PLANT MOISTURE VESSEL

SKPM 1400 Series

Instruction Manual

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Plant Moisture Vessel SKPM 1400

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Acknowledgments:-
We would like to thank Professor P. Jarvis, The University of Edinburgh for his help in the production of this manual.

Plant Moisture Vessel SKPM 1400

Introduction

Thank you for purchasing the SKYE SKPM1400 series Plant Moisture Vessel which is a precision instrument that with care will give many years of service.

There are 6 models in the SKPM 1400 series, this manual covers all models so please ignore portions which are not appropriate to your particular model.

The SKPM 1400/40, 1400/50, 1400/80 models are digital versions, with maximum working pressures of 40 bar, 50 bar and 80 bar respectively.

The SKPM 1405/40, 1405/50, 1405/80 models are analogue versions, with maximum working pressures of 40 bar, 50 bar and 80 bar respectively.

Replacement seals are readily available in a range of sizes to suit all stems. The battery used can be obtained throughout the world, or a rechargeable unit may be used instead.

A variety of compressed gas sources may be employed, a source may have been supplied with the unit. The system may be used with compressed air, nitrogen or carbon dioxide. It must never be used with Oxygen or other gases that may be an explosion hazard.

Each unit has been tested to far greater than its maximum operating pressure by an independent Government-approved Standards Laboratory. 0-40 and 0-50 bar working range models are tested to twice their working pressure, i.e. 80 bar and 100 bar respectively. 0-80 bar working range models are tested to 120 bar. The test certificate is supplied with the unit to which it relates.

Although the SKPM1400 has been designed with safety as the first thought, it is still a pressure vessel and as such must be treated with great respect and care. Many points are stressed in this manual and these must be observed to ensure safety

I THEORY

Wa ter po ten tial

The thermodynamic state of water in a tissue can be defined as the difference between the chemical potential of water in the tissue *(μ)* and the chemical potential of pure, free water at the same temperature and elevation and at atmospheric pressure *(μ_0).* Thus the difference in chemical potential ($\Delta\mu_w$) is ($\mu - \mu_0$). The chemical potential of pure, free water at atmospheric pressure is arbitrarily defined as zero. When water is less available than this because it contains solutes or is contained in a fine matrix of small pores, the chemical potential is less than that of pure free water so that $\Delta\mu_w$ is negative. If water is more available because it is raised up to a height or is under pressure, as in a water pistol, its chemical potential is higher than that of pure free water and $\Delta\mu_w$ is positive.

Chemical potential has units of J/mol but as the most common way of measuring the thermodynamic state of water in plant tissues is the pressure chamber, plant physiologists have preferred to express the state o f water in tissues by the *water potential* (*ψ)* with dimensions of pressure. ψ is defined by:

$$\psi = \Delta\mu_w / \overline{V}_w \qquad (1)$$

where $\overline{V}_w$ is the average partial molar volume of water (′ 18 cm^3/mol). Thus ψ has dimensions of J/m^3 which are the dimensions of pressure (i.e. N/m or Pa or bar; note 10 bar = 1 MPa = 10^6 Pa).

Water potential expresses the general availability of water and the tendency for water to move, because water moves from places of high potential to places of low potential, like electric current. But water potential alone does not tell us how or why water is more or less available. For this, it is necessary to look at the components of water potential at a particular point in the plant or cell.

Measurement of leaf water potential

The pressure chamber is based on the principle that the water potential of a tissue is exactly equal and opposite to the pressure just required to move water out of the tissue. To find the water potential of a leaf we m easure the applied pressure just sufficient to bring water in the xylem to the cut surface in the petiole. This pressure is called the balancing pressure (P_b). At the balance the water potential of the xylem water has been brought exactly equal to zero by the applied pressure. Thus

$$\psi_{xy} + P_b = 0 \quad \text{and} \quad \psi_{xy} = -P_b \qquad (2)$$

The amount of solutes in the xylem water is negligible ($\pi_{xy} > -0.01$ MPa) so that the average water potential of the leaf cells can be taken as equal to ψ_{xy}, i.e.

$$\psi \prime \psi_{xy}.$$

Component potentials

In general the water potential at a point comprises several components, i.e.

$$\psi = \psi_1 + \psi_2 + \psi_3 \ldots \psi_n.$$

In soils and plants we can commonly identify the following component potentials

P - the hydrostatic pressure (turgor pressure) potential (usually + ve)

π - the solute (osmotic pressure) potential (-ve)

t - the matric (capillary pressure) potential (-ve)

G - the gravitational potential ($\rho_w gh$ = 0.01 MPa/m) (-ve) or overburden pressure potential (+ ve).

In plant cells, the main components of water potential are solutes and pressure. Solutes restrained in cells by membranes reduce the chemical potential whereas the pressure of the cell wall against the cell contents and of one cell against another, raise the chemical potential. Thus the water potential of a cell can be expressed as the sum of the solute potential (π) and the pressure potential (P):

$$\psi = \pi + P. \qquad (3)$$

π is negative and P is normally positive in living cells but is negative in xylem cells where the water is under tension. The values of P and π are presumed to be uniform throughout the symplasm, since it is unlikely that any significant pressure gradients can exist across the bounding membranes of the vacuole and cytoplasmic organelles (Tyree & Jarvis, 1982). However, the solutes contributing to the reduction in π in the various symplasmic compartments may be quite different (Tyree & Jarvis, 1982). The pressure potential is often called the turgor pressure; the solute potential is often called the osmotic potential. Both low turgor pressures and low osmotic potentials are damaging to cellular processes.

