Cross-validation on saplings of High-Capacity Tensiometer and Thermocouple Psychrometer for continuous monitoring of xylem water potential

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Highlight

High-Capacity Tensiometer and Thermocouple Psychrometer are proven to measure xylem water potential with high accuracy and adequate response time as demonstrated by simultaneous measurement on sapling stem.

Abstract

The Pressure Chamber, the most popular method used to measure xylem water potential, is a discontinuous and destructive technique and therefore not suitable for automated monitoring. Continuous non-destructive monitoring could only be achieved until very recently via the Thermocouple Psychrometer (TP). We here present the High-Capacity Tensiometer (HCT) as alternative method for continuous non-destructive monitoring. This provided us with a unique chance to cross-validate the two instruments by installing them simultaneously on the same sapling stem. The HCT and the TP showed excellent agreement for xylem water potential < -0.5 MPa. Response to day/night cycles and watering was remarkably in phase, indicating excellent response time of both instruments despite substantially different working principles. For xylem water potential > -0.5 MPa, the discrepancies sometimes observed between the HCT and TP were mainly attributed to the kaolin paste used to establish contact between the xylem and the HCT, which becomes hydraulically poorly conductive in this range of water potential once dried beyond its air-entry value and subsequently re-wetted. Notwithstanding this limitation, which can be overcome by selecting a clay paste with higher air-entry value, the HCT has been shown to represent a valid alternative to the TP.

Keywords

Introduction

The Thermocouple Psychrometer (TP) and the Pressure Chamber are most common instruments used to measure xylem water potential. The Pressure Chamber is an established technique and is considered the reference for the measurement of xylem water potential. However, this technique is destructive and is therefore not suitable for continuous monitoring and/or for monitoring when a relatively small number of leaves is available, which is generally the case in laboratory experiments.

The TP developed by Dixon and Tyree (1984) has been so far the only technique available for continuous and non-destructive monitoring of xylem water potential (Martinez et al. 2011; Yang et al., 2011; Patankar et al. 2013; Wang et al., 2014). The TP measures xylem water potential through equilibrium via vapour phase, i.e. it measures the relative humidity of the air in equilibrium with the xylem water, which is then converted to xylem water potential via the psychrometric law. Since the air acts as a semipermeable barrier, the presence of solutes in the xylem water affects the relative humidity of the air surrounding the xylem water and, hence, the measurement by the TP (Marinho et al., 2008). As a result, the TP does not allow discriminating between the osmotic and the matric components of the potential of the apoplast solution present in the xylem (Boyer, 1995). The common assumption that the osmotic component of xylem water potential is negligible (Jones, 2006) does not always hold (Campbell and Gardner, 1971; Goode and Higgs, 1973).

Like any instrument based on vapour equilibrium, the TP is sensitive to temperature fluctuations and may lose accuracy for air relative humidity close to saturation, i.e. at water potential values close to zero (Bulut and Leong, 2008). In addition, the TP does not allow for the measurement of the water potential for the case where the xylem water pressure becomes positive, which can be recorded under particular conditions, for example water-saturated soil combined with very low transpiration (Charrier et al., 2017). An alternative approach to the TP consists in measuring the matric component of the xylem water potential through equilibrium via the liquid phase. Balling and Zimmermann (1990) have attempted to directly measure xylem water tension using a probe made of a capillary tube filled with water and silicon oil and inserted into a xylem vessel. The tension of the xylem water was transmitted through the liquid and measured by a pressure transducer. However, they could not record xylem water potential below -0.65 MPa (Wei et al., 2001) and were not able to prolong measurement for more than a few hours due to cavitation occurring in the instrument.

A probe somehow similar to the one presented by Balling and Zimmermann (1990) has been developed in the field of geomechanics to measure tension of soil water in the range 0 - 2 MPa. This probe is referred to as High-Capacity Tensiometer (HCT) and has been used extensively for almost 30 years in laboratory and field testing of unsaturated soils (Ridley and Burland, 1993; Tarantino, 2004; Marinho et al., 2008).

