



XYL'EM

INSTRUCTION MANUAL

And

TUTORIAL FOR XYLEM EMBOLISM MEASUREMENTS

Version 1.1
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http://www.bronkhorst.fr/fr/produits/xylem_emboliemetre/

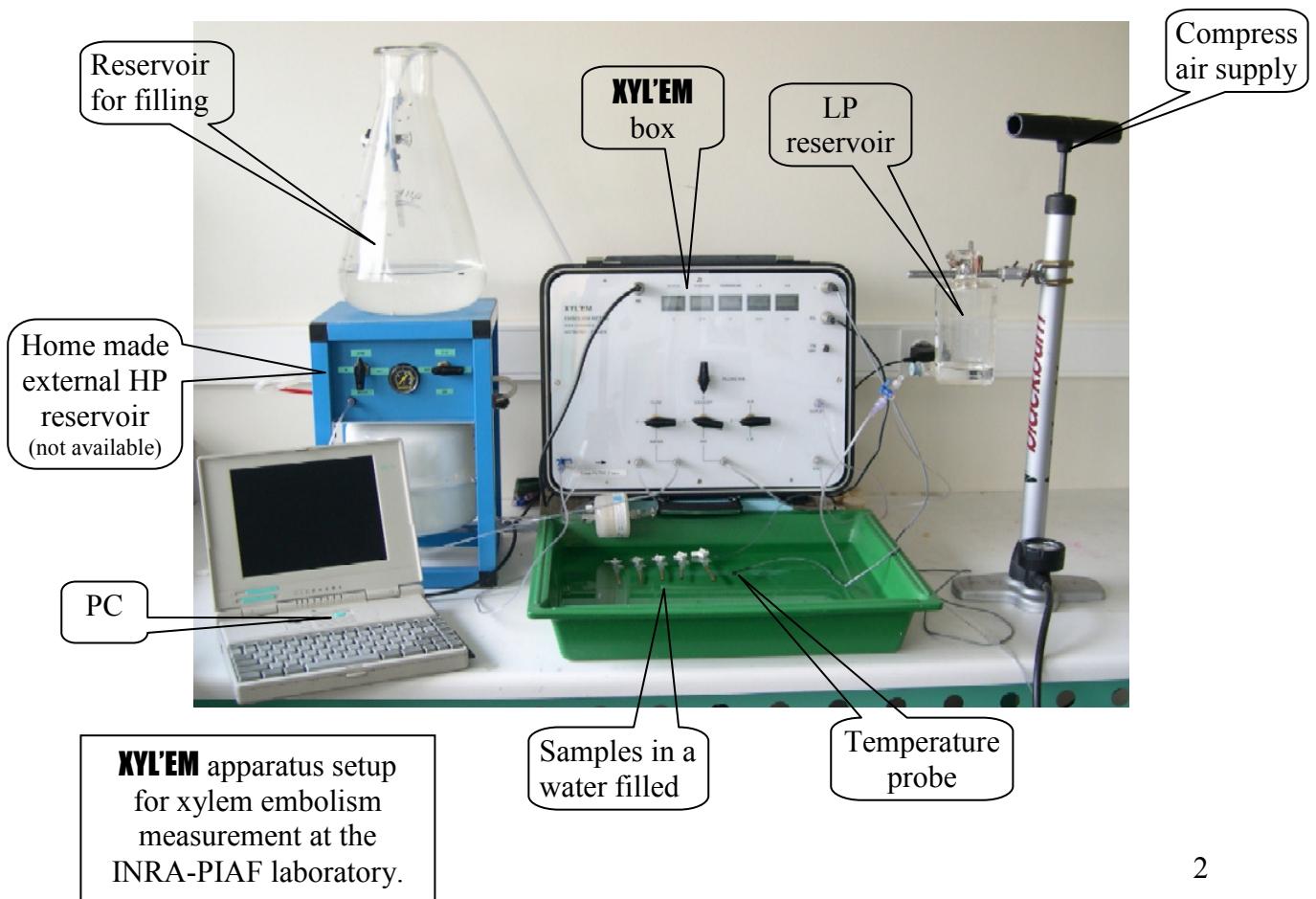
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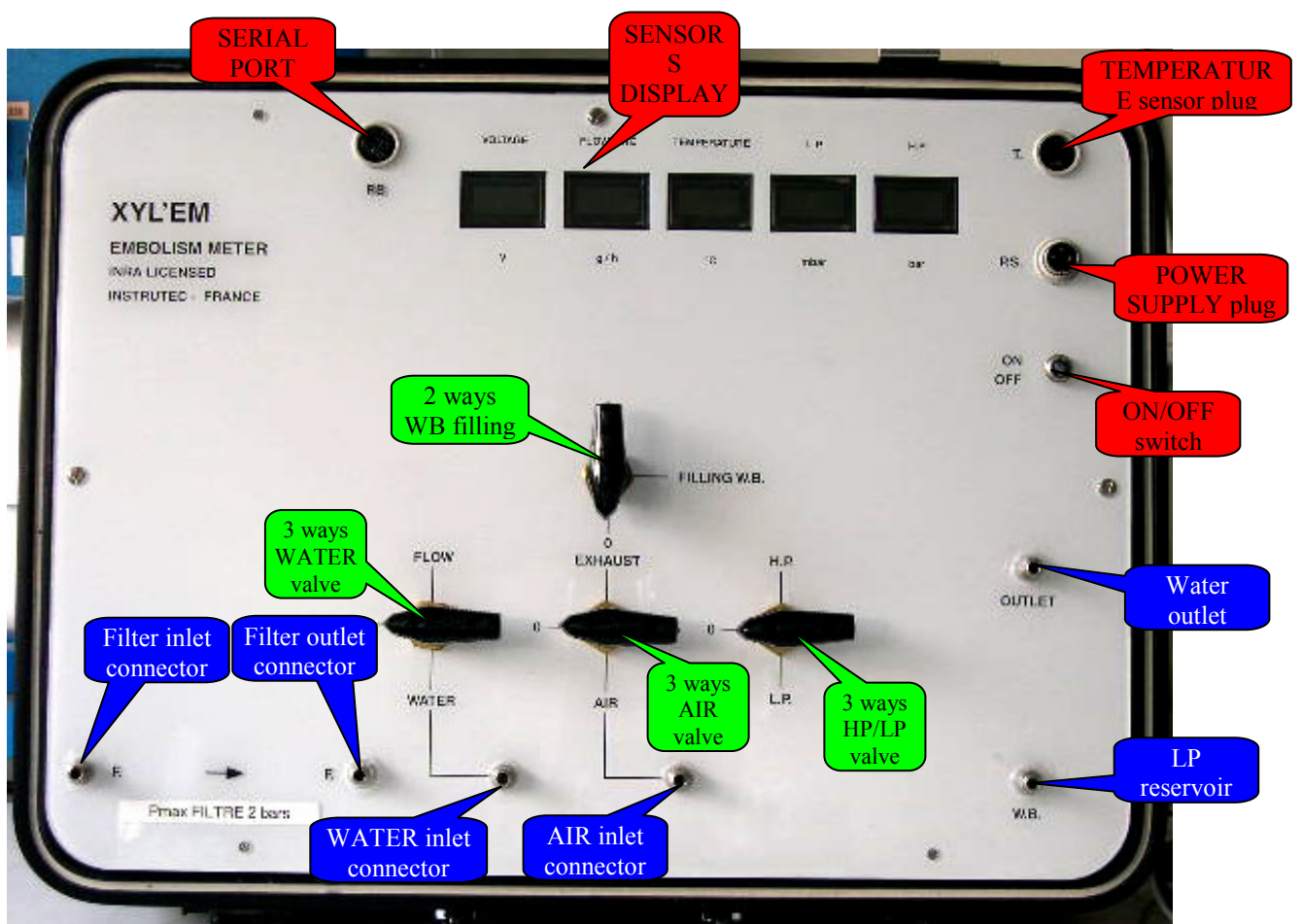
1. Introduction

We thank you for purchasing this **XYL'EM** apparatus, a system primarily designed for measuring the degree of xylem embolism in vascular plants. Embolism blocks the circulation of sap and decreases stem hydraulic conductance. Xylem embolism is now recognized as a major dysfunction that correlates with plant drought resistance and growth performance. The reference 'hydraulic' method was introduced by Sperry et al. in 1988 ⁽¹⁾. The method consists in estimating the hydraulic conductance of small segments (few cm), then re-saturating these samples by successive perfusions under pressure with degassed water. This perfusion evacuates or dissolves air contained in the embolised xylem vessels. The initial conductance/full saturated conductance ratio gives a quantitative value of embolism level. This technique is now wide-spread but remains laborious and delicate as it requires the use of a precision balance, and is almost unusable in the field.

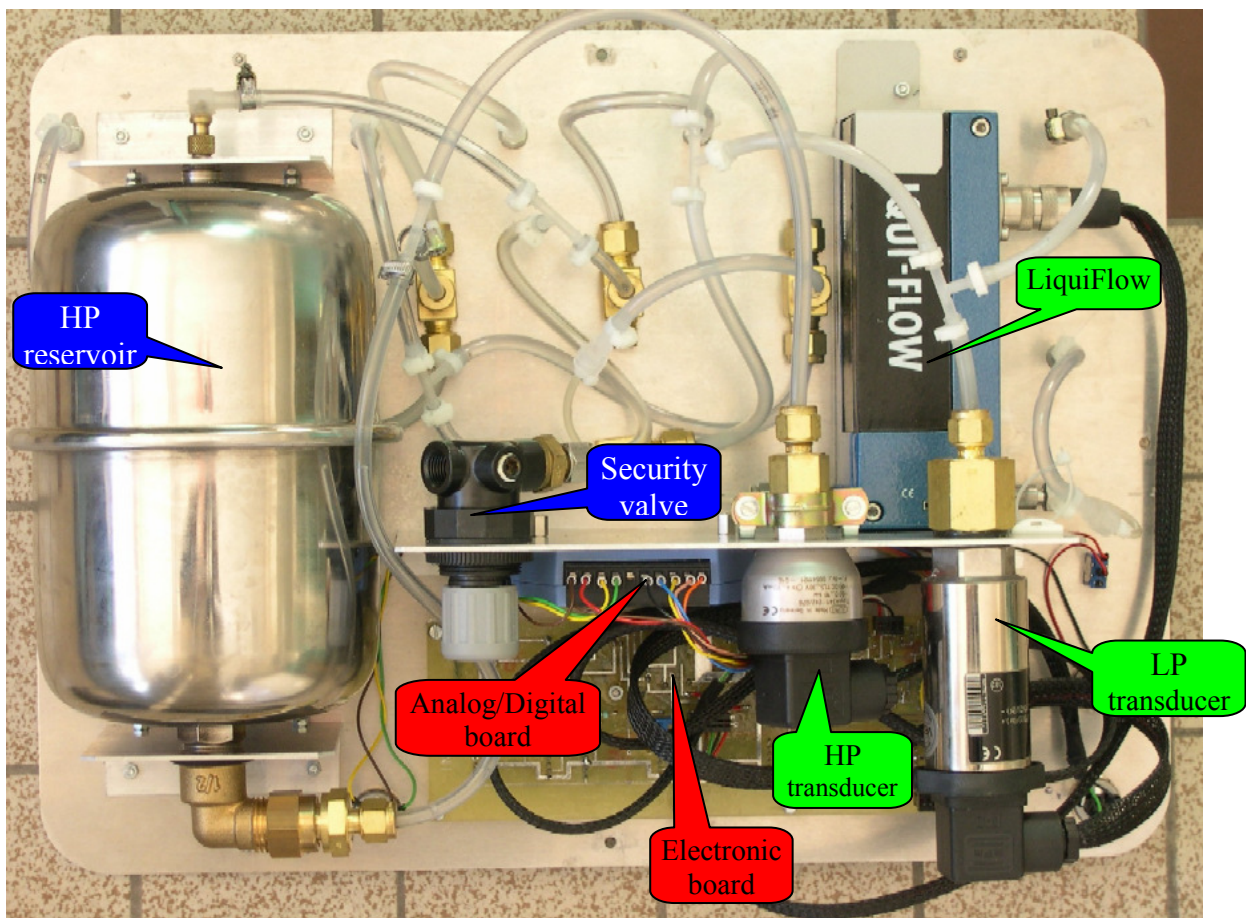
The **XYL'EM** system allows the determination of embolism amount without the need precision balance. Water flows are measured with a high precision liquid flowmeter (Liquiflow, Instrutec). The kit ruggedness allows intensive use in laboratory as well as in field. Simplicity of use makes embolism measurement easiest and fastest. The **XYL'EM** system was developed conjointly by the Instrutec Company, H Cochard and T Améglio from INRA-PIAF Laboratory at Clermont-Ferrand, France. This laboratory has more than 15 years of experience in xylem embolism measurements. Prototypes and commercial versions of the **XYL'EM** system have being tested during the past five years in this laboratory. Measurements of xylem embolism with the **XYL'EM** system have being published in a number of scientific papers (see Appendix II). In the Appendix I of this manual, H Cochard has shared his personal experience with xylem embolism studies. The Instrutec Company can not be taken responsible for this writing. However, we hope this appendix will be useful to people not yet familiar with this type of measurements.

PLEASE READ ENTIRELY THIS MANUAL BEFORE USING THE XYL'EM!

(1) Sperry J.S., Donnelly J.R., Tyree M.T., 1988 - A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell and Environment, 11, 35-40.

