# 6 Theory

## 6.1 Stem Water Potential

Total water potential is the measure of the plants ability to interact with the environment. It consists of four basic components:

$$\Psi = Tp - \Pi - T - g \quad \textbf{Equation 1}$$

**Where:**
Ψ= Total Water Potential
Tp = Turgor Pressure
Π= Osmotic Potential
T= Matric Potential
G = Gravity

The PSY1 Stem Psychrometer is designed to measure water potential with due consideration of ambient temperature and temperature gradients.

### 6.1.1 Turgor pressure

Is the outward pressure that occurs in a plant cell when the cytoplasm and vacuoles, or membrane bound tissues, fill with water and the cell membrane pushes against the cell wall. The more water within the cell wall the greater the osmotic pressure. Turgor pressure and osmotic potential typically dominate the total water potential of the plant and form the basis of the measurement made by the stem Psychrometer.

### 6.1.2 Osmotic potential

Osmotic potential is the result of dissolved solutes in membrane bounded tissues of a plant. It is possible to measure this component of total water potential independently on samples from frozen or crushed plant tissue, usually leaves. This is covered in more detail in section Osmotic Potential.

### 6.1.3 Matric Potential

Matric potential is defined as the energy required to extract water from a porous medium to overcome capillary and absorptive forces and is usually overwhelmed by osmotic potential and turgor pressure as a component of total water potential.

### 6.1.4 Gravity

In most plants gravity has a negligible effect, but in trees of suitable height such as Redwood (*Sequoiadendron giganteum*) gravity can have a significant affect as demonstrated by figure 1 (Page 20) in which the PSY1 measured stem water potential at 88m above ground level at the top of a 91m Redwood tree. The effect of gravity is shown by the night time recovery in stem water potential being offset by approx -0.90 MPa due to the contribution of gravity being equal to approx 101.9m head pressure / MPa.

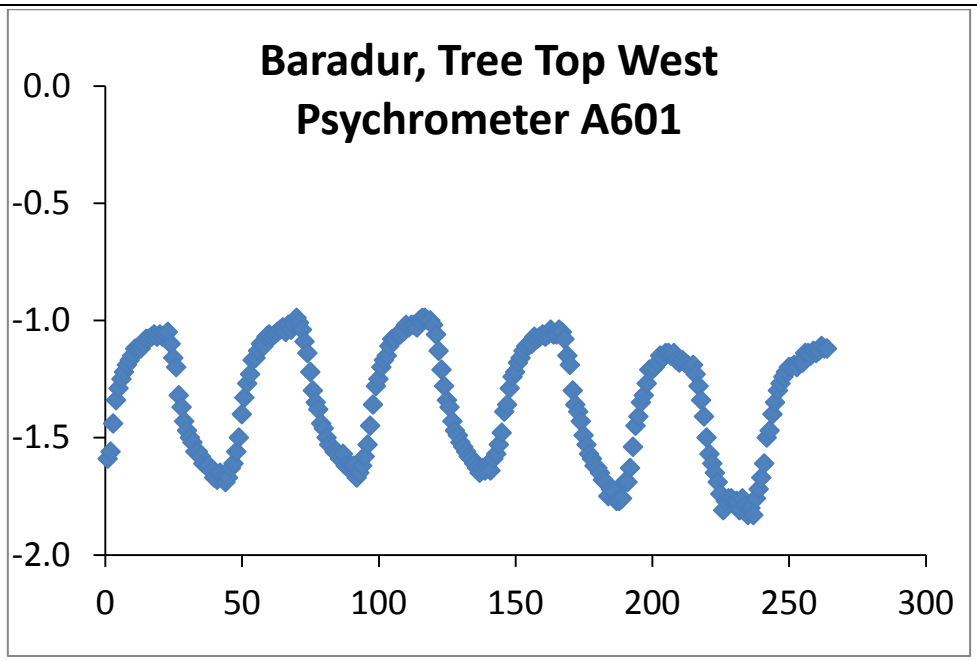



**Figure 1 - 5 daily cycles of water potential measured with ICT psychrometer on a branch at about 88 meter height in a 91 meter tall *Sequoiadendron giganteum* At Whitaker's Forest, California during the period Aug. 13 – Aug. 17, 2010. Unpublished Data - Courtesy George Koch Northern Arizona University**

## 6.2 Psychrometric measurement

Each measured parameter in the psychrometric equation is directly measured by the PSY1 Stem Psychrometer. The stem psychrometer is constructed of chromium plated brass to provide a large heat sink for thermal stability during the psychrometric measurement.