The High-Capacity Tensiometer has been recently proven to be capable of measuring successfully xylem water tension by Dainese and Tarantino (2020). They tested the HCT on a Chestnut tree (in the field) and Pear and Willow saplings (in the laboratory) and validated the HCT measurements against Pressure Chamber measurements.

The accuracy of the diaphragm-based pressure sensor incorporated into the HCT is typically of 1-2 kPa over the entire measurement range (as inferred from the calibration in the positive range) and the effect of ambient temperature fluctuations is negligible. In addition, because the sensing diaphragm behaves symmetrically, the HCT can also measure positive xylem water pressures.

The HCT is a measurement technique for continuous and non-destructive measurement of xylem water potential alternative to the Thermocouple Psychrometer and offers a unique chance to cross-validate these two instruments in terms of accuracy and response time. This paper compares the measurements by the HCT and the Thermocouple Psychrometer installed simultaneously on the stem of four different saplings in the laboratory. The saplings were subjected to day/night light cycles and were tested under both well irrigated and drought conditions. These measurements were compared to discontinuous measurements made with the Pressure Chamber used here as a reference.

Background

Water under tension (absolute negative pressure)

The traditional phase diagram of water (Fig. 1A) reports the conditions of temperature and absolute pressure characterising the solid (ice), liquid, and vapour phases of water. Since vapour pressure cannot be negative, this diagram seems to suggest that water cannot exist in liquid phase under tension (negative absolute water pressure).

However, the phase diagram represents only the stable states of water, while other metastable states are possible without violating the principles of classic thermodynamics (Fig. 1B). The existence of a status of liquid water under tension may be considered through the van der Waals' equation of state of fluids (De Benedetti, 1996). This equation can be used to calculate theoretically the maximum tension that can be sustained by liquid water. For example, the maximum sustainable water tension at 20°C derived from the van der Waals' equation of state is of the order of 100 MPa (Marinho et al., 2008). However, the values of water tension experimentally measured are usually two orders of magnitude smaller than the theoretical one. The difficulty for water to reach the theoretical value is related to the presence of imperfections that lead heterogeneous nucleation (Marinho et al., 2008). Cavitation nuclei originate from air pockets 'invisible at naked eye' that remain entrapped at the boundary between the liquid and the surface of the water container or impurities dispersed in the water.

The challenge of direct measurement of water tension is associated with its metastable state. Water under tension is subject to cavitation, i.e. water tend to move from metastable states where the liquid is under tension (point D' in Fig. 1B) to stable states where liquid and vapour phases coexist under positive absolute pressure (point B in Fig. 1A).

Direct measurement of water tension

The transition from metastable to stable states cannot be prevented but only delayed long enough to allow for long-term measurement of water tension. This is achieved by pre-pressurising water in the measuring instrument to dissolve the majority of cavitation nuclei (Marinho et al., 2008). This is the working principle behind the HCT measurement as first developed by Ridley and Burland (1993). Typical design of the High-Capacity Tensiometer includes a high air-entry porous ceramic filter, a water reservoir, and a strain-gauged diaphragm to convert water tension into an electrical signal (Fig. 2). When the instrument is applied to a sample with a water under tension (negative pore-water pressure), the water is drawn out of the water reservoir and the diaphragm bends changing the electrical resistance of the strain gauge. The water in the reservoir and in the porous ceramic filter can sustain the water tension even if air cavities are present at the ceramic interface, which may form in the clay paste used to ensure hydraulic connection between the porous ceramic filter and the xylem vessel (Fig. 2). These menisci sustain the unbalance between water under tension in the ceramic filter and the atmospheric air pressure in these cavities. The maximum pressure unbalance, referred to as ceramic air-entry value, is inversely proportional to the size of the largest ceramic pores and limits the maximum water tension sustainable by the HCT.

The volume of the water reservoir is generally maintained very small (~4 mm3) as Ridley and Burland (1993) assumed its small size was key to enable sustained measurement of water tension. However, Mendes et al. (2020) have demonstrated that the volume of the reservoir does not play a critical role as generally assumed in the literature.