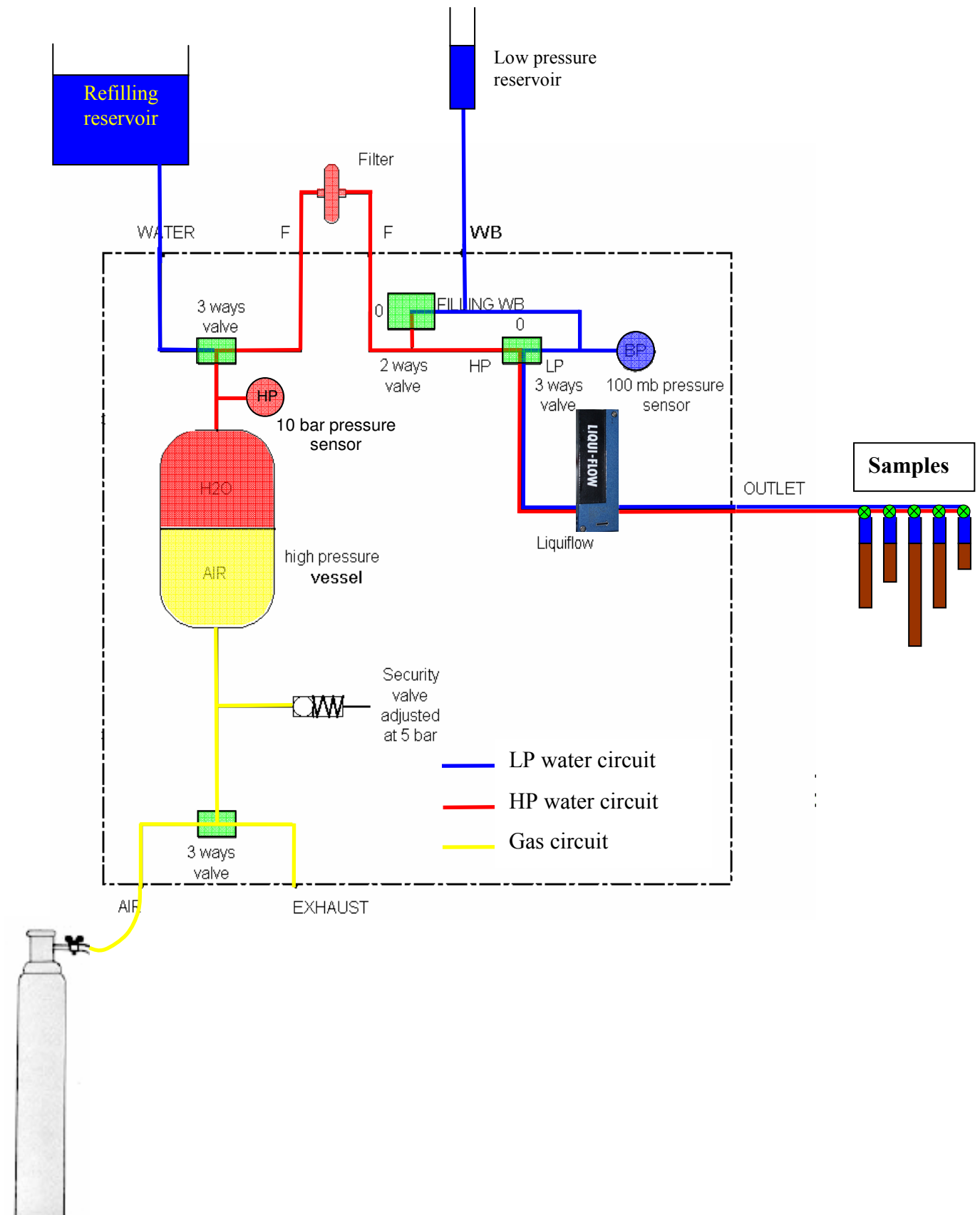


XYL'EM : front panel



XYL'EM : on the inside

XYL'EM fluid diagram



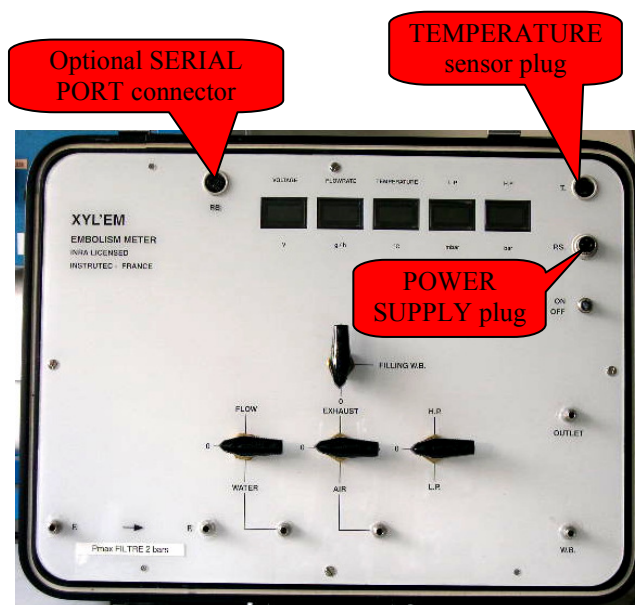
2. Set up for the **XYL'EM**

This section describes the procedure to setup the apparatus for a first use. The following order must be respected. After the initial installation, the user will normally proceed starting at step 3. The **XYL'EM** box normally lay on a horizontal position on a desk. A vertical position is not recommended.

2.1 Cables Connection

Procedure:

1. **Temperature probe.** Connect to Pt100 temperature probe to the **T** plug.
2. **Serial communication.** If the **XYL'EM** was purchased with the DATAXYL option, connect the cable to the **RS** plug on the apparatus and to the COM1 on the computer.
3. **Power supply.** Connect the power supply cable to the **PS** plug. A second cable provided for connection to an external 12V DC power supply battery (typically a car battery). The **blue** wire must be connected to +12V and the **brown** wire to the ground.



2.2 Water filter installation

Filters are used to prevent tiny particles and air bubbles from entering samples. Without these filters the samples rapidly plug and the data are no longer reliable. Five disposable filters are provided with each **XYL'EM** apparatus (maximum pressure 2 bars). Filters are connected to the Luer **F** plugs. We recommend changing the filters before each trial as they may rapidly plug and reduce the high pressure water supply.

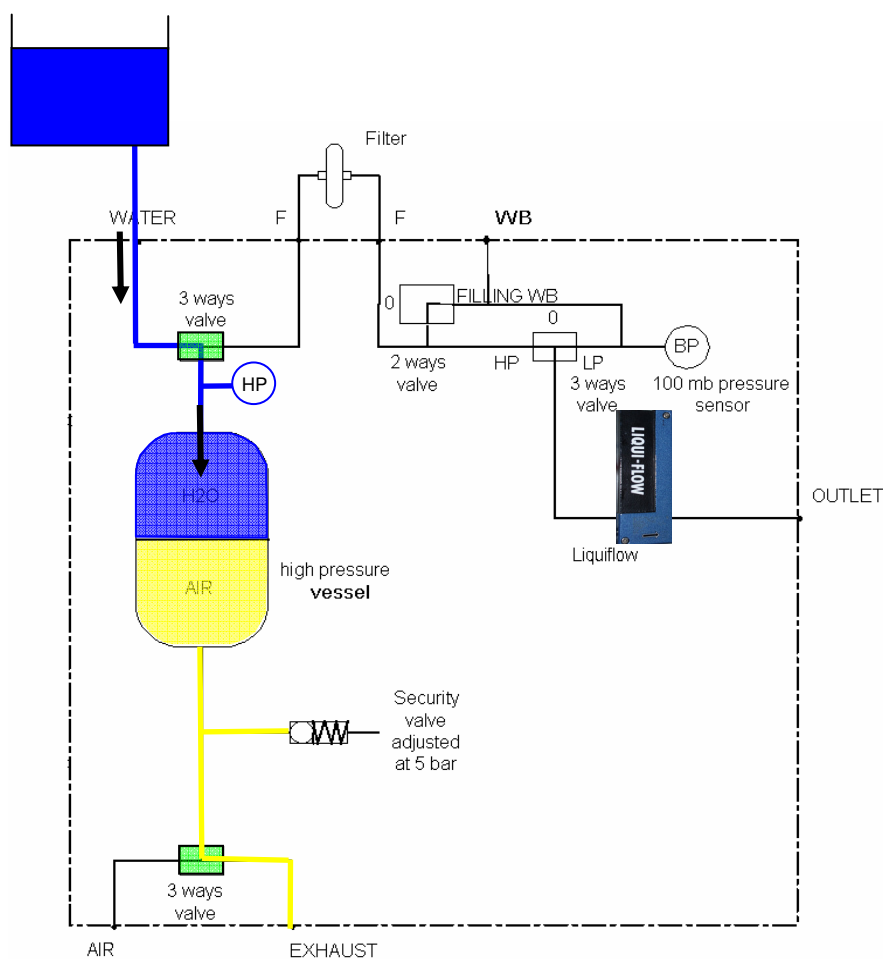
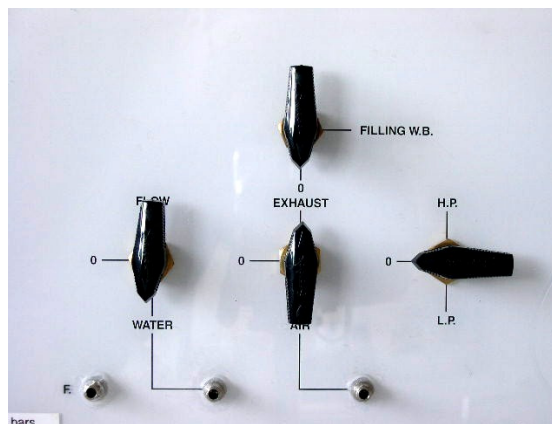
Alternatively, you can connect a disposable filter capsule with a very high surface of filtration. These filters are more expensive than the above disposable filters but they can last a whole season. Make sure to use filters for water filtration not for air filtration! Filter pore size must be 0.2 μm or smaller. Make sure that the maximum pressure the filter can hold is compatible with the pressure you use while operating the **XYL'EM** apparatus.

2.3 Filling the high pressure water reservoir

An HP reservoir is included inside the **XYLEM** box. This reservoir is used for filling the low pressure reservoir, for « flushing » the samples and to measure sample hydraulic conductance in the high pressure mode. The reservoir is a stainless steel captive air tank. A flexible membrane divides the tank in two compartments, one for compressed air, and the other for water. Distilled or deionised ultra pure water **MUST** be used. In no case tap water can be employed (tap water may contain clay particles that will pass through the filter but plug the samples). It is recommended to degas water before use. Water is degassed by running a vacuum pump and active stirring. It is also recommended to add 10mmol of KCl in the water.

Procedure:

1. Connect the reservoir with distilled degas water to Luer Water plug of the **XYLEM** box with a Luer flexible tube.
2. Set the 3 way AIR valve to **EXHAUST** to release the air pressure inside the HP reservoir and allow air exit upon refilling.
3. Set the 3 way WATER valve to **WATER**, and set the 2 remaining valves to the **0**. The pressure head between the water reservoir and the **XYLEM** must be around 1m.
4. Wait until no water flow into the HP reservoir (about 0.5 litres if the reservoir was empty)
5. When the HP reservoir is filled, set all the valves to their **0** position.



2.4 Pressurizing the HP reservoir with compressed gas

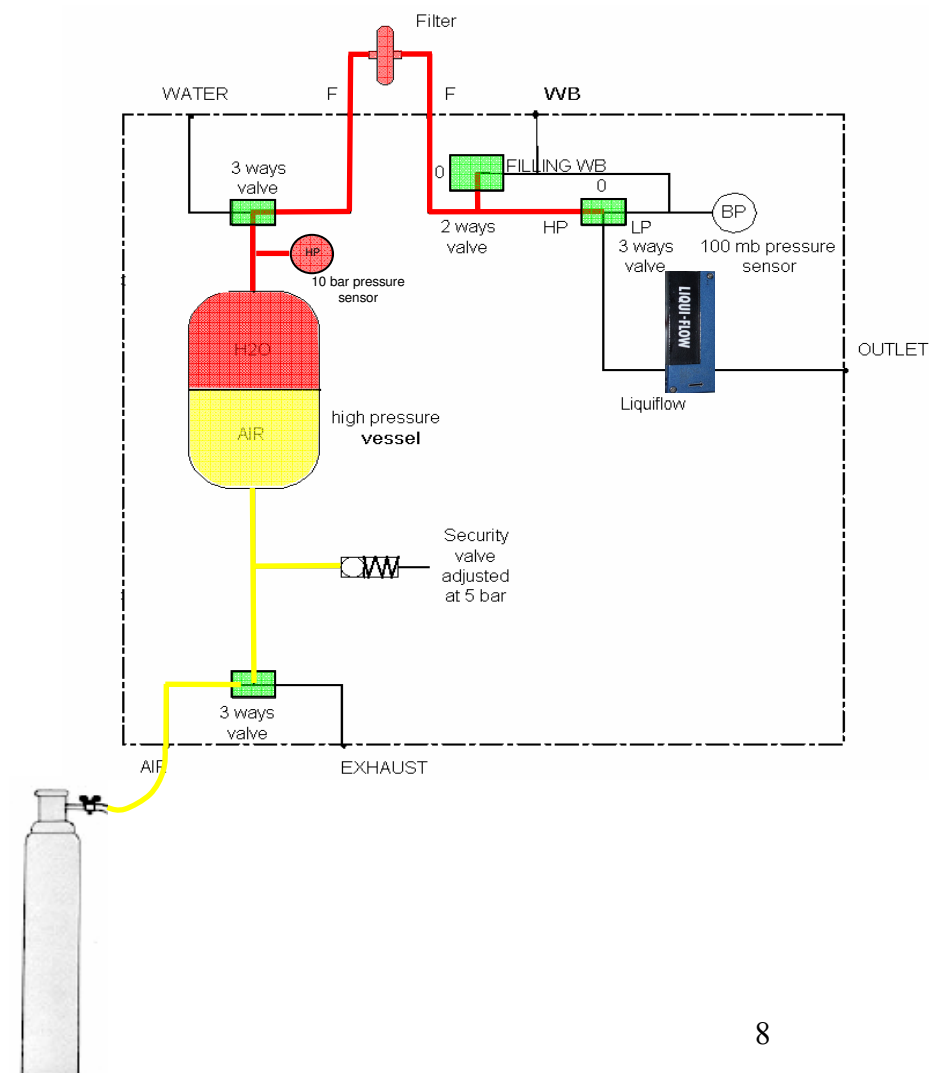
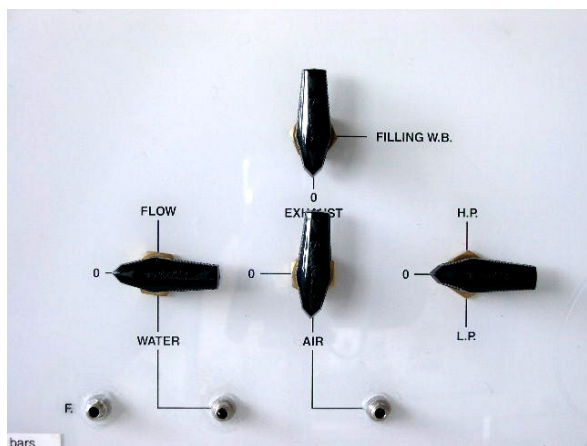
The HP reservoir must be pressurized only if it contains water. As the HP pressure transducer is connected to the water circuit, it will record correct values only if the HP reservoir contains water. Incidentally, if the HP pressure does not rise upon pressurization, this indicates an empty HP reservoir. Similarly, a sudden drop of HP pressure during measurement indicates an empty HP reservoir. The HP tank can be compressed with any non-flammable gas. Air, nitrogen or CO₂ are the most indicated. Any supply delivering at least 2 bars (0.2 MPa, 30 PSI) is suitable (compress tank, electrical or manual pumps etc...).

Procedure:

1. Connect the pressurize air supply to the AIR Luer plug;
2. Set the 3 ways AIR valve to **AIR**.
3. Control the increase in pressure with the HP numerical indicator. Values are indicated in **bar**. (1 bar = 0.1 MPa = 14.5 PSI).
4. Once the desired pressure is obtained (typically 1 or 2 bar), set the AIR valve to **0**. The **XYLEM** includes a security valve adjusted at 5 bars to prevent accidental over pressurizations.

It is recommended to purge slightly the HP reservoir before proceeding further (see chapter IV *Purge of the HP reservoir*). This will remove air trapped in the water compartment of the HP tank. It might be useful to position the **XYLEM** box vertically to facilitate bubbles expulsion.

It is possible to connect an external captive air tank with higher water content (not available from INSTRUTECH) to the **xylem** box. To do so, insert a 3 ways Luer valve between the filter and the filter inlet Luer plug on the box and connect the external tank to the free way of the valve. If the external tank is equipped with its own filter the **XYLEM** filter can be by-passed. Make sure that the 3 ways Water valve is set to FLOW unless the HP sensor will not record the high pressure properly.