The Höfler-Thoday diagram of tissue water relations characteristics

In turgid tissue at equilibrium

$$-P_b = \psi = \overline{P} + \overline{\pi} \qquad (4)$$

where $\overline{P}$ and $\overline{\pi}$ are defined as weight-averaged values of tissue turgor pressure and tissue osmotic potential, respectively. In other words,

$$\overline{P} = \sum_{i=1}^{n} \frac{w_s^i}{W_s} P^i \qquad (5a)$$

and

$$\overline{\pi} = \sum_{i=1}^{n} \frac{w_s^i}{W_s} \pi^i \qquad (5b)$$

where P^i, π^i, and w_s^i are the turgor pressure, osmotic potential, and weight of symplasmic water, respectively, in the i^{th} cell in the tissue, and W_S is the total weight of symplasmic water in the tissue (Tyree & Jarvis, 1982).

From the ideal gas law, Raoult's law and the Van't Hoff approximation

$$\overline{\pi} = -\phi RTN_S/V = -\phi RTN_S\rho_w/W_S \qquad (6)$$

where V is the volume and W_S the weight of symplasmic water, N_S is the number of (os)moles of solute in the cell sap, ϕ is an osmotic coefficient to account for the non-ideal behaviour of the solutes and ρ_w is the density of water in the solution [$= M_w/V$ where M_w is the molar mass of water ($= 18$ g/mol)].

As water is lost from the tissue, P falls to zero at the turgor loss point and at lower potentials

$$-P_b = \psi = \overline{\pi} \qquad (7)$$

Thus below the turgor loss point

$$\frac{1}{P_b} = -\frac{1}{\overline{\pi}} = \frac{W_s}{\phi RTN_s\rho_w}. \qquad (8)$$

and if negative turgor pressures do not occur in the tissue, then at all lower water contents, $\psi = \overline{\pi}$ Hence,

$$\frac{1}{P_b} = -\frac{1}{\bar{\pi}} = \frac{\theta(W_s^0 + W_a^0)}{\phi RTN_s\rho_w} - \frac{W_a}{\phi RTN_s\rho_w} \qquad (9)$$

where W_a is the weight of apoplasmic water in the tissue, W_s^0 and W_a^0 are the weights of symplasmic and apoplasmic water, respectively, in the tissue at saturation, and θ is the relative water content of the tissue (Tyree & Jarvis, 1982). The third equality in Eq. (9) follows from the definition of θ, since

$$\theta = \frac{W_s + W_a}{W_s^0 + W_a^0} \qquad (10)$$

and $$W_s = \theta\,(W_s^0 + W_a^{0)} - W_a \qquad (11)$$

If ϕ and W_a remain constant as θ decreases below the point at which $\bar{P}$ reaches zero, then Eq. (9) describes a linear relationship between $1/\psi$ (or $1/\pi$) and θ. Hence, a plot of $1/\psi$ against θ will yield a straight line in the region where $P = 0$ (Tyree & Jarvis, 1982).

Required tissue water relations characteristics can be obtained from extrapolation to the limit $\theta = 1.0$, at the same time making the assumption that ϕ and W_a do not change with dehydration, and this gives the solute potential at full turgor (π_0) from the intercept on the $1/P_b$-axis as

$$\frac{1}{P_b} = -\frac{1}{\bar{\pi}_0} = \frac{W_s^0}{\phi RTN_s\rho_w} \quad (\text{for } \theta = 1.0) \qquad (12)$$

It is also clear from Eqn (9) that at the other limit of $1/P_b = 0$, the proportion of tissue water that is apoplasmic is given by the intercept on the θ-axis as

$$\theta_a = \frac{W_a}{W_s^0 + W_a^0} \quad (\text{for } 1/P_b = 0) \qquad (13)$$

From the extrapolation of the straight line to $\theta = 1.0$, it is also possible to calculate $\bar{P}$ for any and all values of θ between full hydration and the point at which P reaches zero. Given this calculated relationship between P and θ, together with the measured relationship between ψ and θ, it is then possible to calculate the relationship between $\bar{P}$ and θ in the following way. $\bar{P}$ at any water content is shown by the vertical distance between the curves of $1/\psi$ versus θ and $1/\pi$ versus θ. $\bar{P}$ can therefore be calculated using equation (4) at any water content from the values of ψ and $\bar{P}$ at that value of θ and can also be plotted as a function of θ.

It is now possible to list the following characteristic parameters of the leaf:

$$\pi_0, P_0, \theta_a, \theta^*, \psi^*$$

ψ^* and θ^* are the values at the turgor loss point.

In addition the weight-averaged bulk modulus of elasticity, defined as the change in turgor pressure for a fractional change in symplasmic water content, as follows:

$$\varepsilon = \frac{d\bar{P}}{dW_s / W_s} = \frac{d\bar{P}}{d\theta / \theta_s} \qquad (14)$$

where θ_S is the symplasmic water content ($\theta - \theta_a$), can be determined. To estimate ε plot $\bar{P}$ against θ_s. ε is then calculated as the slope of the line at particular values of θ_s.

Three major assumptions of the pressure chamber technique are: (1) negative tissue turgor pressures do not occur (i.e., $\bar{P} \geq 0$), (2) as water is lost from the tissue, the concentration of solutes increases in an ideal fashion (i.e., ϕ = constant), and (3) all of the water lost from the tissue comes from the symplasm (i.e., W_a = constant = W_a^0). Other

possible sources of error include: (1) systematic and random errors in the measurement of ψ, (2) internal disequilibria in ψ, (3) changes in π over the period of measurements and (4) plastic deformation of the cell walls over the period of measurements (Robichaux et al. 1984).

