = temperature (K); subscripted 's' = sample surface temp.,

$$k = \frac{1}{C^*}\frac{dC^*}{dT},$$

C^* = saturated water vapour concentration at temp. T,
V_w = partial molar volume of water (m^3 mol^{-1}),
ΔT = temperature difference between stem surface and dewpoint measuring junction.

The correction factor (kRT_s/V_w) evaluates to 7.77 MPa K^{-1} at 25 °C.

Results

Following initial equilibration, ψ_{hyg}, as measured by the stem hygrometer, was always lower (0.2–1.0 MPa) than ψ_{xp} before correction for the measured temperature gradient had been taken into account (Fig. 3). As long as the temperature gradient remained constant, the relationship between ψ_{hyg} and ψ_{xp} was linear. Deviations from linearity within experiments usually reflected minor fluctuations in the temperature gradient. Measurement of the gradient and subsequent correction of ψ_{hyg} resulted in the relationship shown in Fig. 4.

The half-time ($t_{1/2}$ = time to reach 50% equilibrium) for vapour pressure equilibration was usually less than 60 s and seemed to vary mainly according to the length of stem protruding from the pressure bomb (the longer the stem, the longer the half-time). Figure 5 shows replicate determinations of equilibration time on the same shoot with 35 mm protruding. The points represent the natural logarithm of the absolute value of the difference between apparent ψ_{hyg} and computed ψ_{xp} *after* the pressure change had been completed.

The time course for temperature equilibration following rapid pressure changes in the bomb is shown in Fig. 6. Generally, the half-time for

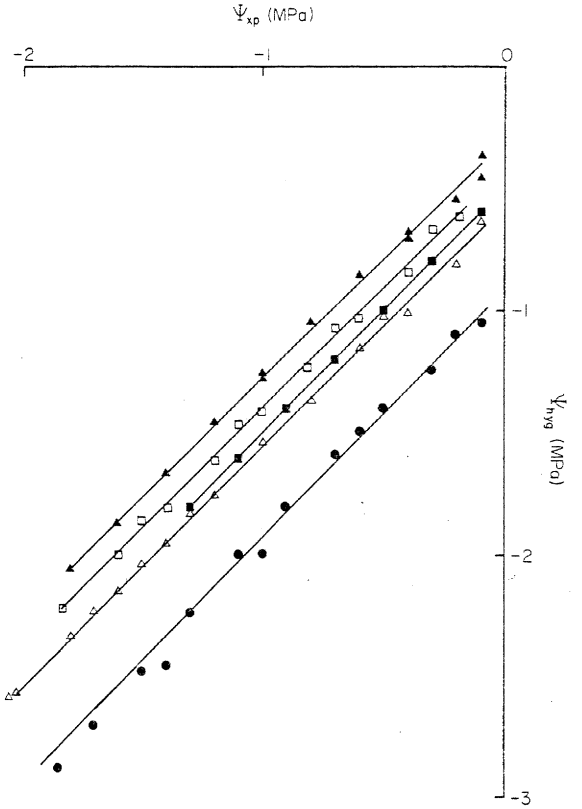

Figure 3. The relationship between computed ψ_{xp} (bomb pressure minus initial balance pressure) and ψ_{hyg} as measured by the stem hygrometer before correction for temperature gradients. Only five experiments are shown to avoid the confusion of coincident data points.

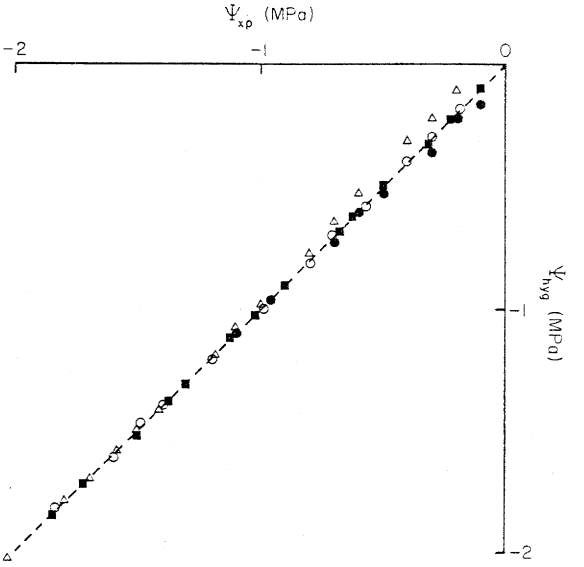

Figure 4. The relationship between computed ψ_{xp} (bomb pressure minus initial balance pressure) and ψ_{hyg} as measured by the stem hygrometer after correction for temperature gradients. The dashed line represents a 1 : 1 correlation.