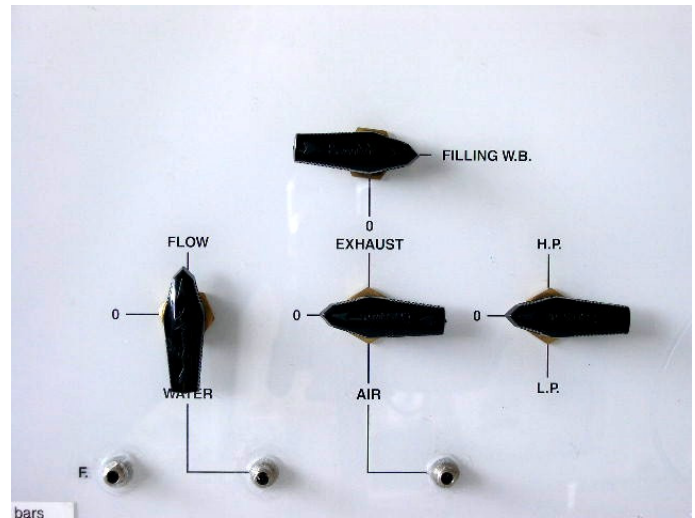


2.5 Filling the LP reservoir for measurements in the LP mode.

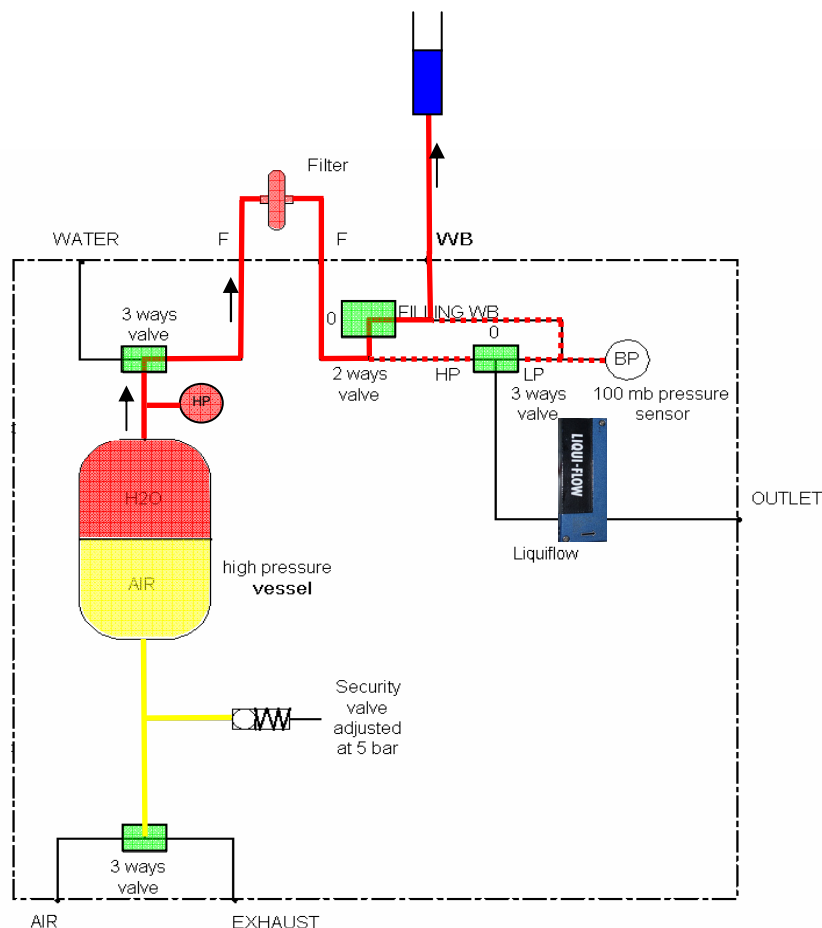
A low pressure reservoir (LP) is provided for measurements in the low pressure mode. The reservoir is filled with the water contained in the HP reservoir. This will ensure that the water in the LP reservoir has been properly degassed and filtered. Any water in the LP reservoir remaining from a previous experiment **MUST** be discarded (possible pollution by bacterial or fungal growth). Make sure that the LP reservoir has a free air event so that its pressure remains atmospheric during measurement (if not the LP values recorded by the LP pressure transducer will be false). A 100ml disposable syringe is appropriate.

Procedure:

1. Connect the LP reservoir to the **WB** Luer plug on the **XYL'EM** box with a flexible Luer tube.
2. Set the 3 ways WATER valve on **FLOW**.
3. Set the 2 ways valve to **FILLING WB**.
4. When the LP reservoir is filled, set the 2 ways valve and the 3 ways WATER valve to their **0** position.



The **XYL'EM** apparatus is now prepared for measurements in LP or HP modes

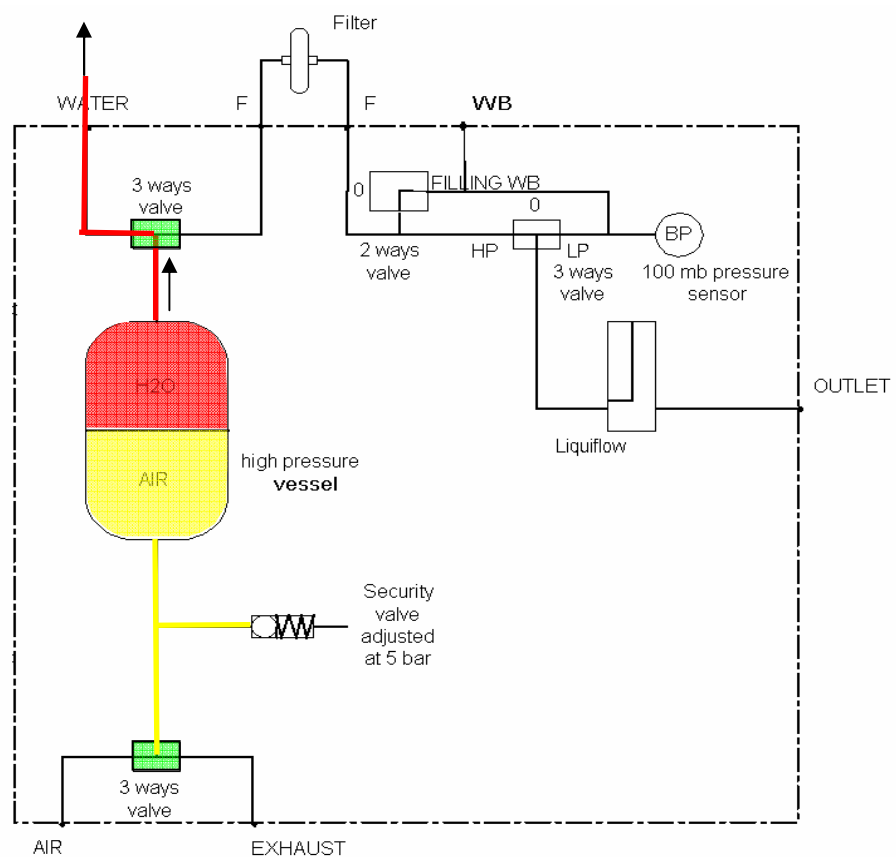
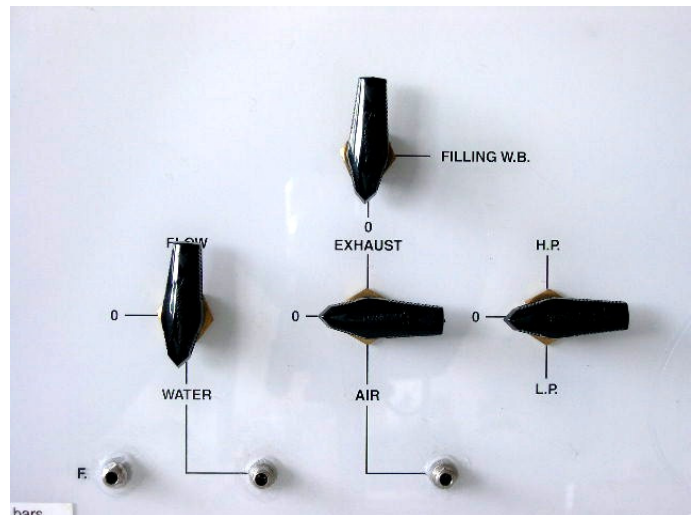


2.6 Purging the HP water reservoir

To prevent bacterial or fungal growth in the HP reservoir, we recommend emptying this reservoir between to sets of measurements. The tank is emptied through its filling connector. Emptying it through the sample outlet connector will use unnecessarily the filter.

Procedure:

1. Start with all the valves at their **0** position.
2. Make sure that the air pressure in the HP reservoir is high enough (~ 2 bar) as indicated by the HP indicator.
3. Connect a Luer tube to the Luer WATER outlet connector. Place the free end of the tube in a sink or a bucket.
4. Set the 3 ways WATER valve to **WATER**.
5. Wait until water flow through the tube stops. If the 3 ways WATER valve is set to **FLOW** the HP indicator must now read 0 bar.
6. Release the air pressure in the HP tank by setting the 3 ways AIR valve to **EXHAUST**.

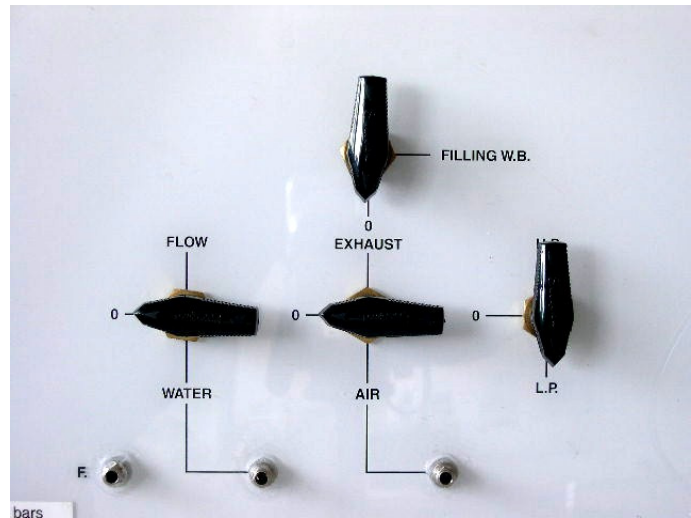


3. Operating the **XYL'EM** in the low pressure (LP) mode

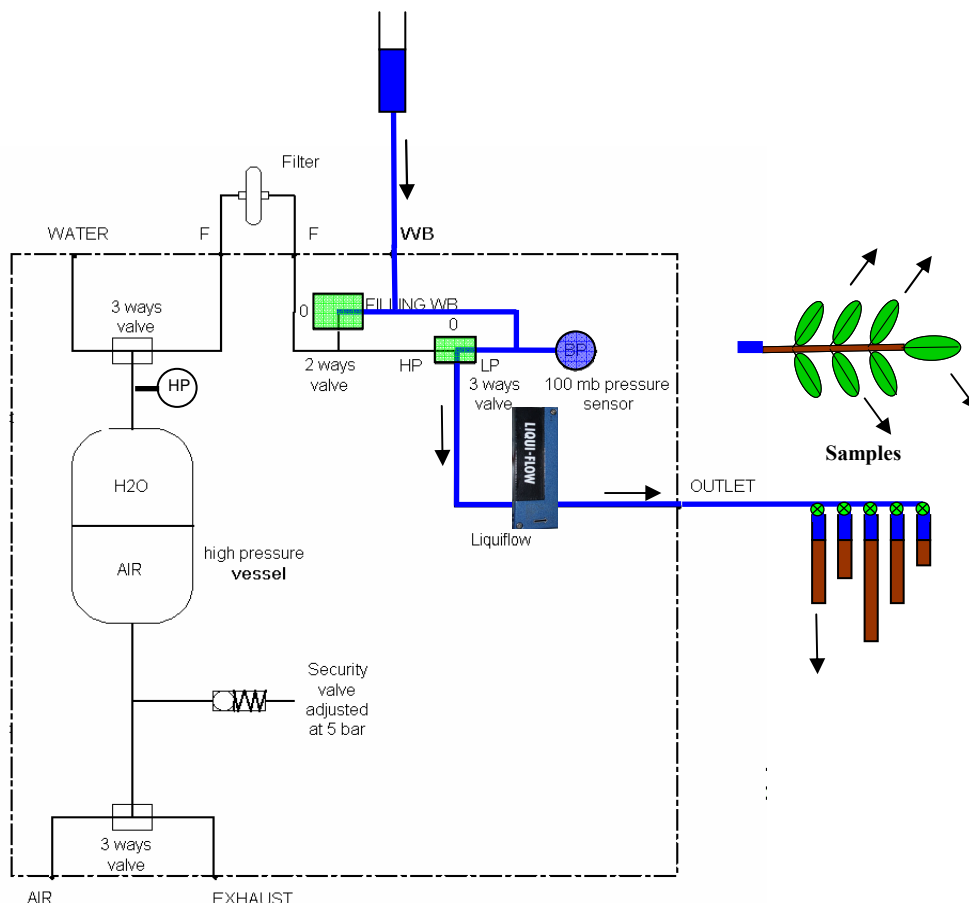
The LP mode is typically used for measuring losses of xylem hydraulic conductance caused by embolism in plant xylem segments. As will be discussed at length in Appendix II, it is essential to measure the initial xylem hydraulic conductance with a very low pressure (typically less than 6kPa or 60 cm H₂O). If the samples are exposed to higher pressures the embolism is removed.

Procedure:

1. The LP reservoir must be connected to the **WB** Luer connector and placed at the desired level above the **XYL'EM** box.
2. Set to HP/BP 3 ways valve to **BP**, all the other valves being set to their **0** position.
3. Connect the sample to the **OUTLET** Luer plug with a Luer tube.
4. The **XYL'EM** apparatus will measure the flow through the sample at the preset LP pressure.



If the flow exceeds the full scale range of the Liquiflow, lower the LP reservoir. Alternatively you may work with longer xylem samples.



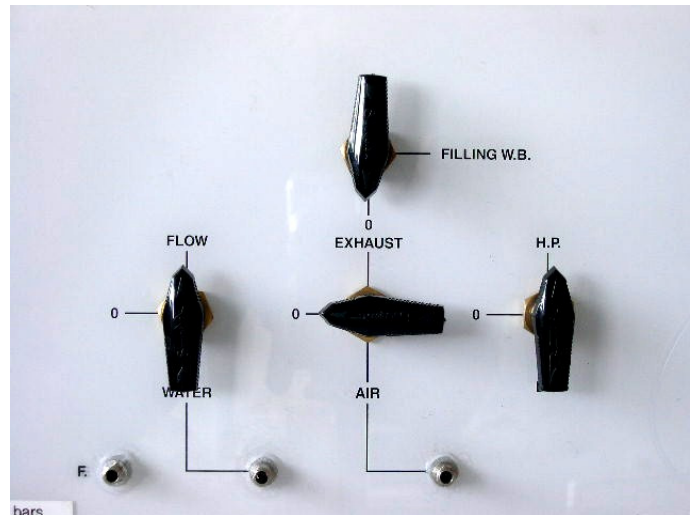
You may also connect a transpiring leafy shoot to the **OUTLET** of the **XYL'EM** box in the LP mode. The **XYL'EM** will measure the water flow entering the shoot, a proxy of the water loss by leaf evaporation.