IIA Description of Controls and Fittings

SKPM 1400 DIGITAL MODELS

1) Main Gas Control

This has three positions, '**Fill Chamber**', '**Vent Chamber**' and a central '**Off**' position. It controls the flow of gas to and from the pressure vessel. In the '**Fill**' position it permits the flow of gas from the quick release inlet through the needle valve and safety pressure release valve into the chamber. In the '**Vent**' position it allows gas to escape from the chamber to the space beneath the panel. The gas inlet is isolated in this position. In the '**Off**' position the chamber is isolated as is the gas inlet.

2) Needle Valve

This controls the rate of gas flow into the chamber when the gas control is set to '**Fill Chamber**'. It is able to virtually stop the flow of gas when screwed fully in, but should not be used as an on - off tap. It should be set to a position by experience that will give a reasonable rate of pressure rise in the vessel. The setting will be different if the final pressure is to be 35 bar rather than 3 bar but for a batch of material the needle valve can be left in a set position, and the main gas tap used to stop and start the flow. It should be noted that this valve **should not be over tightened** as it is possible to deform the 'needle' and spoil it's control characteristics.

3) Quick Release Gas Connector

This is fitted to enable the flexible hose to be removed for transport. It is rated at many times the pressure of the systems safety release pressure and will stand the pressure of the gas inside a pressure bottle. A mating connector is fitted to the flexible hose supplied as an accessory and individual connectors are available to connect to customers own manifolds etc. They have a 3/8" B.S.P. female thread.

4) On - Off Switch

This switch interrupts the power to the electronic circuitry. Be sure to use it or you will waste batteries!

5) Range Switch

This switch sets the full scale of the display meter. Always use the 0 - 20 bar (0-2 MPa)setting when possible as this will give a resolution of 0.01 bar. If the pressure rise in the chamber causes the display to overrange, simply select the 0 - 200 bar range. Such overranging will not harm the unit.

6) Dis play Free and Hold But tons

These are provided in order that the operator need not watch the display at the same time as he or she should be concentrating on the determination of the 'end point' of the test. When the **Hold** button is pressed, the display is frozen until such time as the **Free** button is pressed. The display will then jump to show the current chamber pressure if it has changed in the meantime. Thus the operator need only watch the cut end of the stem and push the hold button when fluids are seen to emerge from the cut end. At any time, if unsure as to whether t he display is held or free, the desired button may be pressed and the display will adopt that mode or remain in that m ode if was already in it.

7) Dis play Zero

This control is provided to set the pressure transducer offset to zero. This offset will change slightly with various factors, mostly temperature. The offset if uncorrected will give rise to constant offset errors of pressure measurement (the sensitivity will not be affected).For example, if the display reads as 0.04 bar when the chamber is fully vented (a zero offset of 0.04 bar), then all pressure readings taken subsequently will have a constant 0.04 bar added to them. To avoid this, set the display to zero before commencing measurements. This is simply done by selecting the 0 - 20 bar range and turning the zero control until the display shows zero. Note that it is possible to show a 'subzero' pressure, and that the correct zero is when the minus sign is flickering on and off occasionally.

8) Batteries

The unit uses nine volt batteries of the PP3 type (or MN1604 or 6LF22 as they are also designated). The unit consumes approximately 12 mA of current and thus an alkaline battery will have a continuous life of about 30

hours. In intermittent use this will be longer and may correspond to many months of use if switched off when not required. Rechargeable nickel-cadmium batteries may also be employed if it is convenient to recharge them.

The circuit has a 'low battery detector' that shows a low battery warning on the display when the voltage drops to around 6.9 volts. If an alkaline cell is in use then there is just about 5-8% of life left when this first shows. If a nickel-cadmium rechargeable cell is in use then it should be recharged as soon as possible after the low battery warning shows as it has less than an hours life left depending on its age and condition. Readings will become inaccurate when the battery voltage falls below 6.9 volts. At this time the zero will change erratically. Be warned that if low cost Leclanche batteries (e.g. Ever Ready Blue types) are used they leak readily and may corrode the holder. Low mercury content alkaline cells are becoming more readily available now, and these are especially recommended on environmental grounds if they can be found.

IIB Description of Controls and Fittings

SKPM 1405 ANALOGUE MODELS

1) Main Gas Control

This has three positions, '**Fill Chamber**', '**Vent Chamber**' and a central '**Off**' position. It controls the flow of gas to and from the pressure vessel. In the '**Fill**' position it permits the flow of gas from the quick release inlet through the needle valve and safety pressure release valve into the chamber. In the '**Vent**' position it allows gas to escape from the chamber to the space beneath the panel. The gas inlet is isolated in this position. In the '**Off**' position the chamber is isolated as is the gas inlet.

2) Needle Valve

This controls the rate of gas flow into the chamber when the gas control is set to '**Fill Chamber**'. It is able to virtually stop the flow of gas when screwed fully in, but should not be used as an on - off tap. It should be set to a position by experience that will give a reasonable rate of pressure rise in the vessel. The setting will be different if the final pressure is to be 35 bar rather than 3 bar but for a batch of material the needle valve can be left in a set position, and the main gas tap used to stop and start the flow. It should be noted that this valve **should not be overtightened** as it is possible to deform the 'needle' and spoil it's control characteristics.