**NOTE 4:** The ambient temperature of the PSY1 can fluctuate within the normal range of diurnal cycles without affecting the measured water potential. However, the temperature within the chamber MUST remain stable throughout the duration of the 20 second measurement period. Rapid temperature changes of even 0.1°C from the start of the measurement to the end of the measurement will cause noise and errors in the measurement of water potential. To ensure a stable thermal environment the Psychrometer chamber MUST be wrapped in an insulating material that dampens the ambient thermal environment.

The chamber houses two chromel/constantan thermocouples in series; one located in the chamber air (Thermocouple-C) and the second, extending above the chamber well to contact the sample surface (Thermocouple-S). The differential output from these two junctions (ΔT) is a measure of the temperature gradient between the sample (Thermocouple-S) and the measuring junction (Thermocouple-C) and allows the correction of the measurement of water potential for the influence of this gradient.

The PSY1 generates a Peltier cooling pulse (of user definable duration, but typically 10 second), to cool Thermocouple-C sufficiently to condense water on the thermocouple. Watch **Video 9** Principle of Operation for a visual example of this process.

The microvolt output of the Thermocouple-C is recorded at a sampling frequency of 10 Hz or 10 times per second. A Psychrometric (Wet-Bulb) depression is read at six (6) seconds after the end of cooling and automatically corrected for any measured temperature gradient between the Thermocouple-C and Thermocouple-S. Finally, these data are automatically processed using the Stem Psychrometer equation applying the chamber specific calibration to yield the precise measurement of the sample's water potential.

A copper/constantan thermocouple is embedded in the chamber body to allow measurement of chamber temperature. This temperature value is recorded by the PSY1 and used to automatically correct the reading to 25°C, an arbitrary standard reference for water potential measurements.

The stem psychrometer provides the means to measure in-situ water potential over a wide range (-0.01 Mpa to -10 Mpa) with accuracy and repeatability. However, certain precautions (as detailed in the following sections) must be adhered to if meaningful results are to be achieved.

**Video 10** presented by Prof. Mike Dixon is a concise explanation of the Stem Psychrometer Theory. Including: the theoretical principle employed by the PSY1 Stem Psychrometer to measure stem water potential; the chamber design; nomenclature used to describe the internal components of the chamber; and the measurement procedure.