4. Operating the **XYL'EM** in the high pressure (HP) mode

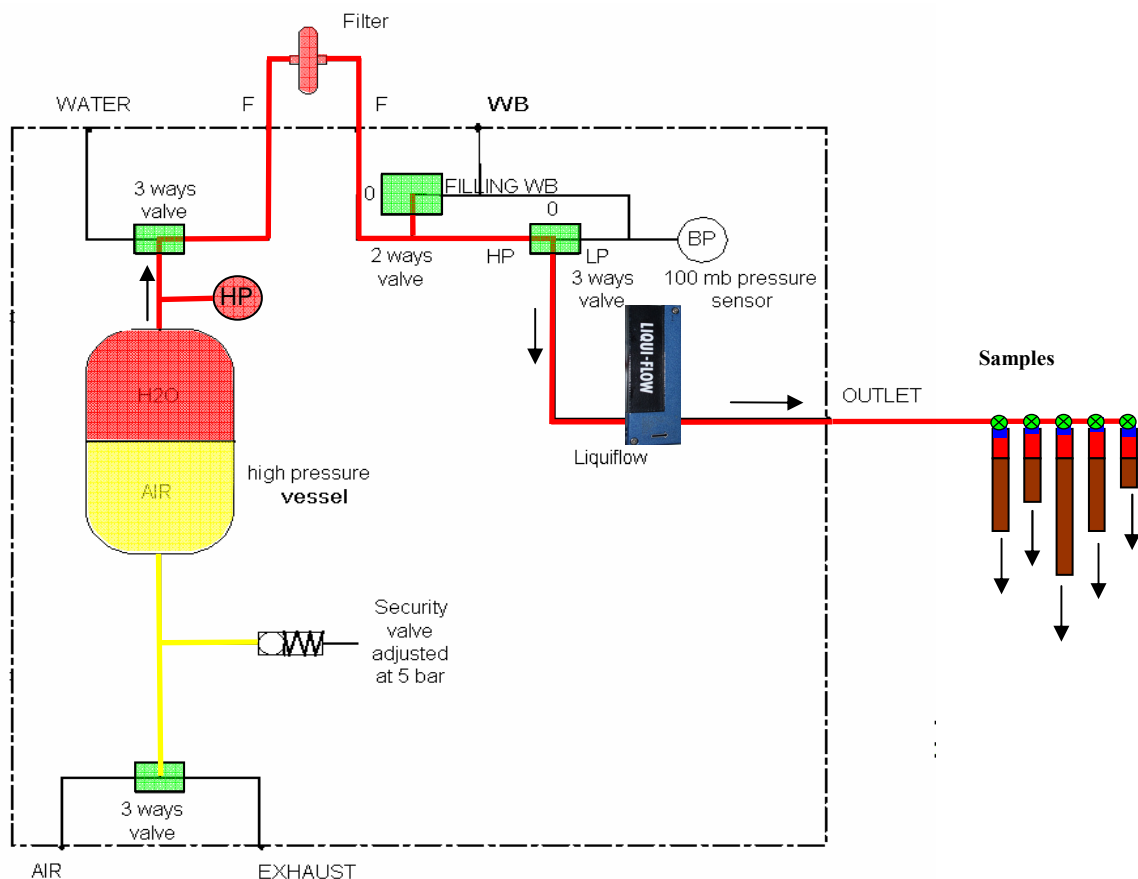
The HP mode is typically used to resaturate (“flush”) samples after initial measurement in the LP mode (procedure 1). It can also be employed to determine the hydraulic conductance of highly resistive plant material such as leaf blades (procedure 1&2).

Procedure 1:

1. Connect the sample to the **OUTLET** Luer plug with a Luer tube.
2. Set the 3 ways HP/LP valve to **HP**.
3. Set the 3 ways WATER valve to the **FLOW** position
4. All the other valves must be at their **0** position.
5. The **XYL'EM** apparatus will measure the flow F through the sample at the preset HP pressure. F/HP is the conductance of the sample.



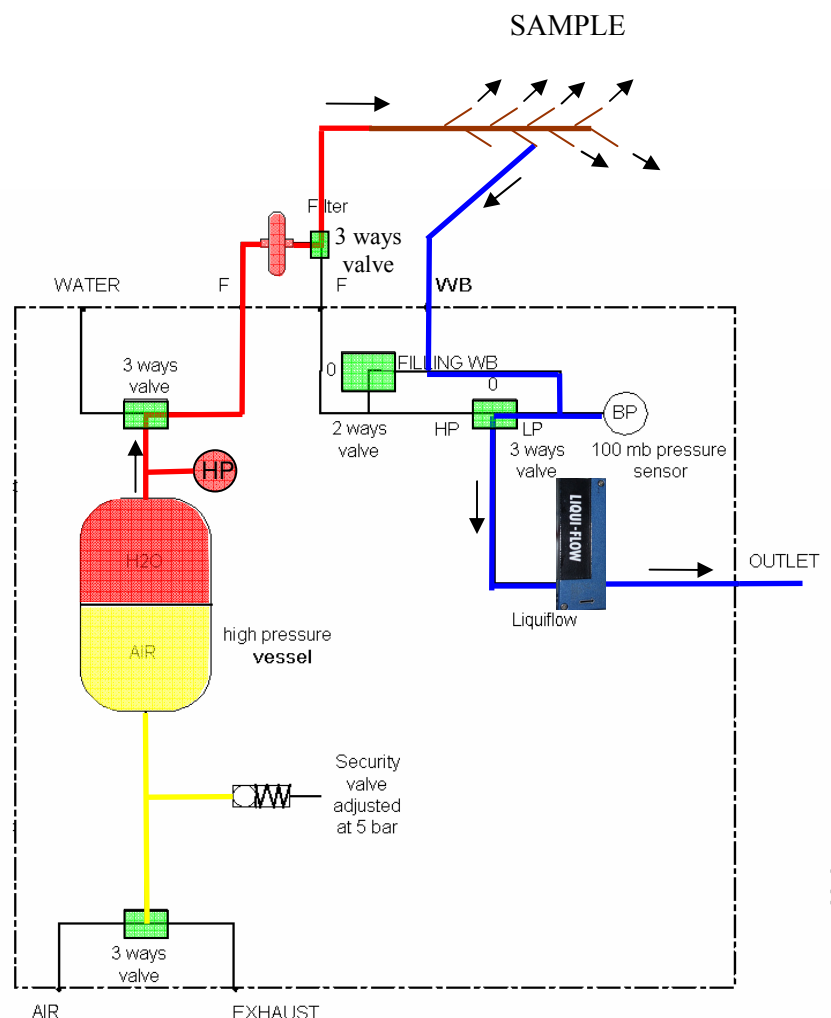
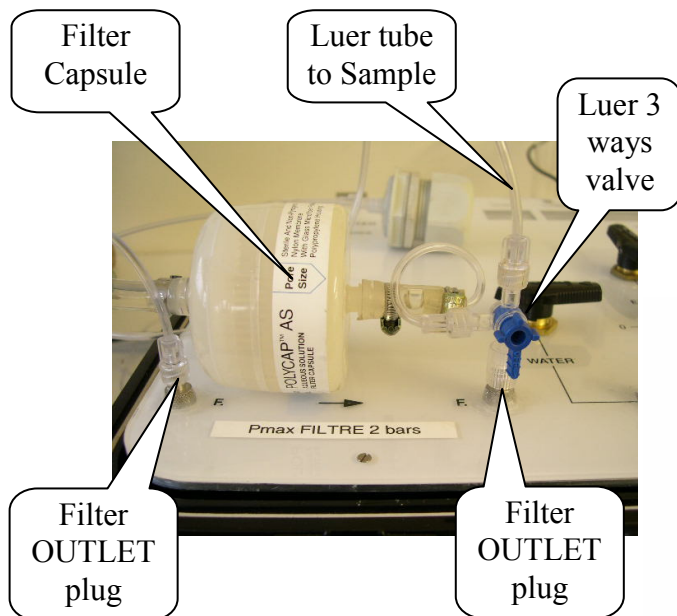
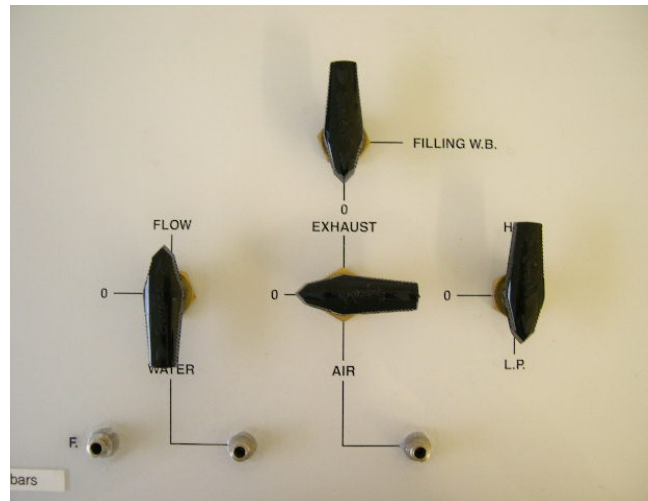
Note that if the xylem conductance is high, flows in the HP mode may exceed the full scale range of the LiquiFlow.



Procedure 2

The second procedure can be useful for measuring the integrated conductance of the sap pathway from a branch base to the petiole of a particular leaf for instance. The base of the branch is connected to the HP tank through a 3 ways Luer valve at the filter outlet. The HP circuit bypasses the Liquiflow meter.

1. Connect the proximal end of the sample (ie base of the branch) to the 3 ways Luer valve at the outlet of the filter with a Luer tube.
2. Set the 3 ways WATER valve to **FLOW**.
3. Connect the distal end of the segment (ie a cut petiole) to the LP reservoir connector (**WB**) with a Luer tube.
4. Set the 3 ways HP/LP valve to the **LP**.
5. All the other valves must be at their **0** position.
6. The **XYLEM** apparatus will measure the flow **F** coming out of the distal end at the preset HP pressure applied at the proximal end. F/HP is the conductance of the sap pathway between the two branch ends.
7. You can then repeat the measurement with another distal end.



5. **KYL'EM** Applications and Theory of Operation

The **KYL'EM** apparatus is designed for measuring hydraulic conductance xylem of plant parts. The pressurization system included in the apparatus permit resaturation of air-filled (embolised) xylem conduits. Thus the system enables also the measure of the percentage of loss of conductance (PLC) due to air blockage.

The **hydraulic conductance** of a xylem segment (K) is defined as the water mass flow (F) per hydrostatic pressure drop ($P_{in}-P_{out}$) across the sample.

$$K = \frac{F}{P_{in} - P_{out}}$$

Units

The pressure unit is in MPa (1MPa = 10bars = 145PSI). The Liquiflow sensor is calibrated in g h⁻¹ and flows are displayed with this unit on the **KYL'EM** box LCD panel. However, the SI standard for mass flow is either in mmol s⁻¹ or in Kg s⁻¹. The two standards are currently used, although for consistence with the units for vapor water flow in plants, mmol s⁻¹ is recommendable. The user can select one of the two units. K is then expressed in mmol s⁻¹MPa⁻¹ or Kg s⁻¹MPa⁻¹. Use the following table to convert units:

mmol s ⁻¹	Kg s ⁻¹	g h ⁻¹
1	1.80E-05	64.8

If the length (L, m) of the xylem segment is known, **hydraulic conductivity** can be computed as: K * L. The unit is in mmol m s⁻¹MPa⁻¹. If leaf area (LA, m²) distal to the segment is measured, the leaf area specific hydraulic conductance (or **LSC**) is computed as: K * L / LA. The unit is in mmol m⁻¹ s⁻¹MPa⁻¹.

Temperature compensation

Hydraulic conductance values are temperature dependent because water viscosity change with temperature. The effect being substantial (about 2.4% per °C), the **KYL'EM** apparatus is equipped with a PT100 temperature probe to account for temperature variations. We recommend installing the samples in a water filled container and to measure the temperature of the water in the container. The software corrects K values for a reference temperature of 20 °C with the following empirical formula, T being the temperature in °C.

$$K_{20^{\circ}\text{C}} = K_T \times 3.4939 \times \frac{9.3252 + \sqrt{54.2176 - T}}{T + 32.7425}$$

The formula above was computed to fit viscosity values for **laboratory conditions** (15°C<T<45°C). For more extreme temperature conditions, substantial deviations from the true

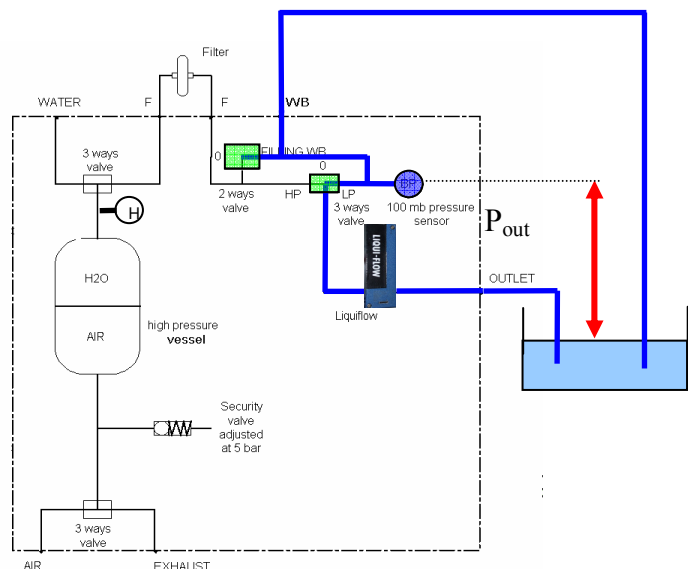
viscosity values might exist. If for some reason sample temperature cannot be measured by the temperature probe (sample out of reach, broken probe...) the temperature can be entered **manually** in the software. As the viscosity of water at 20°C is equal to 1, setting manually the sample temperature to 20°C will result in non temperature-compensated K values.

Pressure drop compensation

According to the above formula, the **pressure drop** across the xylem sample must be known to compute K. The **XYLEM** apparatus measure **only** the pressure at entrance of the sample (P_{in}). However, it is possible to measure P_{out} at the onset of a trial, to memorize P_{out} and to subtract P_{out} to P_{in} during measurements.

The procedure for measuring P_{out} is the following:

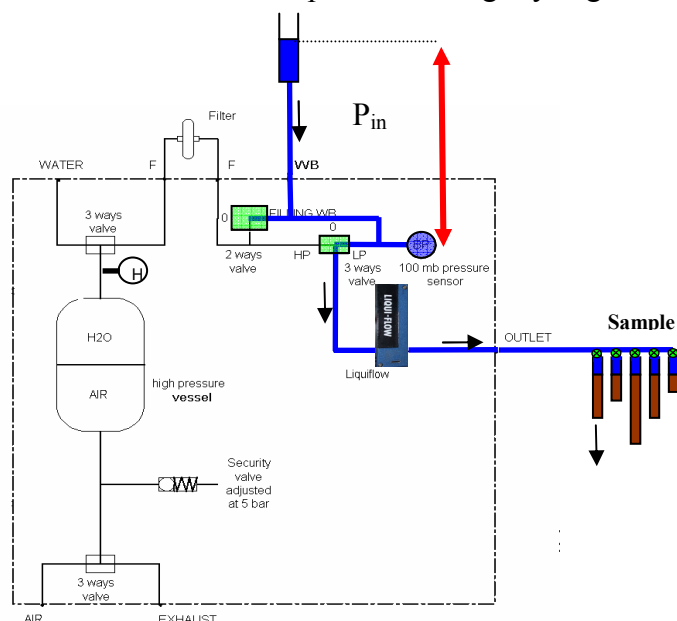
1. Position the container that will receive the samples at its final place with the desired amount of water.
2. Connect a Luer tube to the LP reservoir connector “WB”, and immerse the free end in the container.
3. Connect a Luer tube to the sample “outlet” connector and immersed its free end in the container.
4. Fill the tubes with water (see §2.5 *Filling the LP reservoir* and § 4. *Operating the XYLEM in the HP mode* for the procedures).
5. Set the valves to the LP mode (see below § 3. *Operating the XYLEM in the LP mode* for details).
6. The LP sensor now reads P_{out} , the pressure difference between the sensor head and the water level in the container.
7. Note or memorize this value in the software. The value can be positive or slightly negative.