3) Quick Release Gas Connector

This is fitted to enable the flexible hose to be removed for transport. It is rated at many times the pressure of the systems safety release pressure and will stand the pressure of the gas inside a pressure bottle. A mating connector is fitted to the flexible hose supplied as an accessory and individual connectors are available to connect to customers own manifolds etc. They have a 3/8" B.S.P. female thread.

4) Display

The large, easy to read dial gauge display has two readout pointers. The black pointer shows the current chamber pressure whilst the red pointer indicates the maximum pressure reached. To zero the red pointer fit the black 'peg' which is attached to the main case into the centre of the dial gauge and turn anticlockwise to bring the pointer round to zero.

The purpose of this red pointer is to enable to user to watch the cut end of the stem for the 'endpoint' without having to concentrate on the pressure reading at the same time. If chamber pressure is released by turning the main gas control to OFF at the end point, the red 'maximum pressure' pointer will continue to show the end p oint pressure.

III Operation

The unit is supplied in a black moulded attache case. Keys are supplied to the two locks. When closing the lid of the case the top of the pressure vessel must be removed to allow the lid of the case to close. A threaded boss of metal is mounted on the front of the instrument to hold the top of the vessel when the lid is closed. Before screwing the top to this boss the large 'O' ring that seals the top to the base of the vessel must be placed on the temporary mount to avoid the threads binding. It may be found convenient to store a spare stem seal or two in the space under the vessel top when it is mounted thus.

First ensure that the gas supply is ready, but turned off completely. Make sure that the regulator is able to deliver the maximum pressure that is likely to be required, but remember that the safety release valve in the SKPM1400 is set to 40 bar or approx 600 p.s.i. Be sure that the flexible supply hose is in good condition and that the quick release couplings are sealing correctly. If not, seek advice before proceeding. There must be no pressure in the flexible hose as this will make it impossible to connect it to the unit.

Turn the main gas tap to the 'off' position and screw the needle valve fully clockwise to close it. DO NOT OVERTIGHTEN the needle valve as this will damage it. Only very minimal tightness is required. Connect the flexible hose to the unit and turn the gas supply on at the bottle. Set the regulator (if necessary) to deliver the maximum pressure that is likely to be required. (see manufacturers instructions)

SKPM 1400 DIGITAL MODELS

First check and zero the pressure transducer. Make sure that a battery is fitted to the unit. Switch on, and set the range to 0-20 bar. The display will default to the free setting when first switched on, but if preferred a check can be made. To do this, push the 'display hold' button and then turn the 'set zero' potentiometer. The display should not alter its reading (although it may not be precisely zero unless adjusted prior to the test). Now press the 'display free' button. The display should immediately jump to a new value as it is 'freed'. Next the display should be carefully set to zero, but please ensure that the range is set to 0-20 bar, and that the main gas tap is set to 'vent chamber' and the chamber thus allowed to equilibrate with the atmosphere. The zero should always be set on the mostsensitive range (i.e. 0-20 bar) as this will give the most accurate zero on the less sensitive range.

Set the 'display range' switch to the range appropriate to the work in hand. If in doubt always use the most sensitive range in order to get the best resolution and change ranges when the display is overranged. The system will not be harmed in any way by doing this. Ensure that the display is free, if in doubt press the 'display free' button.

SKPM 1405 ANALOGUE MODELS

First check that the red 'maximum pressure' pointer is set to zero. To do this fit the black 'peg' which is attached to the main case into the centre of the dial gauge and turn anticlockwise to bring the pointer round to zero.

ALL MODELS

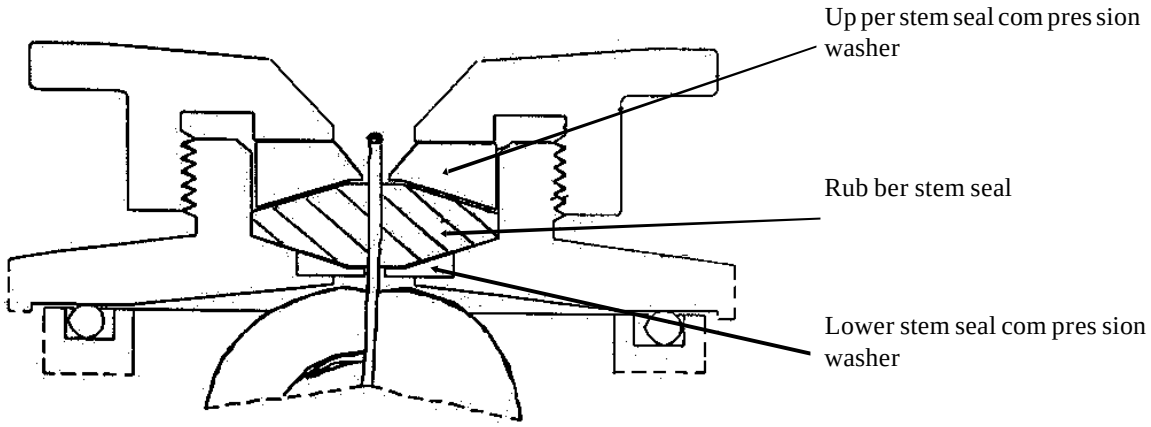
Before proceeding make sure that the large gas tap is set to the position marked 'Vent Chamber'. Put some wet filter paper or tissue in the bottom of the chamber.

Enclose the leaf to be measured in a small polythene bag to prevent water loss. Detach it with a sharp clean razor blade or scalpel making a right angle cut. If the surface is ragged, trim it with another cut but remove as little of the stem or petiole as possible. Take the leaf to the pressure chamber.