The maximum sustainable duration of the measurement can be augmented by imposing cycles of cavitation and re-saturation at high water pressure to 'extract' cavitation nuclei remained undissolved upon simple pressurisation (Tarantino and Mongioví, 2001).

Since the porous ceramic filter does not prevent the diffusion of ions into the water reservoir (Tarantino, 2004), the measurement by the HCT is not liable for differences in concentration between water in the instrument and at the measuring site, i.e. the HCT only measures the matric component of xylem water potential (similarly to the Pressure Chamber).

Materials

Equipment

High-capacity Tensiometer (HCT)

The HCTs used in this study were manufactured according to the design developed at the University of Trento by Tarantino and Mongioví (2002). The tensiometer mounts a ceramic filter with a nominal air-entry value of 1.5 MPa and includes an integral strain gauge diaphragm 0.4 mm thickness. The HCTs used in this study were calibrated in the positive range (0-1.5 MPa) at 20°C using a dead-weight calibration device and performing a full loading-unloading cycle (0.2, 0.4, 0.8, 1.2, 2.2 MPa and reversal). A linear calibration curve was derived by best fitting the calibration data:

$$\Psi = a_{20} + b_{20} \cdot mV \qquad\qquad [1]$$

where Ψ is water potential, mV is the electrical signal in millivolts a_{20} and b_{20} are the intercept and slope respectively of the calibration curve at 20°C. The diaphragm-based pressure sensors showed an accuracy better than 0.003 MPa (standard deviation of the error). The calibration curve was then extrapolated to the negative range according to Tarantino and Mongioví (2003). Saturation of the ceramic filter was achieved by pre-pressurisation at 4MPa.

To investigate the effect of temperature on HCT response, calibration was repeated at 30°C, 40°C and then back to 30°C and 20°C. The error was quantified by comparing the imposed water pressure with the water pressure that would have been estimated using the calibration curve initially derived at 20°C (see Eq. [1]). This error is shown in Fig. 3A and B for two imposed water potential, 0.2 and 2.2 MPa. It can be observed that the error is significant in the sense that it is higher than the standard deviation of the error associated with the calibration at 20°C. However, the error is relatively small (<0.04 MPa) and acceptable in most practical applications.

Thermocouple Psychrometer (TP)

The psychrometer used for this study is manufactured by ICT International (Armidale, NSW, Australia). To measure the relative humidity of the air in equilibrium with the xylem water, a thermocouple is cooled until the temperature drops below the dew point and a drop condenses on the thermocouple junction. Cooling is therefore stopped, and the drop starts evaporating into the air confined between the xylem and the instrument. The rate of evaporation is related to the relative humidity in the chamber, the higher the relative humidity, the longer the drop will take to evaporate (Boyer, 1995).

The response of the psychrometer was calibrated against solutions of known relative humidity derived as shown in the Appendix S1 according to Romero (1999) (Supplementary **Error! Reference source not found.** at JXB online). The thermocouple signal depends on two

parameters, the cooling time and the wait time (the time lag between the start of drop evaporation and the recording of the signal).

The instrument was calibrated at 20°C according to the procedure suggested by the manufacturer (ICT International 2017) after setting the wait time to 6 sec and the cooling time to 8 sec (the same setting was maintained for the measurements). The psychrometer was initially kept in a desiccator overnight to start from a condition of 0% relative humidity. Solutions of NaCl at different concentrations in the range of 0.1-1 molality were prepared to impose known values of relative humidity. These concentrations were selected in order to cover an adequate range of xylem water potential (from -0.45 to -4.55 MPa at 20 °C).

A filter paper disk was soaked with the first solution (1 molality) and placed in the disk holder provided by the manufacturer. The disk holder containing the filter paper disk was fitted to the psychrometer. Once the reading was recorded, the disk holder was removed, a new filter paper was soaked with the second solution, and again fitted to the psychrometer. This procedure was repeated for the remaining four NaCl solutions up to solution associated with 0.1 molality. The procedure was then reversed, i.e. filter paper disks with increasing molality were let to equilibrate with the psychrometer.