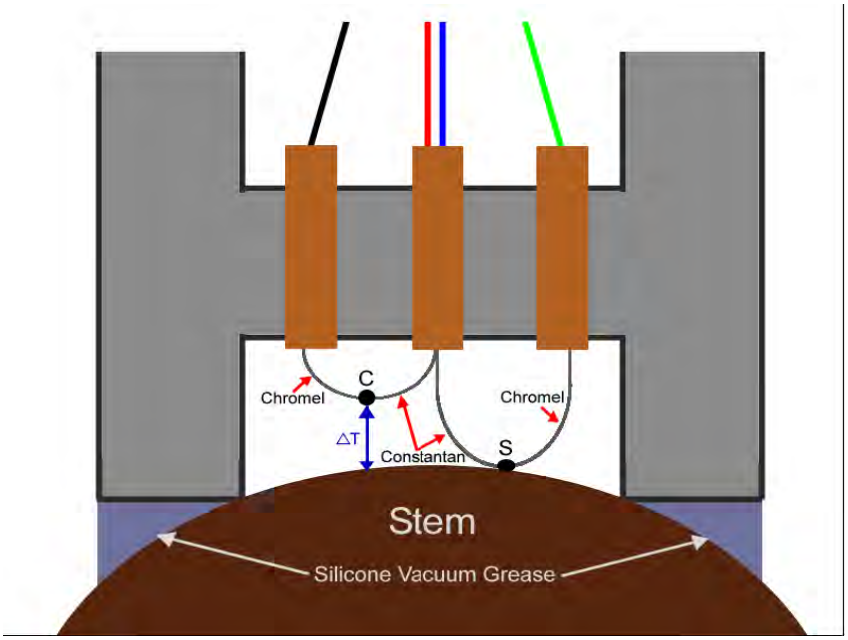
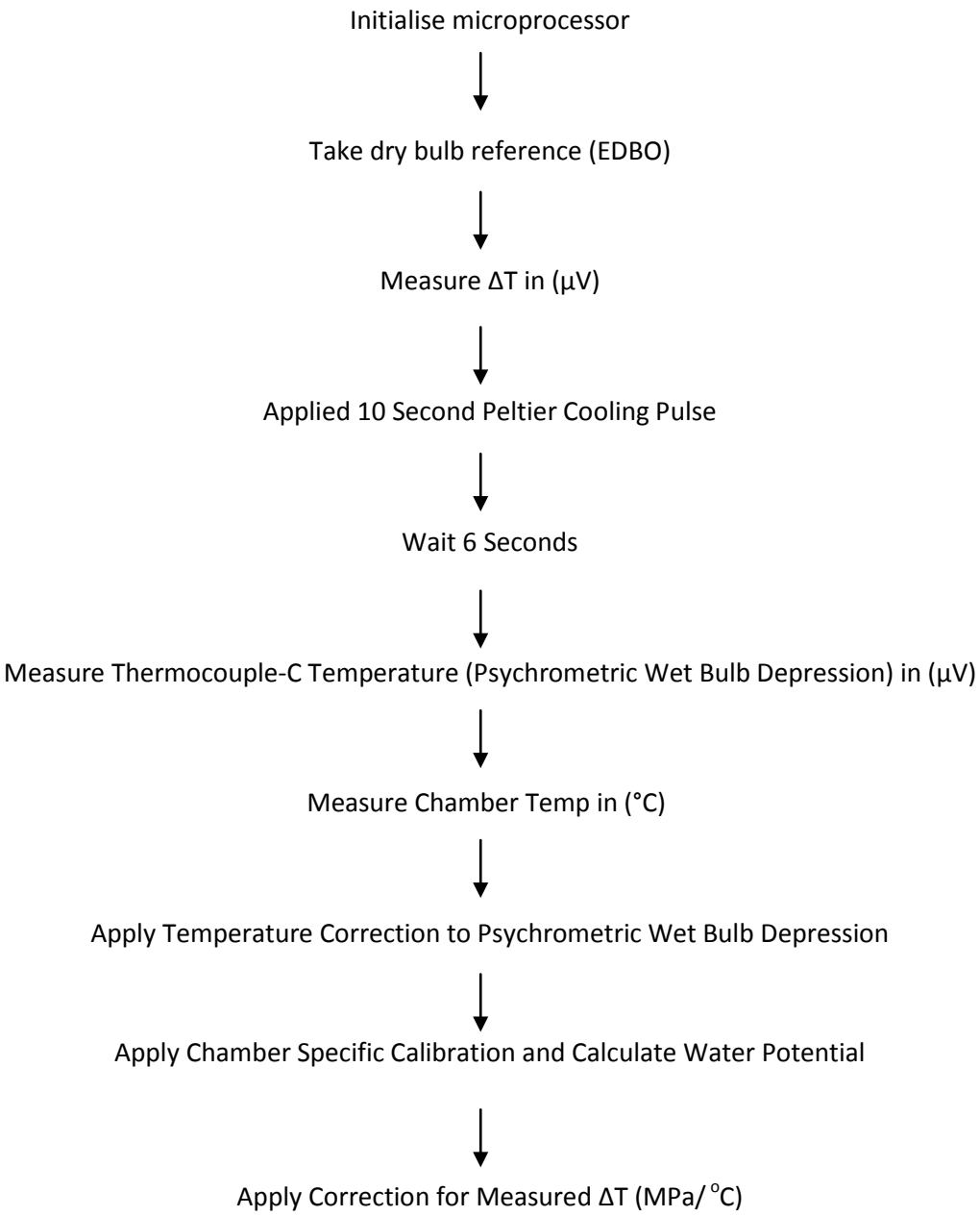