We offer two types of head for the pressure chamber. The first (and original) high pressure head has a three part sealing washer system. This is more suitable for samples of a woody nature. The second low pressure head has just one washer with a suitably matching adaptor and is more suitable for fleshy samples. Decide on a rubber sealing washer that is just about the same size as or slightly larger than the stems of the specimens to be measured. (If they are woody stems then the bark, if it is loose or irregular, is often best removed first to give a more reliable seal.) If the stem is woody it is best to choose a rubber seal that it is a tight push fit in.

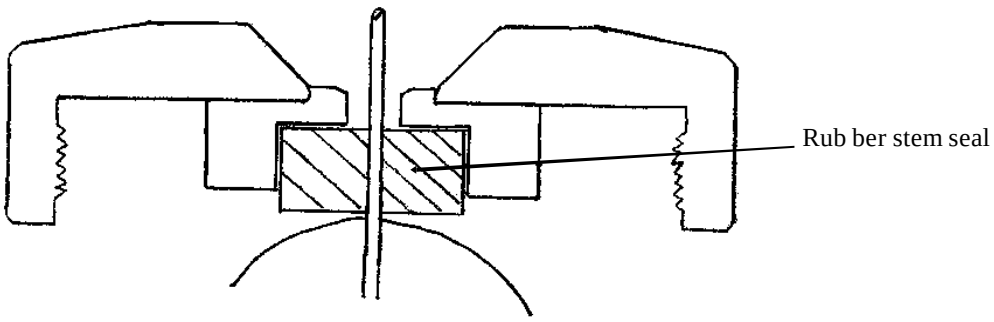
HIGH PRESSURE CHAMBER HEAD FOR WOODY SAMPLES

Seal is made by the com pres sion wash ers when the two part head is screwed to gether



CHAMBER HEAD FOR FLESHY SAMPLES LOW PRESSURE

Seal is made by the gas pres sure in side the ves sel push ing against the soft rub ber stem seal



High Pressure Chamber Head Suitable for Woody Samples

This chamber head is suitable for all measurement pressures from zero to 80 bar, if applicable to your model.

In this type of head the seal is made by the compression washers when the two part head is screwed together. Select an appropriate metal lower stem seal washer and fit in the base of the lid. There are several sizes to suit different rubber sealing washers. The correct one to choose is that which has a central hole just larger than the rubber sealing washer, so that it gives maximum support to the rubber washer under pressure but does not touch the plant stem. The metal lower stem seal washer simply drops into the recess shown in the diagram.

In the same way choose a plastic upper stem seal washer to go on the top of the rubber sealing washer. It is important to use the size that is just a millimetre or so larger than the rubber washer. This will give the best compression of the rubber and also prevent extrusion of the rubber by the pressure within the vessel.

Assemble these three in the vessel lid as shown in the diagram taking care to clean the recesses and the washers first to ensure a reliable seal. Engage the threads of the stem seal clamp and screw it loosely down. DO NOT FORCE the thread. When the threads are engaged correctly it will turn easily.

Insert the cut end of the stem or petiole from the underside of the lid so that it just appears though the central hole of the stem seal clamp and then gently tighten the clamp. Experience will dictate the degree of tightness required to obtain a satisfactory seal with any given material, but it is always possible to increase the sealing pressure by screwing the clamp on a little further.

Low Pressure Chamber Head Suitable for Fleshy Samples

This head is recommended for use with chamber pressures up to 40 bar only. If you are using a 50 bar or 80 bar model, please use the high pressure head for measurement pressures above 40 bar.

This chamber head requires only a rubber washer (wide choice of hole sizes) and an adapter for holding the specimen (choice of central hole or slit). The seal is made by the gas pressure inside the pressure vessel pushing against the soft rubber stem seal.

Choose the appropriate specimen holding adapter for your sample, ideally choose the size which is only slightly bigger than the diameter of the petiole or stem to be measured. These adapters can be interchanged as necessary by unscrewing the 4 screws holding attaching them to the chamber head.

Open the cut in the rubber stem seal and place the petiole or stem along the central groove, close and simply push into the underside of the head. The rubber seal does not need to be a tight fit at this stage as the gas pressure will ease it fully home as the chamber pressure increases.

MAKING A MEASUREMENT

Ensure that the 'O' ring groove on the vessel base is free of dirt and grit etc., and that the large 'O' ring is correctly seated in place. Put the leaf in the chamber still within the polythene bag, holding it carefully if necessary. Screw the lid assembly onto the base taking care that the threads engage correctly. DO NOT FORCE the thread. It may be found helpful to rotate the head in the 'unscrewing' direction for 1/4 or ½ turn before screwing it on so that the threads engage correctly. Tighten the head assembly firmly onto the vessel base.

Before proceeding to pressurise the vessel, clip the perspex safety shield in place on the vessel lid, and put on the safety glasses provided with the system. It is most important to observe these precautions since there is a chance of the plant material being ejected from the vessel under pressure if it is not secured adequately.
When this is done then the vessel can be pressurised.

Turn the main gas tap to the 'fill chamber' position. open the needle valve a turn or so. A faint hiss may be heard as the chamber fills. Watch the pressure rise on the display, but concentrate on the cut end of the specimen. DO NOT LOOK down on or bend over the top of the vessel. Always fit the safety cover so that the open sides are away from the operator.

If using a digital model, keep a finger on the 'display hold' button. The rate of pressure rise may be increased if desired by opening the needle valve further. When the end point is observed push the 'display hold' button and then turn the main gas tap to 'off'.