The following equation was considered for the calibration curve as suggested by the manufacturer

$$\Psi = \frac{\left(\frac{WBD}{C_1 \cdot T_c + C_2} - CI\right)}{CS} + \frac{\Delta T}{k}\,CF_{\Delta T} \qquad [2]$$

where Ψ is the measured water potential, C1 and C2 are empirically derived temperature correction coefficients provided by the manufacturer, and CS and CI are the calibration slope and intercept respectively to be determined via calibration as instrument-specific parameters. The variables measured directly by the instrument are the psychrometric wet bulb depression WBD (μV), the temperature chamber Tc (°C), the temperature differential between the two psychrometer thermocouples ΔT (μV). The chromel-constantan thermocouple output (61 μV/°C) and the ΔT correction for CFΔT (MPa/°C) are also provided by the manufacturer.

The parameters CS and CI were determined by performing the calibration at 20°C (in the range -0.45 to -4.55 MPa according to Supplementary **Error! Reference source not found.**). The thermocouple psychrometer showed an accuracy lower than 0.1 MPa at 20°C (standard deviation of the error).

To investigate the effect of temperature, a filter paper was soaked with NaCl solution at either 0.1 or 1M. The filter paper was placed in the disk holder in turn fitted to the psychrometer. The psychrometer together with the disk holder were placed in a climatic chamber and the

temperature was increased in steps of 5°C from 20 to 40°C and then reversed. A period of 20 min was sufficient for the signal to stabilise after each temperature variation.

The signal was recorded after each temperature increment or decrement and converted into water potential using Eq. [2] with the parameters CI and CS calibrated at 20°C. The measured value was compared with the theoretical value of water potential imposed by the solution of given molarity (see Appendix S1). The error is shown in Fig. 3C and D for two imposed water pressure, -0.4 and -4.5 MPa. It can be observed that the error is significant in the sense that it is higher than the standard deviation of the error associated with the calibration at 20°C. In addition, the error is relatively large (up to 0.66 MPa) and one order of magnitude greater than the HCT.

Pressure chamber

The Pressure Chamber (PC) used in this experimental programme is manufactured by PMS Instrument Company (Model 1515D). It can be used to measure the water tension in the range from 0 to 10 MPa by placing a leaf inside the sealed chamber with the cut end of the petiole protruding through the seal.

The plant material

Four broad-leaved young saplings were selected for the tests, a cherry tree (Bigarreau burlat), an oak tree (Quercus rubra), a pear tree (Pyrus communis, Supplementary data at JXB online), and a lemon tree (Citrus limon). Gymnosperms were avoided because of possible clogging of the HCT porous ceramic filter due to the presence of resin. The saplings (Supplementary **Error! Reference source not found.** and Supplementary **Error! Reference source not found.** both at JXB online), provided by an external nursery, were approximately 3-4 years old and came in pots of loose highly organic soil. Prior to the experiment, both saplings were kept in the laboratory under controlled temperature (20°C) and relative humidity (50%). They were irrigated regularly and kept under a growth lamp (Sylvania Gro-Lux T8, 36W, 3250 lumens).

Methods

High-Capacity Tensiometer

Conditioning

To achieve adequate saturation, the HCT porous ceramic filter was briefly exposed to air in order to generate high water tension and induce cavitation in the porous ceramic filter. The HCT was then saturated at 4 MPa pressure for at least 48 h in a saturation chamber (Tarantino, 2004). The HCTs were then removed from the saturation chamber and placed in water at atmospheric pressure. The porous ceramic filter was again exposed to air and the water tension was let to increase to around 1 MPa before placing it back in free water to release the

water tension generated. This procedure was repeated twice to relieve any residual stresses in the sensing diaphragm caused by the high positive pressure applied during saturation (Tarantino and Mongiovi, 2003). The tensiometers in free water were then zeroed.