**Figure 2: PSY1 Stem Psychrometer principle schematic.**

## 6.3 Psychrometric measurement protocol




The microprocessor begins a water potential measurement by measuring dry bulb reference or Electronic Dry Bulb Offset (EDBO) and appointing it a value of zero. The difference in temperature of the two chromel-constantan thermocouples (C and S - chamber air and sample respectively) is measured. This represents the temperature gradient between the sample and the measuring junction and is converted to $^{o}$C by dividing the reading by 61 (the temperature coefficient of a chromel-constatan thermocouple). It is then multiplied by the correction factor. If, for example, the chamber temperature (i.e. ambient temp.) is 20$^{o}$C, then the correction factor is 8.38 MPa/ $^{o}$C. You now have a measure of the error in the apparent measured water potential as a result of the temperature gradient between the tissue and the measuring junction. The water potential is then automatically measured in the psychrometric mode, applying necessary temperature correction.

## 6.4 Psychrometric equation:

A Psychrometric Wet Bulb Depression (WBD) is measured when a Peltier cooling current condenses water from the atmosphere of the chamber which subsequently evaporates and cools the thermocouple junction. The raw Psychrometric Wet Bulb Depression is corrected for ambient temperature using an empirically derived algorithm. It is then converted to water potential with a calibration slope and intercept derived for the instrument from a six point calibration protocol using solutions of known solute potential (Molality). Finally, a correction for ΔT, or the temperature gradient between the tissue and the measuring junction is applied.

$$\Psi = ((((WBD/((C_1 * T_C) + C_2)) - CI)/CS) + (\Delta T / k * CF)) \quad \textbf{Equation 2}$$

**Where:**

Ψ = Corrected Water Potential

$C_1$ = Empirically derived temperature correction Constant

$C_2$ = Empirically derived temperature correction Constant

CI = Calibration Intercept

CS = Calibration Slope

WBD = (Psychrometric) Wet Bulb Depression (µV)

$T_C$ = Chamber Temperature (°C)

ΔT = Measured temperature difference between Thermocouple-C and Thermocouple-S (µV)

k = Chromel Constantan Thermocouple output /°C

$CF_{\Delta T}$ = Correction for ΔT - MPa/°C

### 6.4.1 Psychrometric Wet Bulb Depression

The Psychrometric Wet Bulb Depression is the temperature to which the Thermocouple-C is cooled when water condensed from the chamber air is allowed to evaporate. The Psychrometric Plateau representing the wet bulb depression is systematically determined by pausing for an empirically determined period (6 sec) following the termination of the Peltier cooling. Once all the condensed water has evaporated the temperature of the thermocouple returns to that of the bulk chamber represented by zero (i.e. No difference between the thermocouple and bulk chamber) (Fig 3)