If using an analogue model, the rate of pressure rise may be increased if desired by opening the needle valve further. When the end point is observed turn the main gas tap to 'off'. The red 'maximum pressure' needle will display the end point reached.

Observe the cut end of the stem or petiole with a good lens or, preferably, with a low power binocular microscope. You are looking for sap to appear at the cut surface of the xylem. The end point required is the "balancing pressure" just required to hold the xylem sap at the cut surface. This balancing pressure is then equal to the xylem pressure potential in the leaf at the time of cutting. The appearance of the sap at the surface can be clearly seen because the surface darkens and reflects light. It may be necessary to adjust the angle of illumination to define good end point. As soon as the sap appears, switch the gas supply to OFF and record the pressure on the display (or press the HOLD button on digital models). If you are uncertain about the end point, or have overshot it, you can repeat the determination of the end point. You can also slow down the rate of rise of pressure so as to define the end point better, if necessary.

If it is required to repeat the reading, then the pressure in the vessel should be reduced below the end point by turning the main gas tap SLOWLY to the 'vent chamber' position. Be careful doing this for the first time as gas will be released very quickly beneath the panel with a considerable noise. Only a little gas need be vented in this way to make a repeat reading, and the tap may then be turned to 'off'. The procedure in the paragraph above may then be repeated.

When the measurements are complete the chamber must be returned completely to atmospheric pressure before the specimen can be removed. To accomplish this the main gas tap must be SLOWLY turned to the 'vent chamber' position until gas escape is heard. Let the system vent slowly at first and do not turn the tap fully to the 'vent chamber' position until the pressure gauge reads almost zero. When the pressure has been released then the lid may be unscrewed and the specimen replaced by reversing and then repeating the process described earlier. Rapid changes in pressure result in large changes in temperature that may harm the leaf or distort measurement.

If the measuring session is complete, and the instrument is to be packed away then the pressure in the flexible hose must be released. Before the last specimen is removed the gas supply must be turned off at the cylinder (see manufacturers instructions). The chamber is then filled with the gas left in the pipe, and subsequently vented. This may have to be repeated several times to reduce the pressure sufficiently. Do not attempt to empty the pipe with the chamber lid removed as this may result in tiny particles of grit etc., being blown in the eyes of the operator.

The flexible hose may now be removed and the vessel lid 'parked' on the threaded boss on the front panel. Be sure to switch off the power on digital models and store the gas bottle, regulator and hose carefully.

IV Use with Grass and Irregular stems

The Plant Moisture Vessel can be supplied with a slit specimen holding adapter for the low pressure chamber head, to enable use of the instrument with small grasses. The blank rubber stem seals should have a slitcut in them for use with these.

The Blank rubber stem seals may also be specially shaped to suit irregularly shaped stems that may be dealt with frequently in one particular investigation. It may be found easiest to freeze the rubber with liquid nitrogen or solid CO2 to make it possible to file it. Half round stems and those with flats on their circumference are best dealt with by this technique.

High Pressure Chamber Head Suitable for Woody Samples

The slit must obviously go through the depth of the washer but should also be a part of a diameter, extending to within about 5mm of the edges. A sharp scalpel and a little detergent as a lubricant will help. Remove all traces of the lubricant before use under pressure.

If the rubber stem seal is squeezed so that the slit opens up, then the grass or flat stem may be inserted centrally in the slit. The lid may then be assembled and screwed onto the vessel base.

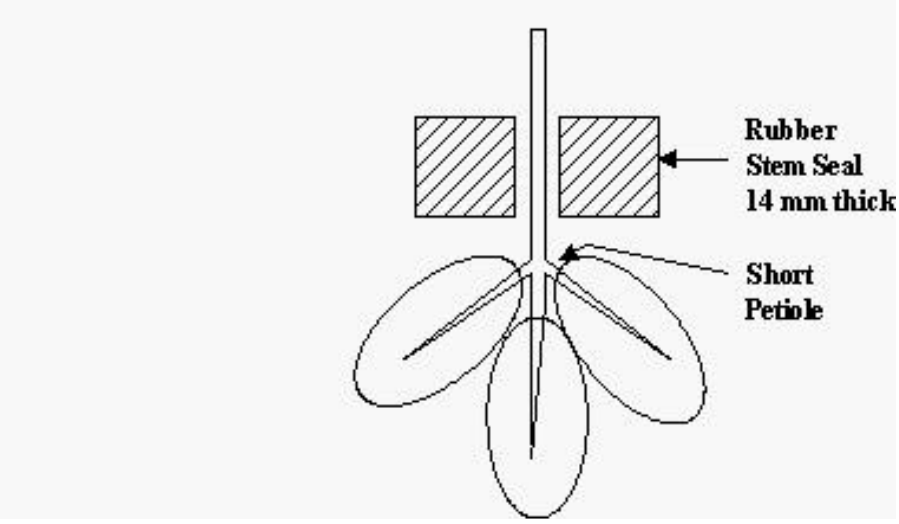
Low Pressure Chamber Head Suitable for Fleshy Samples

Cut a slit right through the depth of the rubber stem seal, across the central diameter but not right to the edge. The seal should not be cut into 2 pieces, but still be joined at one side so that it can be gently opened like a 'mouth' to accept the grass. A sharp scalpel and a little detergent as a lubricant will help. Remove all traces of the lubricant before use under pressure.

Specimens with Short Petioles

It is difficult to seal a short petiole in the standard rubber stem seals supplied by Skye as these are 14 mm thick. It may be possible, with experimentation on your own specimens, to cut down the rubber seals so they are thinner, but this may cause further difficulties, for example, the rubber seal will be difficult to insert and remove from the chamber head, also the rubber may deform under pressure and 'bulge' through the top aperture causing leakage.