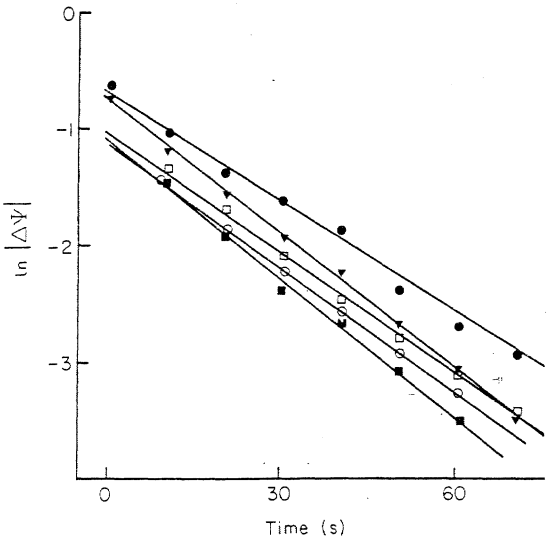

Figure 5. The absolute value of the difference between apparent ψ_{hyg} and computed ψ_{xp} plotted as a natural logarithm against time for a shoot with 35 mm protruding from the pressure bomb. These replicates depict rapid water potential changes in both the positive and negative directions. The average slope is -0.036 ± 0.003 s^{-1}. The half-time for equilibration is 19.3 s.

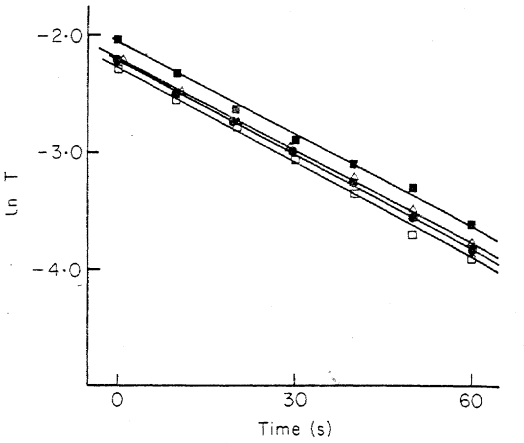

Figure 6. The absolute value of the temperature difference between sample and dewpoint measuring junction plotted as a natural logarithm against time for a shoot with 35 mm protruding from the pressure bomb. These replicates depict temperature changes resulting from rapid bomb pressure changes in both the positive and negative directions. The average slope is -0.026 ± 0.001 s^{-1}. The half-time for equilibration is 26.6 s.

temperature equilibration was roughly equal to that for vapour pressure equilibration. The magnitude of the temperature change at the cut end of the shoot was influenced by the rate of pressure increase or decrease as well as the length of stem protruding from the bomb.

Discussion

Our method of concurrently measuring stem water potential using two independent techniques showed excellent agreement between the two when corrections for temperature gradients were made. It is noteworthy that these gradients persisted even though the stem hygrometer and a significant portion of the stem sample were housed in a temperature-controlled chamber. This was probably the consequence of heat conduction through the rather massive stem from regions outside the temperature-controlled chamber. It is surprising that temperature-corrected hygrometers/psychrometers have not been used more widely. In most chambers designed to take leaf discs or leaf strips the sample is usually completely surrounded by a metallic thermal mass; thus substantial gradients of temperature between the leaf surface and thermocouple junction are less likely than in our systems. However, a temperature-corrected hygrometer might be advantageous when temperature gradients due to the heat of respiration are thought to be substantial (Barrs, 1964). Temperature corrections are clearly needed in stem hygrometers and may be advisable for *in situ* leaf hygrometers, especially when used under field conditions.