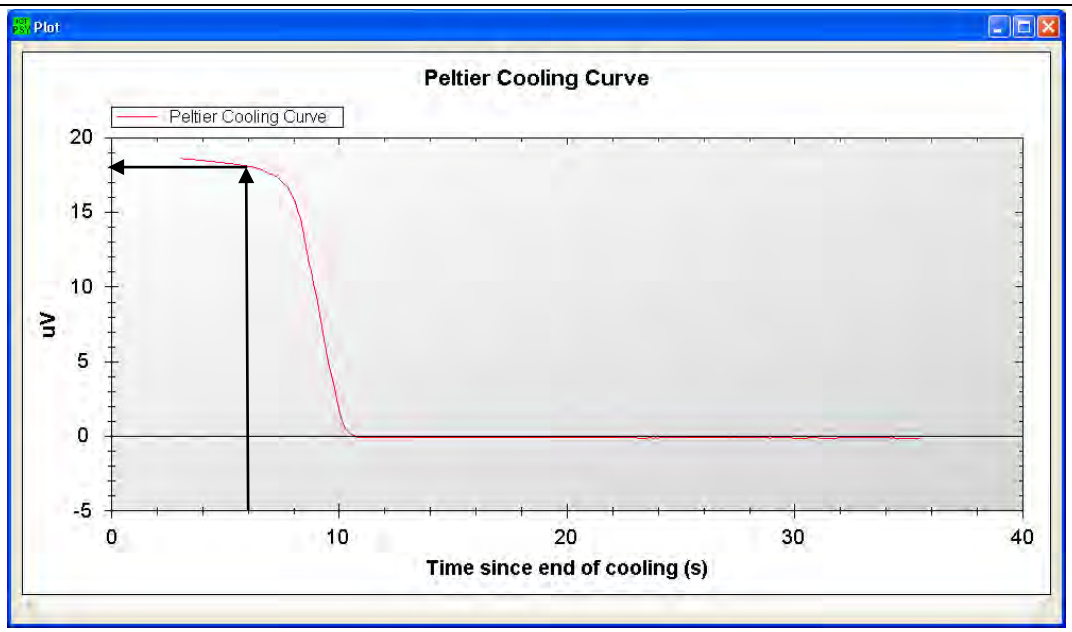



**Figure 3: The Psychrometric Wet Bulb Depression is measured on the Psychrometric plateau at exactly 6 seconds after the end of Peltier cooling.**

### 6.4.2 Chamber Temperature

Chamber temperature is important as this is the ambient temperature at which the psychrometric Wet Bulb Depression was measured. It is used in the temperature correction of the raw psychrometric Wet Bulb depression as well as to determine the correction factor for ΔT based upon the conditions under which that measurement was made.

### 6.4.3 Delta-T (ΔT)

Delta-T is the difference in temperature between the air within the atmosphere of the chamber and the adjacent plant tissue. The temperature of both the chamber air (Thermocouple-C) and the plant tissue (Thermocouple-S) are directly measured to determine ΔT. In an isothermal state this value will be zero. However, in field measurements it is unreasonable to expect that this value will actually be zero, and even small differences in temperature between the two can have a significant effect.

## 6.5 Psychrometric error

Accuracy of the stem psychrometer at the wet end of the spectrum, around zero (0 MPa) is a limitation of the physics of the Psychrometric principle. Where there is little to no drying force, it is difficult to convert liquid water back into vapour phase, and condensation within the chamber is always imminent.

Around zero (0MPa) the error is significantly greater than at water potentials of -0.5 MPa or lower. As soon as there is a drying force acting upon the thermocouples within the chamber to drive evaporation, the error dissipates rapidly and the accuracy improves. Small positives of +0.1 to +0.2 MPa (whilst not theoretically possible to be measured using psychrometry) are not of serious concern, especially when you see good diurnal rhythms, and can be ignored as mathematical artefacts. A positive offset is also not applicable across the whole data set because the error is dissipated very quickly once a drying force is generated as you extend beyond the very wet water potential range.

**NOTE 5:** In the majority of applications the wet end of the water potential spectrum below -0.5 MPa is of only moderate interest as most plants do not exhibit water stress at these levels. The majority of water potential and water relations research is conducted when plants are under stress at values between -0.5 MPa to -2.5 MPa and greater for some arid plants. At water potentials of this range a strong drying force exists and accuracy of a calibrated Stem Psychrometer is very reliable.

## 6.6 Equilibration Time

The stem psychrometer exhibits rapid vapour pressure equilibration. However, chamber temperature gradients, as a result of handling the instrument or ambient fluctuations, generally require more time to dissipate.

Calibration procedures require handling the instrument and usually 15 to 30 minutes are required to re-establish thermal stability under controlled temperature conditions.

Following installation on a stem, significant thermal gradients are usually apparent. Furthermore, disruption of local tissue water potential is likely to have occurred. For these reasons, some hours (2 to 4) should be allowed between the time of installation and the first reading. Using the “Live” mode function you can “watch” in real time the thermal equilibrium occur as the ΔT and Thermocouple-C values are displayed on screen. This data can also be logged to a .csv data file for post processing and analysis.

Provided adequate thermal insulation or temperature control of the installation has been employed, subsequent equilibration to even rapid tissue water potential changes will be dependent only on vapour pressure equilibration. The stem psychrometer exhibits favourable vapour pressure equilibration characteristics due to the absence of significant resistances to vapour exchange (eg. cuticular resistance) between the sample and chamber well.