During measurements in the LP mode (cf §3), the LP sensor reads P_{in} the difference the water level in the LP reservoir and the pressure head. The software subtracts P_{out} to P_{in} to obtain the desired pressure difference between the water levels in the LP reservoir and the sample container.

The vertical position of sample container must not be changed during a trial and its water level must remain constant. In the contrary repeat the procedure above. The position of the LP reservoir can be changed at will.

The relatively high P_{in} values used in the HP mode make the above correction insignificant.



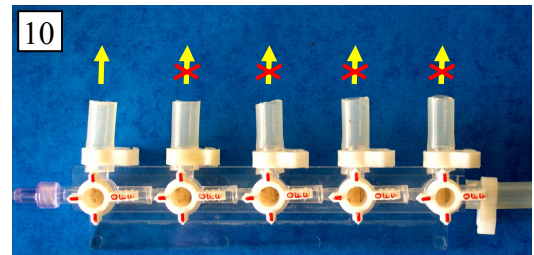
PLC measurements

A typical trial for PLC measurement of xylem segments is the following:

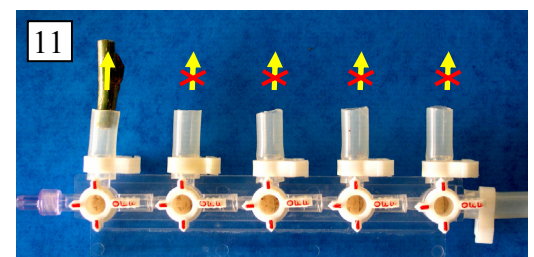
1. Purge the HP reservoir and discard its content (see §2.6 for the procedure)
2. While the HP reservoir is still pressurized (1-2 bars) and the WATER valve set to **WATER**, immerge the Luer tube connected to the WATER plug into the new distilled and degassed solution.
3. Release the HP air pressure by setting the AIR valve to **EXHAUST**. You are now filling the HP reservoir (cf § 2.3).
4. Set the WATER valve to **0** and pressurize the HP reservoir to 1 or 2 bars (cf § 2.4)
5. Set the WATER valve shortly to **WATER** to remove air bubbles (if any) from the HP reservoir.
6. Purge the LP reservoir and fill it with new water (cf § 2.5). It might be useful to rinse several times the LP reservoir with clean water to remove possible particles.
7. “Flush” all the tubes of the sample manifold to remove their vitiated content (cf § 4).
8. Fill the sample container with water and measure P_{out} (see above).
9. Set the **XYLEM** to the LP mode (cf § 3). You are now ready to install samples and measure their PLC values.

Always install samples in the LP measuring mode!

10. Immerge the sample manifold in the container and set all the 3 ways valve of the sample manifold as indicated in the figure on the right. Water must be flowing from the LP reservoir through the first tube of the manifold. Remove air bubbles trapped in the tube.

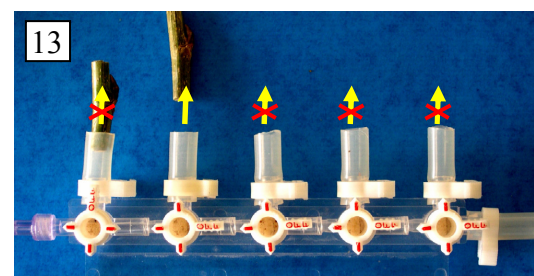


11. Insert **very gently** the first sample in the first tube. If you see bubbles coming out of the cut end then you have removed the embolism before measuring it! If needed, tighten the seal with a collar.

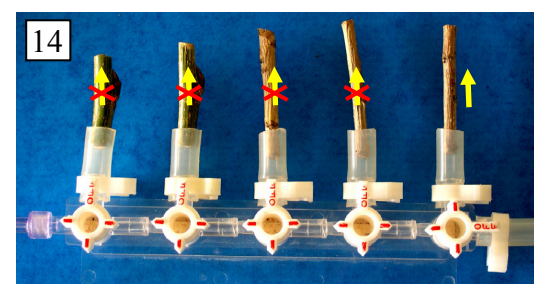


12. Measure the initial hydraulic conductance (K) of the first sample **immediately**. (see § 6 for software operation)

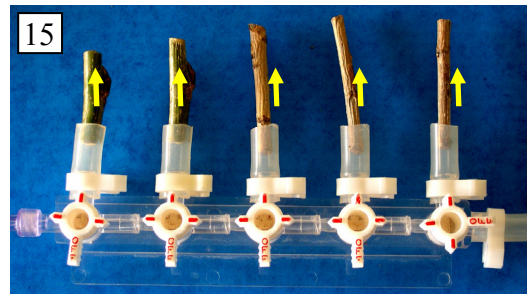
13. Move the first 3 ways valve of the manifold as indicated on the picture and install the second sample in the second tube as above. You will now measure the initial conductance of the second sample.



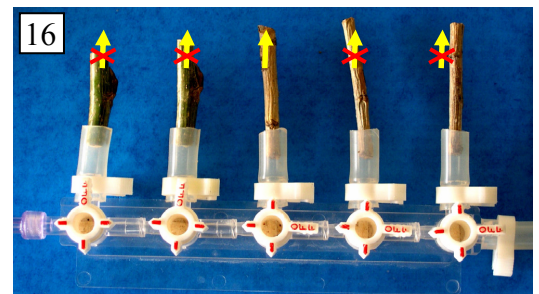
14. Repeat the procedure above for all the samples. The initial conductance of sample #5 is being measured on the right picture.



15. Open all the 3 ways valves of the manifold (but the last one!) and set the **XYL'EM** to the HP mode. You are now re-saturating “flushing” the samples and removing air bubbles from the xylem conduits of all the samples.



16. Set the XYL'EM apparatus to the LP mode and measure sequentially the saturated conductance of each sample as above. Sample #3 is measured on the right picture.



17. “Flush” all the samples for a second time (step 15 above) and repeat the whole procedure until sample conductance no longer increase. You have now determined the saturated hydraulic conductance of each sample (K'). You can compute the percentage loss of hydraulic conductance (PLC) as:

$$PLC = 100(1 - K'/K)$$

For a discussion about possible problems with this technique see Appendix 1.

18. Remove all the samples, measure their length and their diameter if needed. If your trial is finished, empty the HP and LP reservoirs, close all the valves of the **XYL'EM** (0 position) and release the HP air pressure. If you are going to measure new samples go back to step 10 above.

6. Installation and Operation of the **XYL'EM** software

The **XYL'EM** apparatus, when purchased with the **DATAXYL** option, is equipped with a analog/logical board and a RS232 interface. Three softwares are provided to operate the apparatus:

1. **XYL'EM.xls** : a Microsoft Excel spreadsheet that computes conductance and PLC values from flow, pressure and temperature data read on the **XYL'EM** display panel. This spreadsheet will be useful for operating the **XYL'EM** without the **DATAXYL** option or in case of computer failure.
2. **XYL_WIN** is a dedicated software with a graphic interface that will operate on a PC with Windows 95 or higher operating systems. The software logs the data from the **XYL'EM**, computes conductance and PLC values and displays graphics.
3. **XYL_DOS** is a dedicated software that works only in a DOS environment. It will also work on a DOS window in a Window environment. **XYL_DOS** has only a text interface but will run fine on a very old 80x 86 machines! **XYL_DOS** was developed to work on DOS based palmtop computers such as HP100LX.

6.1 Program Installation

- Insert the CD in your PC
- Run « SETUP.EXE » in the directory INSTALL XYLEM
- Wait until installation is completed.

The setup program will install **XYL'EM.xls**, **XYL_DOS.exe** and **XYL_WIN.exe** in the directory you have selected.

6.2 XYL'EM.xls

XYL'EM.xls is a regular and simple Microsoft Excel spreadsheet. A version of this software must be installed on your computer to run **XYL'EM.xls**! When you open the spreadsheet, the tables below will appear blank. Enter values only in the white cells. The blue and green cells contain formulas that will be erased or modified if you enter values into them.

Enter first the P_{out} value in mb as read on the LCD display panel. Install the first sample, and, when the flow is steady, enter the Flow (g/h), LP (mb) and T (°C) values in the corresponding cells of the first line of the "Initial Measurement" table. The temperature corrected conductance is calculated in the blue cell. Repeat for all the samples and perform the first flush. The data for the first flush will be recorded on the "First Flush" table as above. The PLC values appear in the green cells for each sample. Repeat for the second flush. The maximum PLC values for the two flushes are displayed on the PLC Max table with its statistics (mean and standard deviation).

XYL'EM datasheet for conductance and PLC measurements

Initial Measurement				
	Flow g/h	P _{in} mb	Temp °C	Conductance mmol/s/MPa
1	2	30	20	10.0084193
2	1	30	20	5.00420966
3	1.5	30	20.1	7.48788619
4	4	30	20.1	19.9676965
5	6	30	20.1	29.9515448
6	7	30	20.1	34.9434689
7	2	30	20.2	9.95936177
8	8	30	20.2	39.8374471
9	3.7	30	20.2	18.4248193
10	1.3	30	20.2	6.47358515

First flush					
	Flow g/h	P _{in} mb	Temp °C	Conductance mmol/s/MPa	PLC %
1	4	30	20	20.0168386	50
2	1.5	30	20.1	7.48788619	33.169
3	1.6	30	20.1	7.9870786	6.25
4	5	30	20.1	24.9596206	20
5	6.1	30	20.1	30.4507372	1.6393
6	7.5	30	20.1	37.4394309	6.6667
7	4.6	30	20.2	22.9065321	56.522
8	9	30	20.2	44.8171279	11.111
9	4	30	20.2	19.9187235	7.5
10	6	30	20.2	29.8780853	78.333

PLC max %	
1	66.6667
2	33.1693
3	6.25
4	42.8571
5	14.2857
6	22.2222
7	60
8	20
9	26
10	78.3333

P _{out} mb
-1

PLC	
avg	36.9784341
std	24.1670473

Second flush					
	Flow g/h	P _{in} mb	Temp °C	Conductance mmol/s/MPa	PLC %
1	6	30	20	30.025258	66.667
2	1.5	30	20.1	7.48788619	33.169
3	1.5	30	20.1	7.48788619	0
4	7	30	20.1	34.9434689	42.857
5	7	30	20.1	34.9434689	14.286
6	9	30	20.1	44.9273171	22.222
7	5	30	20.2	24.8984044	60
8	10	30	20.2	49.7968088	20
9	5	30	20.2	24.8984044	26
10	6	30	20.2	29.8780853	78.333

6.3 XYL_DOS.exe

6.3.1 Starting XYL_DOS.exe

XYL_DOS is a DOS software. To load the program type **XYL_DOS** at the DOS prompt. In a Windows operating system, simply double click on the file in the Explorer. **XYL_DOS** needs only additional file (XYLEM.SET) that contains the program settings and the calibration factors for the sensors. When working in a Windows environment, you may experience conflicts of use for the serial port between **XYL_DOS** and Windows. If you cannot solve the problem, reboot the computer in the DOS environment. The only advantage for running **XYL_DOS** in a Windows environment is that you can display 50 lines (instead of 25) and thus display the Help or the setup screens simultaneously with the data screen. XYL_DOS has no graphic interface and no mouse support. The program uses keyboard shortcuts instead. The shortcuts are ONE character only, such as 'F' 'V' etc... Unless otherwise specified, the shortcuts can be used in the upper or lower case.

You must switch ON the **XYLEM** before starting **XYL_DOS**, otherwise the program will hang and you will need to reboot your computer. Sorry I couldn't fix this problem! The program is hanged in a loop waiting for a communication with the **XYLEM**. As a reminder, the program prompts you to turn ON the **XYLEM** and will start logging data only when you press any key on the keyboard. The program is configured to communicate at 9600 bds with COM1. If you have to use another serial port you must enter the SETUP screen first to change these parameters. To do so type 'U' when you are prompt to type a key. When the program is loaded, the following screen appears (data screen):

The screenshot shows the XYL_DOS data screen with the following callouts:

- Program version**: points to 'version 16-01-04'.
- Flush duration in sec**: points to '7sec'.
- Current apparatus in use**: points to 'xyl: 1'.
- Variable measured**: points to 'Conductance'.
- mean**: points to '1.930e-02'.
- SD**: points to '8.749e-02'.
- Coefficient of variation**: points to '483.65'.
- Scrutation rate in sec**: points to '0.0'.
- Number of data for the**: points to '10'.
- PLC value**: points to '65.5'.
- mMOL or Kg unit**: points to '0.0'.
- High pressure in bars**: points to '1.86'.
- Temperature in °C**: points to '25.5'.
- Low pressure in cm H2O**: points to '41.2'.
- Zero pressure in cm H2O or bars**: points to '0.0'.
- Warning or information**: points to 'Exit program?'.
- Flow in g/h**: points to '0.01'.
- % full scale flow**: points to '2.3'.
- Last 9 values of the variable measured**: points to the list of values on the right.
- Mean initial value of the variable measured for sample i**: points to '5.46e-02'.
- Mean value of the variable measured for sample i after first flush**: points to '5.08e-03'.
- Sample number**: points to '1'.

Kinit	F1	F2	F3	F4	PLC%
1	5.46e-02	5.08e-03			0.0
2	-7.5e-03	9.43e-04			
3	5.87e-03	1.70e-02			
4	8.78e-02	1.93e-02			
5	--	--			
6	--	--			
7	--	--			
8	--	--			
9	--	--			
0	--	--			

XYLEM Embolus Meter COCHARD/AMEGLIO/BODET INRA
version 16-01-04 Clermont-Fd FRANCE PIAF

1.86 25.5 41.2 0.0

Flow H2O 0.01 2.3

Exit program?

6.3.2 Commands

If the program communicates OK with the **XYL'EM** board, the values with yellow legends will be displayed: HP pressure in bar; Temperature in °C; LP pressure in cm H2O; P_{out} (zero pressure); Flow in g/h; and the % of current flow to full scale flow. At this stage the following operations can be performed by typing the specified keys

- **H/h**: Display the Help Screen. In the 50 line mode the screen appear underneath the data screen.
- **U/u**: Display the Setup Screen. In the 50 line mode the screen appear underneath the data screen. See below for further information on the setup.
- **R/r**: Toggle between different **XYL'EM** apparatus. The characteristics of up to 5 **XYL'EM** can be memorized. This is useful if you use different **XYL'EM** with the same computer or if you purchase a **XYL'EM** with two liquiflow.
- **D/d**: Change the type of measurements. Conductances are normally recorded. However you may also record the LP pressure, the flow and the temperature.
- **O/o**: Change from mmol s⁻¹ to kg s⁻¹ for flow and conductances units.
- **K/k**: Change from Kg s⁻¹ to mmol s⁻¹ for flow and conductances units.
- **S/s**: Enter the scrutation time in seconds. This is the time between two consecutive retrievals of data from the XYL'EM.
- **B/b**: Turn ON or OFF this annoying sound produced when the flow, or the pressure exceed full scale values.
- **M/m**: Enter the number of data for the running mean.
- **T** (upper case only): Toggle between manual and automatic temperature measurements. If Manual is activated data are red. Type 't' (lower case) to enter manually the temperature in °C. You can also type + - * / to change T by 0.1 -0.1 1 -1 °C respectively. You can also type the + - * / during measurement to adjust temperature continuously.
- **P** (upper case only) : Toggle between manual and automatic pressure measurements. Depending on the LP or HP mode either the LP or HP value is displayed in red. Then type **p** (lower case) to enter LP in cm H2O or HP in bars depending on the mode selected.
- **0(zero)**: Save the pressure difference between the **XYL'EM** and the sample. This is Pout as defined in § 5. Pout is displayed in cm H2O or bars in the LP and HP mode respectively.
- **RETURN**: Start measuring data.
- **F/f**: Flush and move to the following column. During a flush the timer is activated.
- **TAB** or **Space bar**: Move between lines to repeat measurements. You can move only between lines, not between columns.
- **V/v**: Save data in a file. This option is activated at the end of a trial to save the conductance values in a file. This creates to files: YYMMDDXX.dat and YYMMDDXX.txt. The first file contains the conductance and PLC values in a text file. You can then import these data in your favourite spreadsheet.