Application to the stem

The current version of the HCT has a diameter of 12 mm and can be installed on stems or branches with diameter of 15 mm at least. The goal of the installation is to make the water in the xylem accessible to the instrument and avoid any localised evaporation from the contact area. The bark was removed, to expose an area of xylem of approximately the same dimension of the HCT (Supplementary **Error! Reference source not found.**A at JXB online). The surface was then cleaned with a few drops of distilled water to remove any remaining living cell. The scratching procedure is the same used for the TP installation (ICT International 2021). However, the exposed xylem surface was kept wet before the installation to avoid desiccation of the xylem tissues. The HCT was installed on the stem using a saturated paste of kaolin to ensure hydraulic contact between the xylem and the porous ceramic filter (Supplementary **Error! Reference source not found.**B). A latex membrane was then used to wrap tightly the area and avoid any evaporation from the paste (Supplementary **Error! Reference source not found.**C). The paste was prepared at approximately its liquid limit and was a compromise between two conflicting requirements. Water content should be sufficiently high to give enough plasticity to the paste and ensure good adherence between the HCT and the xylem. On the other hand, excessive water content would increase considerably the equilibration time due to the amount of water that needs to be sucked out of the paste by the xylem to reach equilibrium.

Measurement data quality check

Following installation of the HCTs, the water tension changes very rapidly due to hydraulic equilibration between the instrument and the xylem. The saturated paste needs to lose water to the xylem until equilibrium is achieved. The HCT readings during the equilibration are not representative of the water status of the plant and are discarded.

The presence of 'stable' air cavities in the porous ceramic filter may affect the measurement of the HCT, generating a differential between the tension in the xylem and the tension in the water reservoir of the instrument (Tarantino, 2004). For this reason, the HCT measurement is crossed-checked by installing two HCTs simultaneously on the same stem. The HCT measurement is considered to be valid if the readings of the two HCTs overlap. If the two measurements diverge, which is likely due to ongoing expansion of air cavities in one of the two HCTs, the measurement of both tensiometers is discarded (since it is generally not possible to recognise which tensiometer generated the faulty measurement). The need of using HCTs in pairs is consistent with the suggestion by Tarantino and Mongioví (2001) when discussing measurement in soil.

If cavitation occurs in the tensiometer, the measurement following cavitation are discarded because water tension is no longer transmitted to the pressure sensor diaphragm. Cavitation is easily detected because of the abrupt rise of water potential to -0.1 MPa.

Post-measurement data quality checks

The presence of 'stable' air cavities in the porous ceramic filter may affect the measurement of the HCT, generating a differential between the tension in the xylem and the tension in the water reservoir of the instrument (Tarantino, 2004). The presence of spurious air cavities is checked at the end of each measurement. If the HCT does not cavitate during the measurement, it is placed in pure water and it is checked that the initial zero water potential is recovered (a residual water potential in the range from 0 to -0.02 MPa is considered acceptable according to Tarantino and Mongioví (2001). If the HCT cavitates during the measurement, it is checked that the water potential rises to -0.1 MPa upon cavitation.

Thermocouple Psychrometer

The integrity of the thermocouple was assessed under stereo microscope before each installation. The installation site on the stem was prepared by removing the cork and the living tissues underneath (cambium). The exposed xylem was cleaned with few drops of distilled water and wiped dry. The psychrometer was then installed on the stem by ensuring that one junction of the thermocouple was in contact with the xylem. The gap between xylem and psychrometer was insulated with Parafilm⊕ and silicon grease to allow the water vapour surrounding the thermocouple to achieve equilibrium with the xylem water. The cooling time was set to 8 seconds and the wait time to 6 seconds consistent with the setting adopted for calibration.

Pressure chamber

Three samples of non-transpiring leaves were taken for each measurement. Each leaf was wrapped in aluminium foil and inserted in a plastic bag at least 2 hours before the leaf was excised and the measurement taken. When the leaf stops transpiring, the water in the leaf equilibrates with the water in the xylem (Lang and Barrs, 1965) and, as a result, the water tension measured in the leaf can be assumed to be equal to the water tension in the branch at the base of the petiole (Richter, 1973).