In some cases the temperature gradient did not account for all the apparent disagreement between the two methods. For example, in Fig. 4, note that the relationship between ψ_{hyg} and computed ψ_{xp} tended to deviate from the 1 : 1 correlation as computed ψ_{xp} increased in one of the trials. Subsequently, it was found that excess water used to soak filter paper inside the bomb had accumulated in a pool on the bottom. The increasing bomb pressure may have caused water to infiltrate the xylem resulting in an error in computed ψ_{xp}; hence the gradual deviation as ψ_{xp} increased.

The stem hygrometer showed rapid vapour pressure and temperature equilibration; half-times never exceeded 60 s (Figs 5 and 6). These favourable characteristics are highly desirable for reliable use of the instrument.

Comparison of equilibration characteristics between our instrument and others reported in the literature is difficult since a standard for comparison is rarely used. Boyer (1972) reported an equilibration half-time of 4 min when using an isopiestic technique (Boyer & Knipling, 1965) on sunflower leaves. This half-time is determined by the combined half-times for tissue and chamber equilibration. Equilibration times, reported in the literature, vary from several minutes to several hours (Millar, 1974; Campbell & Campbell, 1974). A variety of factors influence equilibration time, not least of which is the chamber material. Chambers with rubber seals or oxidized or dirty metal surfaces will display equilibration characteristics dominated by those of the chamber material (Dixon & Grace, 1982). Another factor influencing equilibration is the cuticular resistance of the sample (in the case of leaf tissue). This can be lessened by removal of cuticular waxes (Neumann & Thurtle, 1972) or abrasion of the leaf surface (Brown & Tanner, 1981). Our stem

ygrometer exhibited a very fast equilibration time ecause near optimum chamber-wall materials were sed (Dixon & Grace, 1982), rubber seals were liminated, and cuticular barriers to water evaporaon from the plant surface did not exist. Also, the ssue in equilibrium with the stem hygrometer has a igh hydraulic conductivity and low capacitance elative to leaf tissue. Therefore, water potential quilibration within the stem tissue is quite fast ollowing an increase or decrease in bomb pressure. If the bomb pressure does not exceed the balance ressure then very little water redistribution is eeded inside the shoot to bring about a change in ylem water potential.)

Transient electrical zero offsets are another ignificant source of error in the use of thermocouple sychrometers/hygrometers. It was found that the ero offset of the HR-33T microvoltmeter was ffected by proximity to other electrical equipment nd to human bodies. Poorly earthed equipment and roximity of a.c. mains cables to the hygrometer utput leads was found to cause significant zero ffsets and errors in ψ_{hyg}. Shielding the wires did not liminate but, in fact, accentuated the problem in ome cases. Spatial isolation from other electrical quipment of the microvoltmeter and wires within at least a 2 m radius minimized these errors. Strict adherence to experimental protocol enhanced the reproducibility of data.

We conclude from our tests and calibrations that our version of the stem hygrometer is a reliable and accurate instrument for measuring *in situ* stem water potentials. Of course, certain precautions must be observed, especially when employing this technique in the field.

Avoiding exposure of the installation to direct adiation is helpful in reducing the magnitude of hermal gradients. Cotton wool and Styrofoam are ppropriate insulating materials. A final reflective ayer of aluminium foil is recommended as well. Elaborate temperature control, although sometimes possible in the field, is difficult to achieve. Sufficient nsulation and careful measurements of chamber emperature should produce reliable results when orrected for temperature.

In the tests reported here the hygrometer was ttached to the cross-sectional cut end of the sample. his was achieved using a variation of the clamping evice depicted in Fig. 1. This mode of attachment is ot necessary and is, in fact, not recommended in any cases (e.g. resinous species, such as some pines, nd species which have a significant proportion of iving cells mingled with their xylem conduits). Contamination of the chamber with symplasm or esin will adversely affect readings. The alternative is ttachment to the side of the stem. Careful reparation of the sample by removing the bark, hloem and cambium to expose an appropriate sized rea of the sapwood is required. After the area has een cleaned and wiped dry the instrument can be attached and the remaining exposed area sealed with silicone grease. As long as the sapwood is not wounded there should be no problems. Obviously this technique is limited to those species which allow access to a portion of undamaged xylem.

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