A .dat file typically looks like this:

Kinit	Flush#0	Flush#1	PLC
1	1.54e+00	1.50e+00	0.00
2	1.49e+00	1.53e+00	2.29
3	1.44e+00	1.52e+00	5.18
4	1.49e+00	1.56e+00	4.55
5	1.49e+00	1.50e+00	0.36

XYLEM Enbolus Meter COCHARD/AMEGLIO/BODET version 16-01-04 Clermont-Fd FRANCE						INRA PIAF
	Kinit	F1 10sec	F2	F3	F4	PLCZ
1	1.54e+00	1.50e+00				0.0
2	1.49e+00	1.53e+00				2.3
3	1.44e+00	1.52e+00				5.2
4	1.49e+00	1.56e+00				4.5
5	1.49e+00	1.50e+00				0.4
6	--	--	--	--	--	--
7	--	--	--	--	--	--
8	--	--	--	--	--	--
9	--	--	--	--	--	--
0	--	--	--	--	--	--

R|Xyl: 1

SCRU 1

MEAN 10

SAVE RAZ

EXIT MOL

P 1.46

T 25.1

P 41.9

0 0.0

The second file is a copy of the screen. An illustration is shown on the left. File name is made out of the date of the day (year-month-day) and two incremental digits (XX). The name of the file is displayed on the information box (lower right corner).

- **Z/z**: Erase all data for a new experiment. You are prompt to save the data if you forgot it.
- **X/x**: Exit program. Validate the command by Y or y. You are prompt to save the data if you forgot it.

6.3.3 SETUP window.

The SETUP menu lets you define the calibration factors of the different sensors for the **XYL'EM** apparatus currently selected. Press the number or the letter at the beginning of each line to modify the parameter.

- **1**: Define the calibration factors for the Temperature sensor. You are prompt to enter the slope (span) and the offset in $^{\circ}\text{C mV}^{-1}$ (typically 0.1 and 0)
- **2**: Define the calibration factors for the Low Pressure sensor. You are prompt to enter the slope (span) and the offset in MPa mV^{-1} (typically $2\text{e-}6$ and 0)
- **3**: Define the calibration factors for the High Pressure sensor. You are prompt to enter the slope (span) and the offset in MPa mV^{-1} (typically $2\text{e-}4$ and 0)
- **4**: Define the calibration factors for the flowmeter. You are prompt to enter the slope (span) and the offset in $\text{gh}^{-1} \text{mV}^{-1}$ (typically $4\text{e-}3$ and 0 for a 20g/h)
- **5**: Change the serial communication port. (typically COM1)
- **9**: Change the serial communication baud rate. (typically 9600)
- **6**: Switch Manual/Automatic pressure measurements. (typically Automatic)
- **7**: Switch Manual/Automatic temperature measurements. (typically Automatic)
- **8**: Save transient values in a file. The file is named after the current date (YYMMDD) and has a ".trs" extension. The file contains all the values displayed during measurements. A typical file looks like this:

```
Measuring Sample# 1 Flush# 0
TIME(HH.MM)  Low P(kPa)  High P(MPa)    Temp(°C)  Flow(Kg/s)    Kance (Kg/s/MPa)
21.6575      4.03      1.56e-01      25.36     1.639e-07     3.591e-05
21.6581      4.04      1.46e-01      25.16     1.531e-07     3.362e-05
21.6586      4.06      1.36e-01      25.16     1.631e-07     3.563e-05
```

The column is the current time in decimal hours (20h30min is 20.5h). The units for flow and conductance (Kance) are in Kg/s or mmol/s according to the program setup.

- **a**: Use the value of LOW or HIGH pressure sensor to measure conductance. This switches between the LP and HP modes.
- **s**: Switch between a 25 and a 50 lines screen. 50 lines will work fine only in a DOS window.
- **x**: Save the modifications you have made and go back to the data screen.
- **0**: Exit the setup screen **without** saving the modification and go back to the data screen.

A typical **XYL'EM**.set file looks like this (the text is not included in the file).

1	XYL'EM apparatus currently in use	
10	Number of data for the running mean	
1	Flow unit. Mmol/s of 1, Kg/s if 0	
0	Serial COM PORT. COM1 if 0, COM2 if 1 etc.	
9600	Serial BAUDRATE.	
0	Type of measurements: 0=Conductance 1=Flow 2=Pressure 3=Temp	
1.000000e+00	Data scrutation rate in secondes	
1	Screen size. 0=50 lines 1=25 lines	
4.000000e-03	XYL'EM 1 Flowmeter Span	This is a 20g/h
0.000000e+00	XYL'EM 1 Flowmeter offset	
2.000000e-06	XYL'EM 1 Low Pressure Span	
0.000000e+00	XYL'EM 1 Low Pressure offset	
1.000000e-01	XYL'EM 1 Temperature Span	
0.000000e+00	XYL'EM 1 Temperature offset	
2.000000e-04	XYL'EM 1 High Pressure Span	
0.000000e+00	XYL'EM 1 High Pressure offset	
1.000000e-03	XYL'EM 2 Flowmeter Span	This is a 5g/h
0.000000e+00	XYL'EM 2 Flowmeter offset	
2.000000e-06	XYL'EM 2 Low Pressure Span	
0.000000e+00	XYL'EM 2 Low Pressure offset	
1.000000e-01	XYL'EM 2 Temperature Span	
0.000000e+00	XYL'EM 2 Temperature offset	
2.000000e-04	XYL'EM 2 High Pressure Span	
0.000000e+00	XYL'EM 2 High Pressure offset	
0.000000e+00	XYL'EM 3 Flowmeter Span	Not used
0.000000e+00	XYL'EM 3 Flowmeter offset	
0.000000e+00	XYL'EM 3 Low Pressure Span	
0.000000e+00	XYL'EM 3 Low Pressure offset	
0.000000e+00	XYL'EM 3 Temperature Span	
0.000000e+00	XYL'EM 3 Temperature offset	
0.000000e+00	XYL'EM 3 High Pressure Span	
0.000000e+00	XYL'EM 3 High Pressure offset	
0.000000e+00	XYL'EM 4 Flowmeter Span	Not used
0.000000e+00	XYL'EM 4 Flowmeter offset	
0.000000e+00	XYL'EM 4 Low Pressure Span	
0.000000e+00	XYL'EM 4 Low Pressure offset	
0.000000e+00	XYL'EM 4 Temperature Span	
0.000000e+00	XYL'EM 4 Temperature offset	
0.000000e+00	XYL'EM 4 High Pressure Span	
0.000000e+00	XYL'EM 4 High Pressure offset	
0.000000e+00	XYL'EM 5 Flowmeter Span	Not used
0.000000e+00	XYL'EM 5 Flowmeter offset	
0.000000e+00	XYL'EM 5 Low Pressure Span	
0.000000e+00	XYL'EM 5 Low Pressure offset	
0.000000e+00	XYL'EM 5 Temperature Span	
0.000000e+00	XYL'EM 5 Temperature offset	
0.000000e+00	XYL'EM 5 High Pressure Span	
0.000000e+00	XYL'EM 5 High Pressure offset	
0	Save transient values if 1	
1	LP mode of 1, HP mode if 0	

The file can be edited with a Text editor such as NotePad.exe

6.3.4 Program operation.

Once you have setup the program, the configuration is saved and all the parameters are reloaded when the program is re-opened. A typical procedure for a PLC trial is the following:

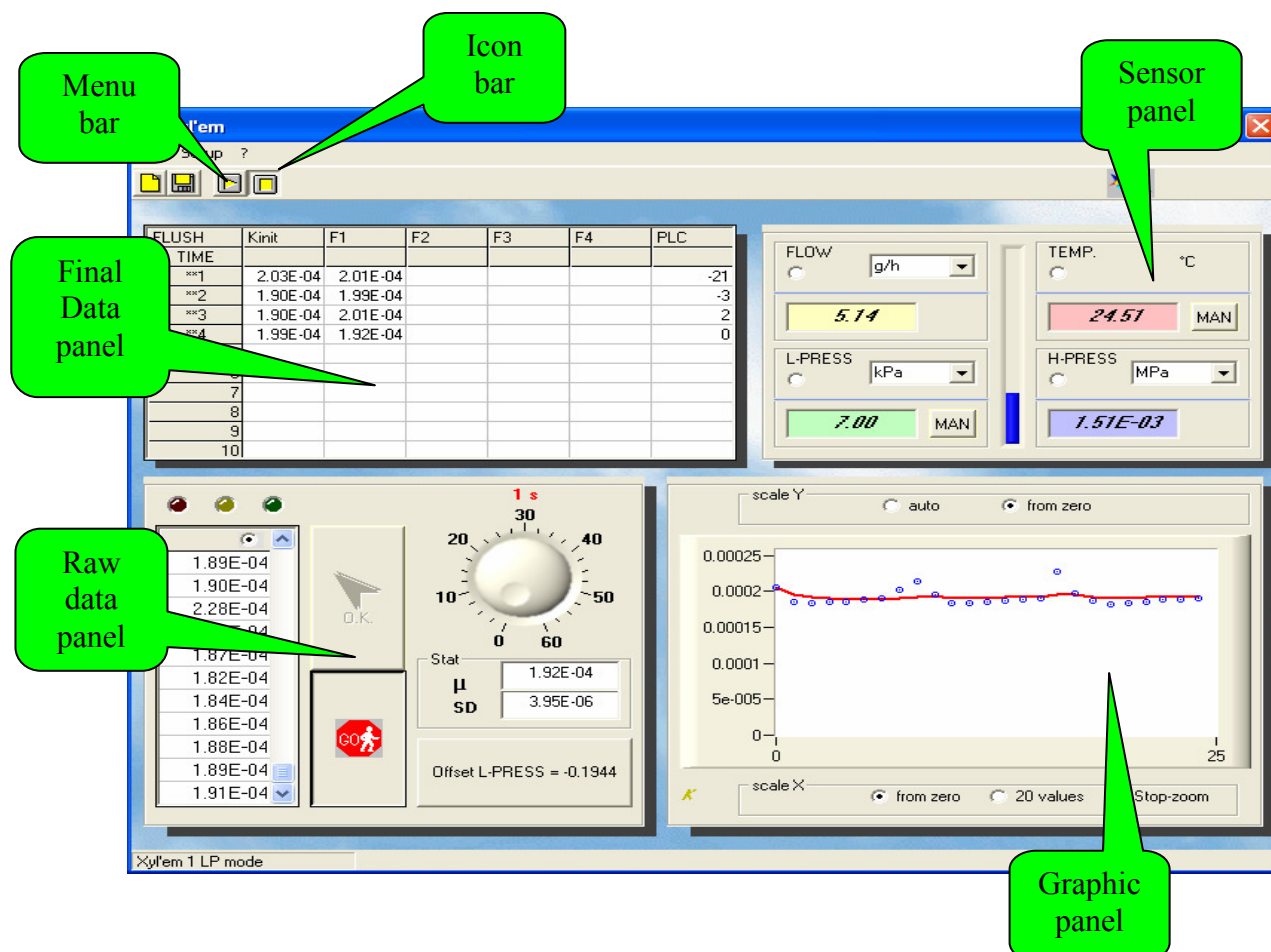
1. Set the **XYL'EM** and **XYL_DOS** to the LP mode.
2. Install sample 1. While the sample is being installed bips are produced because the flow exceeds the full scale. If no sound is produced this may signify the LP reservoir is empty, or more likely, that the valves are not correctly set. If this is the case there is a high risk of embolism removal upon sample insertion into the tubing.
3. Once the sample is installed, the bips may continue because of high a flow. The %full scale flow box will show ">>>" if this is the case. Check the seal at the sample level to detect any leak. If the sample is correctly installed, lower the LP reservoir to lower the flow.
4. Press the 'RETURN' key to start measurements. "MEASURE" is displayed in the data box.
5. Wait until the flow becomes steady, typically when the coefficient of variation is less than 1%. If the flow is small, you may not obtain cv values less than 1%.
6. Press 'ENTER' to stop measurements and store the mean value in the data box.
7. Install the second sample as above and repeat for all the samples (up to 10).
8. If for some reason you want to redo the initial measurement of one sample, use the TAB key and highlight the desired sample. Press ENTER to re-measure. You are prompt to enter 'y' or 'Y' to confirm.
9. When the initial conductance of the last sample has been measured, 'flush' all the samples. Press 'F' or 'f' at the same time you switch the **XYL'EM** HP/LP valve from LP to HP. This will start the chronometer.
10. When the flush is finish, press ENTER to stop the chronometer.
11. Set the **XYL'EM** to the LP mode and select the first sample for measurement.
12. Measure the conductance of sample 1 after first flush as above. The PLC is displayed in the PLC box.
13. Repeat for all the samples.
14. Repeat a second flush as above.
15. If the conductance significantly increased between F1 and F2, perform a third flush and a forth one if necessary. You may increase the duration of the flushes.
16. When the final conductance of the last sample is measured, save the data (V or v).
17. Press 'z' or 'Z' to erase all the data and start a new trial or 'x' or 'X' to exit the program.

For an undetermined reason, the program can sometime hang (runtime error). **Therefore we recommend to write down the data on a paper sheet for safety** (you can use hardcopies of the **XYL'EM.xls** spreadsheet or of blank YYMMDDXX.txt text files. Alternatively you can use the transient data file (if activated) to recover your data.

6.4 XYL_WIN.exe

6.4.1 Starting XYL_WIN.exe

XYL_WIN runs under Windows 95 and upper versions. To load the program double click on **XYL_WIN.exe** in the Explorer. When the program is launched the following screen appears:



6.4.2 Commands

Four different panels compose the window.

- The “final data panel” (upper left) is a table where final conductances values of each sample are stored. The number of samples (up to ten) is defined by pressing the appropriate number in the left most table column.
- The “sensor panel” displays the current values for the Flow, Temperature, Low Pressure and High Pressure. The vertical bar indicates the current flow level relative to the full scale flow. When the flow exceeds the full scale the bar turns red. For each sensor you can select the most convenient unit. Click on the triangle to display the different units. Whatever the unit displayed, conductances are always calculated in $\text{Kg s}^{-1} \text{MPa}^{-1}$ or $\text{mmol s}^{-1} \text{MPa}^{-1}$ (see SETUP menu). Click on the button to graph the desired parameter in the graphic panel.
- The “transient data” panel displays the instantaneous conductance values and their statistics (mean and coefficient of variation). The scrutation rate can be adjusted with the knob scroll bar between 1 and 60 seconds. Click on the STOP/GO box to start and stop measurements. Click on the ARROW box to store the final conductance in the final data panel. The three flashing diodes indicate the stability of the K values. When

the green diode flashes the cv is less than 1% and the value can be stored. Press the “Offset L-Press” box to store the pressure difference between the **XYLEM** and the samples (Pout)

- The “graphic panel” displays the time course for the selected variable. The time courses of K are typically displayed. You can graph all the values or the last 20ones. You can also zoom on a selectable part of the graph. When K values are plotted, the individual values appear as open blue circles and the running mean as a plain red line.