Instrument configuration

Cherry sapling

The position of the instruments is shown in Fig. 4A. The HCTs and the TP were spaced by around 10 cm. The installation sites were selected in order to have a stem diameter wide enough to allow the installation of the instruments. There were no junctions of secondary branches between the instruments. The tensiometers HCT5 and HCT6 and the psychrometer PSY1 were installed at the beginning of the test. When the HCTs cavitated, these were replaced with the tensiometers HCT2 and HCT4 installed at slightly different heights.

The sapling was kept well irrigated before the test. During the test, the sapling was kept in a laboratory at constant temperature and relative humidity, in proximity of a growth lamp to mimic solar radiation (the lamp was switched on from 6 am to 8 pm and switched off from 8 pm to 6 am). The sapling was let to enter a condition of drought over the first 18 days by stopping any irrigation. Water was then added on day 18 and on day 27. These different conditions were imposed in order to explore different ranges of xylem water potential. A few Pressure Chamber measurements were taken throughout the test as a reference.

Oak sapling

The instruments were installed with a spacing of approximately 10 cm (Fig. 4B) with the TP between the two HCTs. At the beginning of the test, two HCTs were installed at 84 cm (medium HCT) and 102 cm (high HCT) from the level of the soil respectively. On day 13, a new HCT was installed at 71 cm (low HCT).

There were no junctions of secondary branches between the TP and the medium HCT. On the other hand, there was a junction of secondary branch between these two instruments and the low and high HCTs respectively. However, the experimental data presented in the next section have shown that the low and high HCT (positioned below and above junctions respectively) and the medium HCT (positioned between two consecutive junctions) did not exhibit significant differences (lower than 0.073 MPa on average as shown in Supplementary **Error! Reference source not found.** at JXB online) and, hence, the presence of the junction did not affect the measurements.

The tree was not irrigated for 19 days and this generated low xylem water potential making the HCTs more prone to cavitation. The third HCT was then added to increases the probability of having at least two active HCTs on the stem at the same time. When a HCT cavitated, it was removed for re-saturation and substituted with a freshly saturated HCT. The new HCT was placed on the same installation site after removing a further xylem layer to expose fresh xylem.

Pressure chamber readings were taken approximately every 3 days during the first 14 days and once a day afterwards (twice a day when the water potential was at its minimum).

Before the test, the oak sapling was kept in the lab and irrigated regularly. During the test, it was kept in a laboratory at constant temperature and relative humidity, in proximity of a growth lamp to mimic solar radiation (the lamp was switched on from 6 am to 8 pm and switched off from 8 pm to 6 am). Irrigation was stopped during the first part of the test to achieve drought conditions. On day 19 the soil was submerged with water and kept fully saturated until day 25. Afterwards, the water was allowed to drain freely from its bottom.

Lemon sapling

The instruments were installed with spacing between 3 and 8 cm (Fig. 4C). At the beginning of the test, three HCTs were installed at 6 cm (Low HCT), 11 cm (Medium HCT) and 22 cm (High HCT) from the level of the soil respectively. The Thermocouple Psychrometer, PSY1, was installed between the Medium and High HCTs at 14cm from the soil surface.

When a HCT cavitated, it was removed, re-saturated for at least 24hrs and re-installed on the same installation site after removing a further xylem layer to expose fresh xylem. The medium and high HCTs were removed and reinstalled on day 22 without re-saturating them and only exposing fresh xylem.

The sapling was kept well irrigated before the test. During the test, the sapling was kept in a laboratory at constant temperature and relative humidity, in proximity of a growth lamp to mimic solar radiation (the lamp was switched on from 6 am to 8 pm and switched off from 8 pm to 6 am). The sapling was let to enter a condition of drought over the first 8 days by stopping any irrigation. Water was then added on day 8, 16, 28, 29, 32 and 35 in different amounts to explore the response of the instruments upon different increments in water potential. A few Pressure Chamber measurements were taken throughout the test as a reference.