## 6.7 Zero Offsets:

On occasion you will find variable zero offsets, especially when operating the psychrometers on potted plants. The exact cause for this has not been determined but it has been found to be mitigated by placing the pots on wooden insulators to keep them off the moist floor, ground or bench. Grounding of the chamber body by any means tends to induce spurious offsets and confound psychrometer operation. This phenomenon is generally obvious so easy to diagnose and take steps to correct. Also, direct sunlight on the psychrometer installation causes large temperature gradients even with insulation and reflective foil coverings on the instruments.

**NOTE 6**: If you discover a large variation it is usually indicative of some spurious electrical effect (e.g. static) or condensation in the chamber. The safest remedy in all cases is to remove the psychrometer, clean it and reinstall at a new site, being careful to completely seal the old site with silicon grease.

## 6.8 Temperature Gradient

The temperature gradient which results in measurement errors is that between the measuring junction and the tissue. The assumption that isothermal conditions prevail within the chamber and adjacent tissue almost never holds for in-situ measurements. The stem psychrometer compensates for this error by direct measurement. Gentle nudging of the 'sample thermocouple' (Thermocouple-S) into position is often necessary to ensure that it is extended sufficiently to make contact with the sample surface. This should be done with the aid of a hand lens or dissection microscope and fine forceps. See Handling the Instrument.

The integrated microprocessor measures the temperature gradient, within the chamber between Thermocouple-C (chamber air) and Thermocouple-S (sample temperature). A positive gradient indicates that the sample is cooler than the chamber air. In this case, the absolute value of the calculated error is subtracted from the absolute value of the measured water potential. In other words the sample is at a higher (less negative) water potential than the value determined by the C thermocouple in the chamber air which is at a slightly warmer temperature.

Calculation of the correction in water potential measurement is automatically calculated by converting the measured voltage to temperature. The calibration coefficient for chromel-constantan thermocouples is 61µV/°C. Having assessed the temperature gradient which causes the error in measured water potential, the correction factor, which is itself a temperature dependent variable, is applied. This factor is 8.20 MPa/°C at 25$^{o}$C and increases to 8.38 MPa/°C at 20$^{o}$C. The temperature dependence of this factor is dynamically recorded and applied to all readings making the measurements directly applicable across a wide range of variable ambient temperatures.

**NOTE 8:** The psychrometric reading of water potential is temperature dependent.

If the psychrometer is used at ambient temperatures which differ from the calibration temperature by more than ten degrees, then corrections using the formula should not be strictly relied upon. For example, it is common to calibrate the psychrometer at 25$^{o}$C. If one routinely uses the instrument at, say, 15$^{o}$C and applies the correction, an unknown potential error could be introduced. It would be

advisable to calibrate the instrument at or near the temperature at which it will normally be used. A very useful exercise would be to calibrate the instrument at a variety of temperatures and assess the relationship between temperature and calibration coefficient for the individual instruments. This will enhance the reliability of the instrument.

## 6.9 Condensation:

Temperature gradients which induce condensation on the inner chamber walls should be avoided. For every bar (0.1 MPa) of measured water potential a temperature gradient of 0.012$^{o}$C or more will induce condensation. If that gradient is such that the sample tissue is cooler than the chamber body, then condensation will occur on the sample and most likely be absorbed and redistributed. If, however, the reverse gradient is the case, then condensation will form on the inner chamber walls and introduce an unknown error in measurements. Generally this problem can be spotted before it seriously affects interpretation of measurements.

**NOTE 8 :** If a gradient favouring condensation on the chamber walls persists (i.e. a negative gradient from Thermocouple-C to Thermocouple-S) then measurements of apparent water potential will tend to rise and approach zero and not vary much between measurements. When it is obvious that this has occurred, remove the instrument, clean and reinstall it.

Under experimental conditions which favour undesirable temperature gradients, such as the cool early hours of the morning before sunrise through until mid morning, the heater can be used to mitigate these problems. It is a 12 volt (DC) resistive heater embedded into the back of the psychrometer chamber. The heating protocol can be adjusted by the user and is automatically controlled by the PSY1.