6.4.3 SETUP menu

To enter the SETUP menu select “setup” in the menu bar.

- In the SETUP menu you can also define up to 3 additional columns in the data table. These columns might be use for storing sample diameter, leaf area or any text.
- The mode of operation (LP versus HP mode) is also defined in the SETUP menu. In the LP mode the program uses the Low Pressure values to compute K. HP is used in the HP mode.
- Use the SETUP menu to select the **XYLEM** apparatus you are going to use. The characteristics of up to 5 apparatus can be defined. The current apparatus and the LP/HP mode are displayed in the lower left corner of the program window.
- Select “save raw data” to save all the raw data in a text file. You are prompted to select or create a file for saving data. If the file already exists, the data are appended at the file end.

A typical file looks like this:

Time	Flow (g/h)	L-PRESS (kPa)	TEMP (°C)	H-PRESS (MPa)	K
13:15:18	5.28	6.81	24.42	1.66E-03	10.82195
13:15:19	4.87	6.81	24.42	1.66E-03	9.975977
13:15:20	4.83	6.81	24.42	1.61E-03	9.89597
13:15:21	4.84	6.81	24.42	1.61E-03	9.921269
13:15:22	4.91	6.81	24.42	1.61E-03	10.05467
13:15:23	4.97	6.80	24.42	1.56E-03	10.19073
13:15:24	5.78	6.81	24.42	1.56E-03	11.85549
13:15:29	4.89	6.81	24.45	1.66E-03	10.01298
13:15:30	4.95	6.80	24.42	1.66E-03	10.15818
13:15:31	5.81	6.81	24.42	1.66E-03	11.90795
13:15:32	5.02	6.81	24.45	1.70E-03	10.28165
13:15:33	4.82	6.81	24.48	1.66E-03	9.870503

6.4.3 CONFIG window

To enter the configuration window select “SETUP” and “CONFIG” in the menu bar. This window allows you to:

- select the unit for the flow and conductance values (Kgs^{-1} or mmol s^{-1}),
- modify the calibration coefficients for the different sensors
- select the file format (dat or xls)
- select the number of data used for computing running means and statistics

Typical values for the different sensors are listed below:

Liquid flow meter configuration :

Enter the liquiflow full scale (for example 15g/h).

The full-scale value of the A/D converter is 5V. Therefore, $15\text{g/h} = 5000\text{mV}$. The span will be 3.10^{-3} g/h mV . Add an offset to compensate zero drift.

Temperature probe configuration:

Span: 0.1°C mV . Add an offset to compensate zero drift.

Low pressure measurement configuration :

The pressure sensor has a full-scale of 100 mb (0.01 MPa) related to 5000mV. The slope will be $0.01/5000=2.10^{-6}$ MPa/mV. Add an offset to compensate zero drift.






High pressure measurement configuration:

The pressure sensor has a full scale of 10 bar (1MPa) related to 5000mV. The slope will be $1/5000 = 2.10^{-4}$ MPa/mV. Add an offset to compensate zero drift.

Press “OK” to save value and exit the SETUP window.

6.4.4 Program operation.

Once you have setup the program correctly, the configuration is saved and all the parameters are reloaded when the program is re-opened. A typical procedure for a PLC trial is the following:

1. Set the **XYL'EM** and **XYL_WIN** to the LP mode.
2. Select the number of samples.
3. Install sample 1.
4. Once the sample is installed, check if the flow does not exceed the full scale range. This is indicated by a **red** vertical bar in the sensor panel. If this is not the case, check the seal at the sample level to detect any leak. If the sample is correctly installed, lower the LP reservoir to lower the flow.
5. Press the start icon  in the icon bar to start communication with the **XYL'EM**.
6. Wait until the flow becomes steady, typically when the coefficient of variation is less than 1%. This will be indicated by a green flashing diode. If the flow is small, you may not obtain cv values less than 1%.
7. Press the arrow box  to stop measurements and store the mean value in the data box.
8. Install the second sample as above and press the GO box  to start measurements
9. Repeat for all the samples (up to 10).
10. If for some reason you want to redo the initial measurement of one sample, click the desired sample
11. When the initial conductance of the last sample has been measured, ‘flush’ all the samples by switching the **XYL'EM** HP/LP valve from LP to HP.
12. When the flush is finish, set the **XYL'EM** to the LP mode and select the first sample for measurement.
13. Measure the conductance of sample 1 after first flush as above. The PLC is displayed in the PLC box.
14. Repeat for all the samples.
15. Repeat a second flush as above.
16. If the conductance significantly increased between F1 and F2, perform a third flush and a forth one if necessary. You may increase the duration of the flushes.
17. When the final conductance of the last sample is measured, save the data by pressing the save icon. 
18. To star a new trial press reset icon  to erase all the data and start a new trial or quit to exit the program.

For undetermined reasons, the program can sometime hang (runtime error). **Therefore we recommend to write down the data on a paper sheet for safety** (you can use hardcopies of the **XYL'EM.xls** spreadsheet. Alternatively you can edit the raw data file (if activated) to recover your data.

Appendix 1

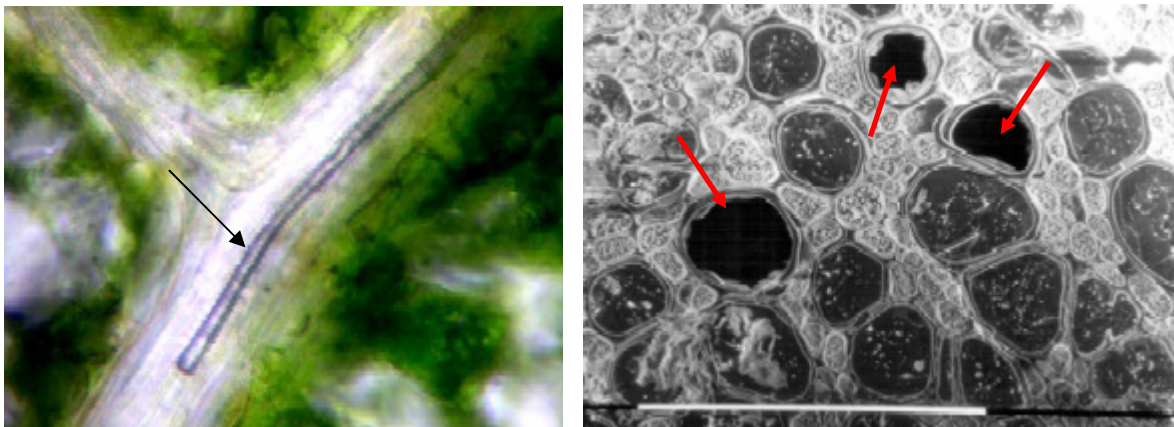
Tips and cautions for using the **XYL'EM**

A1.1 Foreword

This chapter was written by H Cochard UMR-PIAF, INRA, Clermont-Ferrand, France (cochard@clermont.inra.fr) with the intention of helping new users with their first determination of xylem embolism values. Although the principle of the technique is rather simple, several conditions must be respected in order to obtain reliable data. Our objective is to share our experience with the technique and to describe the major difficulties we have encountered. These are only hints and recommendations that you may follow or just ignore. We can guarantee that the **XYL'EM** apparatus is measuring pressures, flow and temperature correctly but we can absolutely not guarantee that your embolism measurements will be accurate if the recommendations below are not followed.

A1.2 Cavitation and embolism in plants

Functional xylem conduits (vessels and tracheids) normally contain water and are hydraulically connected to upstream and downstream conduits. Under some circumstances (drought or frost stresses for instance), xylem conduits can cavitate and consequently become embolised (see pictures below). Xylem cavitation corresponds to the rapid breakdown of the water column in the conduits and to the formation of a cavity filled with vapor water (near vacuum pressure). Rapidly air degasses into this cavity which increases pressure to atmospheric. At this stage the whole conduit lumen contains a large air bubble that block the water flow through the conduit. An air embolism has formed.



Direct observations of air-filled conduits in the vascular system of upper plants. The picture on the left shows an embolised conduit in the vascular system of a walnut leaf blade. On the right, three embolised vessels are visible on this cross section of a walnut petiole observed with a cryo-SEM. (photos : H Cochard)

A1.3 Principle of the technique

The principle of the **XYL'EM** apparatus is to measure the relative decrease in xylem hydraulic conductance caused by the presence of air in the conduits. Contrary to other techniques (dye coloration, acoustic emissions, cryo-SEM observations) this technique is quantitative, i.e., it quantifies the amount of loss of xylem functionality at any given time. John Sperry first proposed the technique in the 80's (Sperry et al 1988).

Let us assume a xylem segment (a petiole, a shoot internode, a root segment etc...) fully functional (no embolism) having a hydraulic conductance equal to K ($\text{mmol s}^{-1} \text{MPa}^{-1}$ or $\text{kg s}^{-1} \text{MPa}^{-1}$). Following a drought or a frost stress, embolism forms which reduces K to K' . The percentage of loss of xylem conductance (PLC) can then be computed as:

$$\text{PLC} = 100 * (1 - K'/K)$$

If $\text{PLC} = 0\%$, $K' = K$, i.e., none of the conduits were embolised. If $\text{PLC} = 100\%$, $K' = 0$, i.e., all the conduits were embolised.

Therefore, to compute PLC, we need to measure K and K' . However, it is usually not possible to measure K for the trivial reason that the technique is destructive. When a sample is collected on an experimental plant, it is likely to contain embolism. Hence, K' is measured first. To estimate K , the idea is to resaturate the sample in order to dissolve any air bubble that may have formed during the treatment. To remove embolism, the segment is perfused ("flushed") with water at a relatively high pressure (0.1 to 0.2 MPa or 1 to 2 bars). Degassing water facilitates dissolution. This is the reason why the **XYL'EM** contains a captive air tank. After the flush, the conductance of the xylem segment is K'' . PLC is then computed as:

$$\text{PLC} = 100 * (1 - K'/K'')$$

It is easy to see that the technique will meet our expectation only if $K'' = K$, i.e., if the xylem conductance of the resaturated segment equals the conductance of the same segment before treatment.

Our experience with many woody species and several herbaceous ones shows that this condition is generally satisfied. We will discuss below the situation when PLC values might be misleading, for a proper usage of the **XYL'EM** apparatus.

A1.3.1- Possible problems with K'

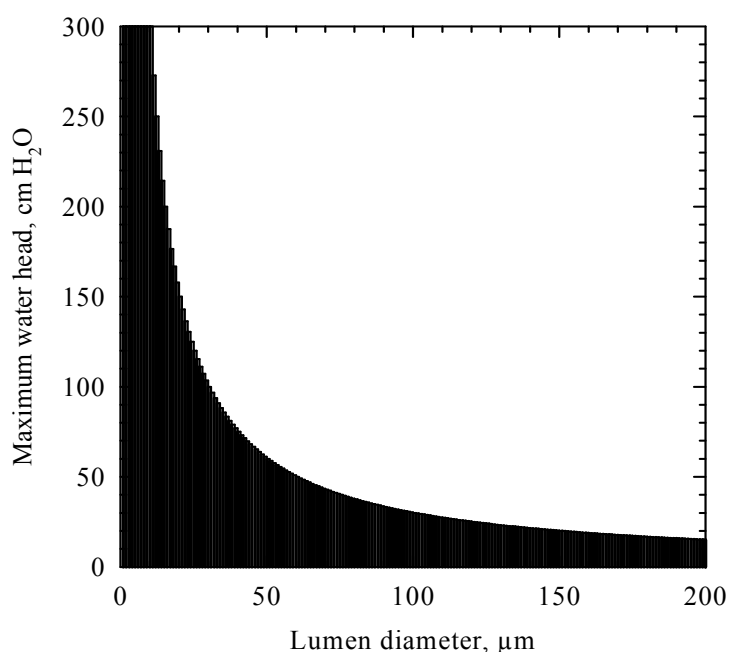
K' is suppose to correspond to the hydraulic conductance of the xylem segment *in planta*, i.e., before it was excised. By the time the sample was collected and measured no embolism must have formed or dissolved, unless the value will be artifactual.

A1.3.1.1 Problems during sample collection

Xylem vessels *in planta* are usually exposed to large negative pressures. Therefore, when a cut is made in the xylem, water in the open vessels is exposed to atmospheric pressure and will thus be sucked upward and downward into the xylem in a few seconds. The capillary pressure that develops at the cut end of the conduits is far too small to maintain intact the air-water meniscus (see figure 1). Sap will be sucked back the entire length of the conduits. This length represents a few millimeters for tracheids but several meters in some large vessel bearing plants (ringporous trees for instance). Therefore, it is essential to know the maximum vessel length if a xylem segment is to be cut in air. The PLC value of a segment containing cut open vessels will be an artifact of the sampling procedure. Experimental procedures exist to measure vessel length (air injection, paint perfusion, vessel microcasting, etc). We will describe another technique that uses the **XYL'EM** apparatus below. It is sometimes possible to excise samples on the plant under water, which greatly reduces this problem.

A1.3.1.2 Problems due to accidental embolism dissolution

Some authors have argued that the hydraulic determination of xylem embolism greatly underestimates the actual levels *in planta* for the reason that xylem pressure has to be released to atmospheric or supra-atmospheric values during measurements. The problem could be potentially acute if xylem conduits remained saturated with vapor pressure (not air) for a long period. Using a spinning experiment (see Cochard et al 2000 and Cochard 2002 for details), we have established that this underestimation was very unlikely in walnut petiole. However, for very tiny samples (leaf petioles, or leaf veins for instance) having small diameter lumens (and thus high capillary pressures) one may observe a rapid embolism dissolution under near zero xylem pressure. This will translate into a progressive increase in sample conductance during the initial K' measurement. One way around this problem is to place the sample in a hydraulic circuit in order to expose it to a negative pressure during measurement.



Maximum water head for measuring the conductance of an embolised vessel according to its internal (lumen) diameter. The computation assumes that vessels are behaving like perfect pipes and follow the capillary equation. For a proper estimate of xylem embolism, the hydrostatic pressure difference between the two ends of the sample must be in the black area.

The major risk of embolism dissolution is not this passive refilling during measurement. Rather, the critical phase is when samples are inserted into the manifold. Indeed, it is very easy to displace the air bubbles trapped in an open vessel during this phase. The figure above gives the theoretical threshold water head (in cm of H₂O) that will displace an air/water meniscus in a conduit of a given diameter. One can see that a water head of 60 cm is enough to refill an embolised open vessel having a 50 microns lumen diameter. For larger conduits, the threshold pressure can be as low as 10 cm. Therefore, one must be sure that the water head used for the initial K' estimate is lower than this threshold value. This threshold pressure can be determined experimentally as follows: prepare a representative xylem segment as described above (excised under water). Connect the sample to an air filled tubing and pressurize for a minute at ca 0.1 MPa. This will cause air to enter the vessels and induce 100PLC. Connect carefully the sample to the **XYLEM** manifold with a pressure head as small as possible (a few centimeters as indicated by the low-pressure transducer). Measure K', which should, in theory, be close to zero. Move up the low-pressure reservoir and note when K' rapidly increases. You should note air bubbles coming out of the sample free end at this moment. This is the threshold pressure you do not want to exceed. Working with longer segments decreases the number of open vessels and thus the risk of refilling

described above. However, longer samples are more difficult to resaturate! We describe below a procedure to test if your PLC data are trustworthy.