Results

Cherry sapling

The measurements of xylem water potential on the oak sapling via the HCT and the TP are compared in Fig. 5. The measurements by the Pressure Chamber are also reported on the same figure. Daily cycles are clearly visible in the HCT and TP continuous measurement, which are consistent with the cycles imposed by the growth lamp. The xylem water potential reached its minimum around 3pm when the lamp was on and reached its maximum at around 6am when the lamp was off. The daily fluctuations were quite limited in the first 10 days of the test (~0.08 MPa) and amplified afterwards (~0.15 MPa).

The psychrometer measured significantly higher values of xylem water potential than the HCTs in the first 5 days (when xylem water potential was relatively high). From day 5 to day 16, only the psychrometer measurement is available. Its measurement was significantly higher than

that of the pressure chamber although the differential tended to reduce when the psychrometer readings started reducing due to prolonged drought.

From day 16 onward, the HCT and TP measurements were very consistent in terms of both values measured and response time (measurement differential was 0.065±0.021 MPa, Supplementary **Error! Reference source not found.**). The HCTs and the TP also responded promptly to watering on day 17 and day 26. HCT and TP measurements were higher than the pressure chamber although the differential tended again to reduce at lower xylem water potentials (from ~0.3 MPa on day 18 to <0.1 MPa on day 27)

Oak sapling

The measurements of xylem water potential on the oak tree via the HCTs and the TP are compared in Fig. 6. The measurements by the Pressure Chamber are also reported on the same figure. The soil was initially under well-watered conditions and was then let to enter drought conditions by stopping any watering for the first 18 days. The minimum xylem water potential was reached at day 18.

The soil was then submerged on day 18 and kept submerged under water until day 25 to release the water tension in the xylem (large grey area in the graph). The instruments responded promptly to the submersion on day 18 showing a sudden increase in water potential in the first 10 hours following submersion. From day 19 to the end of the test, the xylem water potential kept increasing at a slower rate.

Daily cycles are clearly visible in both HCT and TP measurements. However, the daily fluctuations appear to be relatively small over the first five days under well-watered conditions and between day 15 and 18 under drought conditions when the xylem water potential dropped below -1.5 MPa. Daily cycles were consistent with the cycles imposed by the growth lamp, with maximum values of xylem water pressure recorded around 6 am (the growth lamp was switched on at 6am).

When comparing the HCTs and the TP, three intervals can be clearly identified in Fig. 6. In interval I, the measurement of the TP showed higher water potential values than the HCTs (measurement differential was 0.176±0.102 MPa, Supplementary **Error! Reference source not found.**). Measurements by the pressure chamber were not always consistent with those by the HCTs and the TP, i.e. the pressure chamber matched the HCT on days 2 and 3 and the TP on days 5.5. and 7.5.

In interval II, the HCTs and the TP were very consistent in terms of both values measured and response time (measurement differential was 0.028±0.045 MPa, Supplementary **Error! Reference source not found.**). In this interval, the pressure chamber measurements matched both HCT and TP before water submersion whereas the pressure chamber returned values of

water potential higher than both HCT and psychrometer after water submersion. In this interval, the water potential measured by the TP remains lower than -0.5 MPa. It should also be noted that after submersion (day 18 to day 22), the HCT and the TP are very consistent suggesting that the measurement of xylem water potential is reliable. Nonetheless, the water potential measured by the pressure chamber appears to be higher than both HCT and TP.

In interval III, the water potential recorded by the TP and the HCTs deviated significantly (measurement differential was 0.382±0.151 MPa, Supplementary **Error! Reference source not found.**). The TP kept increasing until returning 'out-of-range' positive values. At the same time, the pressure chamber measurements during and after submersion were again higher than the HCTs. The trend of the HCT and pressure chamber measurements appear to be very similar, as if they are both driven by the same 'boundary condition'. The measurement by the TP follows an entirely different trend compared to the pressure chamber and the HCT.