Our suggestion is to seal on the next longest stem, further up the plant. This may mean introducing several leaves into the vessel, as shown below. Although you are now not strictly measuring the leaf potential, many users have found this the only reliable way to measure leaves with short petioles.



V Measurements

A leaf and petiole, or stem with leaves or needles attached is sealed in the top of the vessel (see separate instructions elsewhere) with the cut end of the stem or petiole exposed to the atmosphere. When attached to the plant the column of water would be under a tension related to the water potential and when detached the fluid will drawn back into the xylem etc. The pressure in the body of the vessel is slowly increased so that this column of water is again brought to the cut surface of the stem or petiole. It will be seen first as a moistening of the surface and then as a small bead of water on the end of the stem. The water potential end point is the pressure at which the water just appears at the cut end of the stem.

The readings obtained from the instrument are pressures, read in units of bar. This is related to other units of pressure as follows.....

1 bar = 0.1 MPa = 10^5 Pa ' 14.509 p.s.i. etc.

This reading of pressure is equal but opposite in sign to the xylem pressure potential of the plant material under test. This reading is based on the principal that the water potential of a tissue is equal and opposite to the pressure required to return water to the cut surface of the tissue. It is important that no water is actually removed from the tissue, the pressure corresponding to the xylem pressure potential of the plant tissue is that pressure just required to force water to the cut end of the stem or petiole of the tissue under test.

If the pressure rise is halted at a point before water is lost from the end of the stem, then when the pressure is reduced the water will return to the plant material and repeat measurements may be made.

The xylem pressure potential closely approximates the average water potential of the cells in the leaf. At a steady state, the water potential of the leaf cells will be slightly lower than the xylem pressure potential of the xylem sap. This is usually very small, not more than -0.02 MPa.

The water potential can be related to the water content of a tissue and the pressure vessel may be used to dehydrate a leaf in measured steps by collecting and measuring the expressed fluid. For more details of the theory and techniques the user is referred to the literature. Some relevant references are quoted below.

Cheung, Y.I.V.S., Tyree, M.T. and Dainty, J., 1975. *Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations*. Can. J. Bot. 53: 1342-46

Hallkvist, H., Richards, G.P. and Jarvis, P.G., 1974. *Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber*. J.Appl.Ecol. 11: 637-688.

Jones, H.G. 1983 *Plants and Microclimate.* pp. 64-71, C.U.P.

Ritchie, G.A. and Hinckley, T.M. 1975. *The pressure chamber as an instrument for ecological research*. Adv. in Ecol. Res. 9: 165-254

Robichaux, R.H. 1984. *Variation in the tissue water relations of two sympatric Hawaiian Daubautia species and their natural hybrid.* Oecologia (Berlin) 65:75-81

Robichaux, R.H. et al 1984. *Tissue water deficits and plant growth in wet tropical environments*. (note the appendix). In Physiological Ecology of Plants of the Wet Tropics eds. E. Medina, H.A. Mooney & C. Vazquez-Yanes, pp 99-112. D.W.Junk Publishers, The Hague

Scholander, P.F. et al., 1965. *Sap pressure in vascular plants*. Science, N.Y.148: 339-346.

Tyree, M.T., Dainty, J. and Bevis, M., 1973. *The water relations of hemlock (Tsuga canadensis). I. Some equilibrium water relations as measured by the pressure bomb technique*. Can. J.Bot. 51: 1471-88

Tyree, M.T. 1976. *Physical parameters of the soil-plant-atmosphere system: breeding for drought resistance characteristics that might increase wood yield*. In: "Tree Physiology and Yield Improvement", ed. M.G.R. Cannel & F.T. Last. pp. 329-348. Academic Press

Tyree, M.T. and Jarvis, P.G. 1982. Water *in tissues and cells*. In: Encyclopaedia of Plant Physiology Vol. 12B: Physiological Plant Ecology II" ed. O.L. Lange, P.S.Nobel, C.B.Osmond & H. Ziegler. Springer-Verlag, pp 35-77

Waring, R.H. and Cleary, B.D., 1967. *Plant moisture stress: evaluation by pressure bomb*. Science, N.Y. 155: 1248-54

VI General Operating Observations

In order to minimise water loss from plant material it should be wrapped in a moist environment whilst waiting for measurements to be made. Some workers dealing with delicate leaves will wrap them gently in 'cling film' or similar to avoid water loss. A damp cloth or tissue in the bottom of the vessel chamber in order to keep the gas inside moist is favoured by many workers. The pressure transducer, dial gauge or chamber will not be harmed by condensation since they are stainless steel and chromed brass respectively. Avoid covering the gas or pressure transducer inlet with the tissue.

If the instrument is to be used in the field then it is often important to use a minimum of gas. The amount of gas used depends linearly on both volume and pressure. The pressure cannot be altered, but the volume can. If only small specimens are being measured then part of the chamber may be filled with inert material to occupy volume and thus reduce the volume of gas required to reach any given pressure. Specially shaped volume reducers are available as accessories. These will fill the bottom or sides of the chamber, depending on the space requirements of the plant tissue. Alternatively, WASHED stones or pebbles may be used, but take care to wash them to remove sand, grit etc. Take care also not to deform excessively the plant tissues.