The exact protocol must remain a subject of trial and error depending on the specific conditions experienced. However, a reasonable approach is to routinely pulse the 12 volt heater for periods of 15 seconds to 1 minute between measurements immediately following a measurement to allow sufficient time for the heat introduced to the chamber to dissipate and return to equilibrium before the next measurement. The appropriate protocol is one which maintains conditions such that condensation will not occur on the chamber walls (i.e. the chamber is warmer than the sample). Allow enough time for extraordinary gradients caused by the heater to dissipate before attempting a measurement. See Measurement Protocols for details on the setting of the chamber heating protocol.

## 6.10 Osmotic Potential

Osmotic potentials can be measured on extracted sap samples or destructively sampled leaf tissue or leaf discs. These measurements can be made in the lab or in the field. Samples are placed in the calibration lid of the PSY1 stem psychrometer.

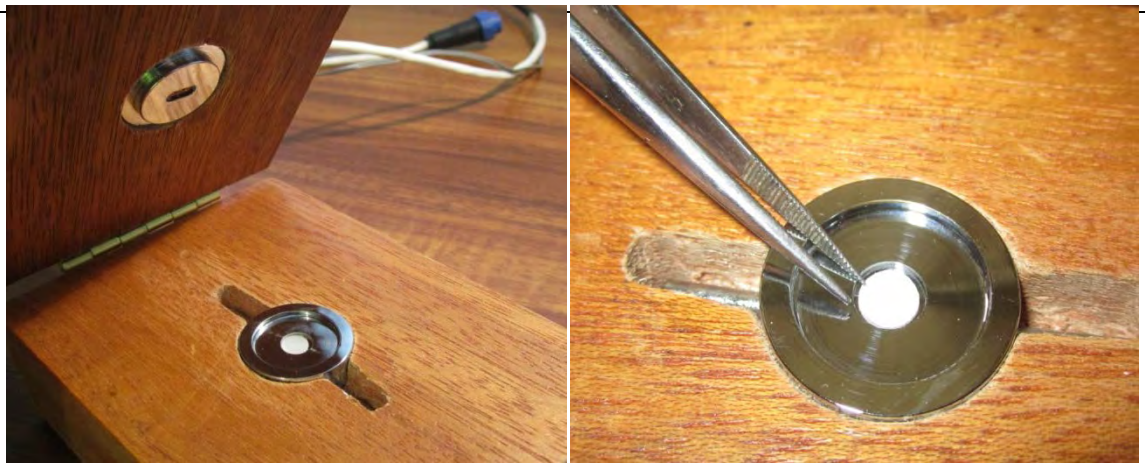
The psychrometer chamber is housed in the Osmotic Potential Insulator (OPI) to provide a thermal insulating jacket around the chamber. This eliminates introduction of thermal gradients caused by a need to handle the chamber to load samples and provides a stable insulated thermal buffer from ambient temperature gradients within the surrounding environment. This enables a very rapid equilibration time between samples.

**Photo 4**: **PSY1 Stem Psychrometer & Osmotic Potential Insulator**

Osmotic potential measurements are typically performed in a manual process. Using the Graphical User Interface (GUI) the PSY1 provides the user with a "Live" mode or a Manual mode to facilitate osmotic potential measurements.

### 6.10.1 Collecting an Extracted Sap Sample

An abraded leaf disc or filter paper disc (saturated with extracted sap exudates from a suitably prepared sample using a freezing and physical disruption protocol to separate the symplastic fluid from the cells of the leaf), are placed in the calibration lid. Wrap the leaf in a foil envelope and include a filter paper disk which will become saturated with the expressed cell contents. Place in liquid Nitrogen to freeze then crush it in a vice to physically and mechanically disrupt the cell walls. Place the saturated filter paper disk in the psychrometer calibration lid and measure the osmotic potential following thermal equilibration/stabilisation of the psychrometer chamber.



(a) (b)

**Photo 5 (a) the psychrometer chamber mounted inside the Osmotic Potential Insulator (OPI) and (b) loading a filter paper disc soaked in an extracted sap solution to measure osmotic potential**