A1.3.2- Possible problems with K''

K'' is the xylem conductance of the resaturated segment. K'' is supposed to be an estimate of K , the conductance of the same segment before treatment. However, under certain circumstances, or for several species, K'' can be very different of K .

A1.3.2.1 Situations where K'' may overestimate K

We have encountered two situations where K'' was greatly overestimating K . The first situation was found in *Festuca lamina* (see Martre et al 2001). In this species, intercellular air spaces are well developed in the leaves and form continuous pipe in the axial direction. When leaves are flushed, the air spaces become water filled and conduct water! Therefore, very high PLC values were noted in the leaf lamina of this species, even for control plants, but this was because K'' was overestimating K . There is not a practical solution to this problem, and K must be estimated statistically (see below).

A more common overestimation of K is found in species having a high native state of embolism. Temperate ring porous species are illustrative of this problem. We know that large vessels embolise during winter are never refilled (see for instance Cochard and Tyree 1990 or Cochard et al 1997.). Therefore, large vessels conduct water only in the current year ring in such species. Older rings have non-functional air filled vessels. When such a sample is flushed, these >1 yr old vessels are refilled and conduct water. The PLC value can then be quite high, especially for old samples. But this does not necessary mean that the sample has experienced any drought stress! To overcome this problem, work on current-year shoots, or follow the trends in embolism during a whole year.

A1.3.2.2 Situations where K'' underestimate K

These situations are of common occurrence. They are found when xylem conduits get plugged for one or more reasons.

First, segment conductance may decline continuously during K measurements or after each flush. This may indicate a change in xylem conductance caused by the perfusion solution itself. This might be due to the presence of tiny particles in the solution that can pass through the pores in the filters (usually > 0.2 microns) but not through the pores in the pit membrane (usually < 0.2 microns). It is critical to use distilled or dionised water, to change periodically the filters and to clean the tubing frequently to avoid such problems. Change in K values might also be caused by the presence or the absence of certain ions in the solution. Usually when a small concentration of KCl is added to the solution (10 mmol per liter) a constant K is achieved. But different species may require different concentrations or different ions. We used to add HCl or oxalic acid to lower the pH and prevent bacterial growth. We now prefer to use neutral water and change the solution more often. It is easy to understand that these plugging problems will be exacerbated if a lot of solution has to pass through the sample (long flushes for instance). This is why we prefer to work on relatively short samples (about 2cm) and used very short flushes (a few seconds). Short samples will have many open vessels that will be easy to refill. However, they are more difficult to install for the same reason! Long samples will contain many bubbles trapped into close vessels and will require prolonged flushes to refill (and hence will be more likely to plug).

Species with resin or latex or gums in their xylem will also cause plugging problems. Removing the bark can help, but not always.

Another source of plugging is the formation of tyloses or gums in embolised vessels. Tyloses are invaginations of contact cells into vessel lumens (usually only in large vessels). We know that vessels have to be embolised for tyloses to form. Tyloses will obviously lower the hydraulic conductance of these vessels once refilled. The extreme situation is when all embolised vessels are entirely filled with tyloses. Then the water flow through these vessels will remain null even after

many flushes. The PLC values for such samples will always be close to zero whatever the actual degree of embolism *in planta*. A similar situation is found in conifers where pit membranes become permanently aspirated against the cell walls of embolised tracheids. These membranes cannot return to their initial position and flow through these tracheids is permanently disrupted. To identify and then overcome these problems is rather difficult. First, it is always wise to do simple dye coloration (with safranin or phloxine B for instance) to find out if flushed samples are indeed entirely resaturated. Second, you may compute the sample xylem-area-specific conductivity of the resaturated samples and compare it to values obtained for control segments. If the saturated specific conductivity decreases with the intensity of the treatment or with time then you may have identified a plugging problem. This may lead to incorrect interpretation of what is going on in your plant. You may think that as the PLC values are decreasing over time the species is recovering or is reversing its embolism but it may just be that tyloses are being formed, or that new functional vessels are being added! This is where the statistical technique can be very useful. The principle is to establish a reference relationship between segment xylem area and segment conductivity for control plants and use this relationship to predict saturated K values for experimental samples. This technique is obviously less precise but you may have no other options. In most instances you will rapidly learn how to avoid the problems listed above and you will rapidly learn to avoid troublesome species!

A.1.3.3 Test experiments

We describe below "test" experiments that we normally perform when we start a study on a new species or when we are training people to this technique. The idea is to do PLC measurements on samples having known or predictable PLC values. They are applicable to vessel bearing species (not for conifers). The first two experiments are just for practice, the third one is always useful.

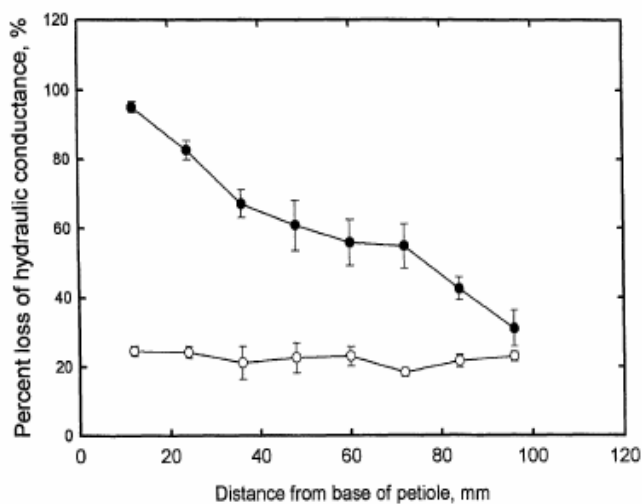
1- Collect an intact branch (leafy or leafless) and connect its cut end to the **XYL'EM** outlet. Immerge the branch under water and flush it with a 0.2 MPa pressure for one hour. This should be enough to remove any embolism in the branch. Collect samples as you would normally do and determine their PLC values. Repeat until you find something very close to zero!

2- Cut in the air a small segment from a plant. Blow 0.1 MPa air on both sides and connect carefully the sample to the **XYL'EM** manifold to determine its PLC value. Repeat until you find something very close to 100PLC!

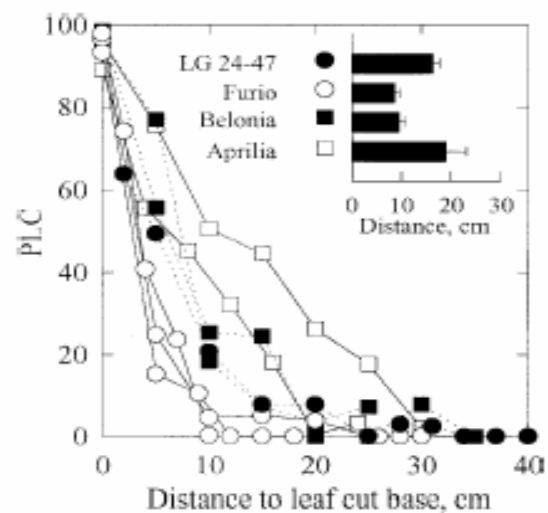
3- First determine the maximum vessel length. For this, the easiest way is to use the air perfusion technique: cut a branch on a plant and blow air at 0.1MPa through the cut end. Then successively remove branch parts starting at the most distal position (leaf petiole for instance). The remaining branch segment will thus become smaller and smaller. Put the terminal cut end in a beaker with tap water and notice if air is bubbling out of a vessel. If not, recut the branch a few more centimeters and repeat the operation. When you see a continuous flow of air bubbles emerging from the terminal end this means that at least one vessel was cut open on its both sides. The branch length will then be an estimate of the maximum vessel length for this sample. You will need to repeat the operation with different branches because maximum vessel length can vary substantially between samples. You may need to perfuse the branch with water first in order to reestablish all the air / water meniscus in the pit membranes. You can also use the paint perfusion technique but this is more laborious (see Tyree and Zimmerman for instructions). Second, cut a similar intact leafy branch in the air from a plant and let it transpire for a couple of minutes. This will be enough to empty all the lumens of the vessels cut open. Immerge the plant under water, excise different samples and note their location relative to the cut end. You want to collect sample from the cut end up to a distance longer than the maximum vessel length measured above. Measure the PLC values for these samples. You expect to find PLC values as follow: The proximal segment (that includes the cut end) will have a PLC value close to 100%. The PLC

values will then progressively decrease in the apical direction. Then the PLC values become constant. This value corresponds to the native state of embolism. It can be close to zero or relatively high if the branch has experienced a drought or a frost stress. The length at which the values become constant should correspond to the maximum vessel length determined above. If your results look different, then the PLC values were not properly determined!

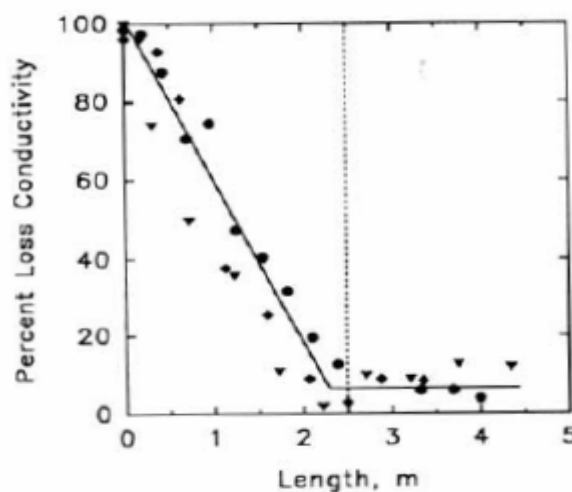
Here are some illustrations of this ‘test’ experiment for different species. Note that the x axis varies considerably between species because of different vessel length.



Helianthus annuus. The open symbols are for samples cut under water and represent the native state of embolism (Tyree & Cochard, 2003)



Zea mais leaf blades (Cochard, 2002)



Rhipidocladum racemiflorum,
a tropical bamboo
(Cochard et al, 1994)

A.1.4 Techniques for obtaining “vulnerability curves”

Vulnerability curves (VCs) are graphs of PLC values versus xylem pressure (P_x). The rationale for relation PLC to P_x is that according to the “air-seeding” hypothesis, cavitations occur when air/water meniscus are disrupted at the level of pores in conduit pit membranes. According to the hypothesis, it is the hydrostatic pressure difference across the pit pore that will cause the meniscus breakdown. *In planta*, the air pressure at the meniscus level is atmospheric ($P_{\text{air}} = 0$ MPa), hence the risk of cavitation is determined by P_x . Several techniques have been employed to generate VCs. The most widespread ones are listed below.

A1.4.1 Bench drying technique

This is the simplest and the reference technique for generating VCs. We recommend to systematically control the other technique below with this technique. The principle is to dehydrate large branches on a bench, to measure the xylem pressure and determine the corresponding PLC values. Xylem pressures are usually assessed with a Scholander pressure chamber. (The PMS Instrument Company produces different models of pressure chamber <http://www.pmsinstrument.com>). Leaves must be bagged for a proper estimate of P_x . When the branch has reached the desired xylem pressure, collect xylem samples under water and measure their PLC immediately. If the branch is large enough you can dehydrate it further and measure a new set of samples. Alternatively you can dehydrate different branches to different P_x values. You will finally end up with a number of PLC values between 0 and 100% corresponding to a specific range of xylem pressure. The technique is straightforward but it is sometime difficult to control branch dehydration and obtain values for the whole PLC range.

A1.4.2 Air pressurization technique

According to the “air seeding” hypothesis, the pressure difference across pit pores determine the formation of embolism. It has been demonstrated (Cochard et al 1992) that increasing the air pressure while maintaining the xylem pressure close to 0MPa result in the same vulnerability curves. Air pressurization can be employed in different manners:

A1.4.2.1 Single end pressure chamber

The principle is to pressurize a leafy branch in a large pressure chamber with the cut end protruding out of the chamber. The pressure in the chamber must be maintained at the preset value (P_{air}) until sap no longer exudes from the branch cut end (several hours might be needed). Release slowly the air pressure and let the branch in a closed bag (to prevent transpiration) for several hours. This is essential to release the air pressure in the intercellular spaces or in the embolised vessels to atmospheric. Unless, air bubbles will come out of the samples during conductance measurements, which will cause erroneous values. Collect and measure samples as indicated for the bench drying technique. VCs are constructed by plotting $-P_{\text{air}}$ versus PLC. The great advantage of this technique is that you can control very precisely branch dehydration and thus construct VCs with a limited number of branches. Our experience with this technique shows that it correlates very well with the reference procedure described above. It is the technique that we normally use to construct VCs in our laboratory. The inconvenient is that VCs are constructed with different branches (The PMS “super-chamber” is well adapted for dehydrating large samples <http://www.pmsinstrument.com/superchamber.html>).

A1.4.2.1 Double end pressure chamber

With this technique, entire VCs are constructed with one xylem segment. Samples are inserted in a dedicated pressure chamber with the 2 ends protruding out of the chamber (such a chamber is available from the PMS company at <http://www.pmsinstrument.com/cavitat.htm>). First, the initial sample conductance is determined. Then, the air pressure is increased to a first small pressure (eg 0.5 MPa) and maintained constant for several minutes. The pressure is gently reduced to atmospheric and the

xylem conductance is measured anew. The procedure is repeated by a stepwise increase of the air pressure inside the chamber until 100PLC is obtained.

Our experience with this technique suggests that it does not always correlates nicely with the two techniques above. Several problems have been encountered. First, it is important to wait until no bubbles come out of the cut end while measuring K. Unless the values will be biased. Second, it is sometime necessary to remove the bark or to incise the xylem facilitate air entry into the samples. Finally, we found that when working on plants with long vessels, the technique sometime overestimate the PLC values (embolism is induced for less negative equivalent xylem pressures). It is probably wise to first construct a reference VC with the two techniques above to confirm the data with this latter technique. This will help defining the proper way to employ it.

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Appendix 2

References to the **XYL'EM** system

(Last update: January 2004)

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The papers can be downloaded from <http://herve.cochard.free.fr/>

Appendix 3

XYL'EM specifications

Flow measurement:

- Principle: Thermal mass flow measurement
- Measuring range : 0.1-5 g/h, 0.2-10 g/h, 0.4-20g/h, 1-50 g/h, 2-100 g/h H₂O (to be specified on the order).
- Accuracy: +/- 1% full scale
- Rangeability : 1 to 50

Low pressure generation and measurement:

- Principle: Water column
- Measuring range :1 .. 7 kPa typ. (10 kPa max)
- Accuracy: +/- 0,2 kPa

High pressure generation and measurement:

- Principle: Pressurised vessel
- Measuring range: up to 3 bar typ, 7 bar max.
- Accuracy: +/- 1 % full scale

Temperature

- Sensor: Pt100 probe
- Measuring range :0°C to 50°C
- Accuracy: +/- 0.2 °C

Water supply:

High pressure:

- Vessel capacity: 1 l
- Max vessel pressure: 3 bars, (7 bars possible if filter is removed).

Low pressure:

- Vessel capacity: 100 ml
- Max vessel pressure: 1 meter water high
- Water filtration: 0,2 µm.

Mechanical characteristics

- Suitcase : IP65
- Dimension : 461x347x206 mm
- Weight (empty vessel): 10 kg

Electrical characteristics

- Power supply : 12Vdc
- Consumption : 900mA
- Electrical connection : Din 3 pins

Computer connection (option):

- Analog to digital conversion (15 bits resolution) and RS232 interface for data transfer to a computer.
- XYL'EM software for DOS and windows environments. This software collects the data, calculates the corrections and determines embolism factor.