It takes practice to make rapid, accurate measurements. It is easy to overshoot the end point and lose plant moisture. If many measurements are to be made on the same type of material it may be found helpful to measure the first rather slowly to avoid missing the end point. On subsequent specimens the rate of rise of pressure in the vessel can be made faster to a pressure just below the expected end point, and then the rate slowed so as not to miss it.

It is also possible that if the rate of pressure rise is too great, the fluid within the plant tissue literally does not have time to get to the cut surface of stem before the true end point pressure is greatly exceeded. This is exemplified by a vigorous bubbling on the cut end of the stem that does not subside until the pressure is reduced well below the point at which it started. In such a case, a significant amount of moisture may be lost so that a repeat measurement may be not be accurate.

It is possible to make valid measurements with some specimens that will not make a perfect seal. The pressure indicated is that in the chamber and thus if the leak is not too great, work can continue, albeit with the wastage of some gas.

VII Routine Care and Maintenance of the Unit

The unit should be kept clean and free of soil and mud as far as possible. In particular the large sealing 'o ring', it's groove in the vessel base and the under surface of the lid must be kept free of all contaminants. Similarly the stem sealing washer and it's seating must also be kept clean.

A small amount of silicone grease or similar may be applied to the threads of the lid and clamp to ease tightening them. ON NO ACCOUNT APPLY ANY GREASE TO THE INSIDE OR SEALING SURFACES OF THE VESSEL. This may seem over-cautious in view of the relatively low pressures and types of gases used, but is none the less sensible and good practice.

If the 'o ring' or rubber stem seal become worn, torn or otherwise damaged then they should be replaced. Do not use increasingly greater force to make a damaged sealing surface make a good seal.

As the surfaces bed in inside the main gas tap, it may require slight adjustment to prevent gas leakage. To do this use the Allen key provided to remove the tap operating lever (having first fully vented the system to atmospheric pressure) and tighten the brass ring now exposed. Use an Allen key in each hole and a screwdriver as a 'tommy bar' between them. Only a small part of a turn will be required. Replace the operating lever. This adjustment may be required occasionally or maybe not at all depending on pressures used.

Be very sure that the flexible gas supply hose is not damaged. If there is any sign of external damage then its replacement should be arranged.

VIII List of Parts and Accessories / Checklist

Basic System

- ❑ SKPM 1400 Plant Moisture Vessel
- ❑ Low pressure chamber head ❑ High pressure chamber head
- ❑ 2 off Large 'O' Rings
- ❑ 1 off Alkaline 9V Battery *(digital models only)*
- ❑ Perspex Safety Screen
- ❑ Safety Glasses
- ❑ 2 off Allen Key $^{3}/_{32}$"

Accessories for low pressure chamber head

- ❑ SKPM 1445 Specimen holding adapter - 6 or 10mm central hole or 17mm slit
- ❑ SKPM 1447 Rubber stem seals - sizes from 1.5 to 5.5mm in 0.5mm steps, or blank

Accessories for high pressure chamber head

- ❑ Upper Stem Seal Washer - 4mm
- ❑ Lower Stem Seal Washer - 3mm
- ❑ Rubber Sealing Washer - blank

Common Accessories

- ❑ SKPM 1420 Volume Reducer Kit - comprises two pieces, 1 cylindrical sections to reduce vessel diameter and 1 thick discs to reduce vessel depth. They can be used separately or in combination as dictated by the type of plant material to be tested
- ❑ SKPM 1430 Portable Gas Cylinder - usually 12.2 litres .
- ❑ SKPM 1435 Portable Gas Cylinder 3 litres
- ❑ SKPM 1437 Regulator - fits both the above cylinders and has an outlet pressure of 40 bar
- ❑ .SKPM 1440 Flexible hose to connect PMS to $^{3}/_{8}$" BSP cone fitting. (Standard for commercial UK & some overseas regulators
- ❑ SKPM 1470 Spare 'O' Rings - pack of five rings
- ❑ SKPM 1480 Alkaline Battery - 9v non recharge able *(digital models only)*
- ❑ SKPM 1485 Recharge able Battery - 9v Nickel Cadmium *(digital models only)*
- ❑ SKPM 1490 Recharger for above - state whether 110v or 240v *(digital models only)*

IX Instructions for Accessories

PORTABLE PRESSURE BOTTLES.

These are always supplied empty, and should not be sent through the post or by carrier when full.

There are two sizes available, the two sizes usually supplied are 2.0 and 9.2 litre. On occasions a 10.4 litre may be supplied as a replacement of a 9.2 litre.

This capacity refers to the volume of the bottle. The amount of air it contains will depend on the pressure used. The maximum filling pressure is 232 bar which is marked on the cylinder. (Fig. 1)

The bottle can be filled by a small compressor unit, or in any diving shop of outlet. These are found in almost all University towns and many coastal towns. Use only compressed air in these bottles.

All bottles supplied have been tested to a pressure much higher than 232 bar and will require periodic retesting, usually every 2 or 3 years, depending on the country it is filled in. It is the customers responsibility to ensure that this testing is carried out. Most diving shops are authorised to test bottles to nationally defined standards.

We supply only the best quality Aluminium bottles, that with care will last a lifetime. They will be damaged however by dropping or denting them. Also avoid damage to the pillar valve, do not lift the bottles by this or over tighten it when turning off.

Do not attempt to unscrew the pillar valve.

Fig. 1.

Bottle size.	2	9.2	10.4
No. of Chamber fills - empty chamber and filled to 40 bar. (Worst case!!)	21	98	111
No. of Chamber fills - half filled chamber OR filled to 20 bar.	42	196	222
3/4 filled chamber or to 10 bar pressure.	84	392	444