Lemon sapling

The measurements of xylem water potential on the lemon tree via the HCTs and the TP are compared in Fig. 7. The measurements by the Pressure Chamber are also reported on the same figure. The soil was initially under well-watered conditions and was then let to enter drought conditions by stopping any watering for the first 8 days. In total, 6 watering events took place during the 42 days of this experiment, on days 8, 16, 28, 29, 32 and 35. All sensors (HCTs and TC) responded immediately to watering events.

Daily cycles are clearly visible in the HCT and psychrometer continuous measurement, which are consistent with the cycles imposed by the growth lamp. The xylem water potential reached its minimum around 3pm when the lamp was on and reached its maximum at around 6am when the lamp was off.

Before the first watering on day 8 (Interval I), the HCT and TP measurements were consistent in terms of both values measured and response time, with TC measurements slightly higher than the HCTs (measurement differential was 0.081±0.030 MPa, Supplementary **Error! Reference source not found.**). Those measurements were also very similar to the pressure chamber measurements taken on day 1 and day 8 (before watering).

After the first watering on day 8 following the drop of water potential to ~ -1MPa and second watering on day 16 following the drop of water potential to ~ -1.3MPa (Interval II), water potential measured by the TP increased much more than the HCTs. The discrepancy remained over the entire Interval II with the exception of the period from day 15 to day 16 where the water potential measured by the TP dropped to values lower than -0.5 MPa (differential in this time interval was 0.521±0.315 MPa, Supplementary **Error! Reference source not found.**). Pressure chamber measurements in Interval II are lower than TP (consistent with transpiration-induced xylem water flow) and higher than the HCTs.

On day 22 (Interval III), the Medium and High HCTs were removed and re-installed straightway on the same installation site after removing a further xylem layer to expose fresh xylem. A new fresh kaolin paste was added to establish hydraulic connection between the HCT and the xylem water. The two re-installed HCTs (Medium and High HCTs) immediately aligned with the TP. The Low HCT (not removed from the xylem) measured water potential significantly lower than the TP and the other two HCTs until their measurement dropped below -0.5 MPa on day 25. Again, as the value measured by the TP reduced below -0.5 MPa, discrepancies between TP and the Low HCT also vanished. The differential between the TP and the average of Medium and High HCTs was 0.050±0.100 MPa in this time interval (Supplementary **Error! Reference source not found.**).

After watering on day 28 following the drop of water potential to ~ -1.7 MPa (Interval IV), this behaviour is again observed. As the value measured by the TP reduced below -0.5 MPa, discrepancies between TP and HCTs essentially vanished (differential for TP< -0.5MPa was 0.026±0.143 MPa, Supplementary **Error! Reference source not found.**). After watering on days 28, 32, and 35 with the water potential measured by TP raising above -0.5 MPa, discrepancies reappeared vanished (differential for TP≥ -0.5MPa was 0.143±0.087 MPa, Supplementary **Error! Reference source not found.**).

Discussion

Response time of TP and HCT

The response time of the HCT and the TP are controlled by very different mechanisms. Equilibration time is controlled by the flow of liquid water to and from the paste for the case of the HCT whereas it is controlled by the water vapour transfer from and to the air gap adjacent to the xylem for the TP. Since the HCT and the TP were found to respond remarkably in phase to changes in boundary conditions, in particular, they responded very promptly to watering, it can be concluded that response time of both instruments is adequate to capture hourly variations of xylem water potential. This is a major outcome of this study achieved thanks to the real-time comparison of these two instruments.

Measurement precision of TP and HCT at low values water potential

The precision of the HCT and TP measurement needs to be discussed separately depending on whether the water potential measured by the TP is lower or higher than ~ -0.5 MPa. For the case where the water potential is lower than ~ -0.5 MPa, the two instruments return very similar measurements as shown in Fig. 5, Fig. 6, and Fig. 7. This is a second major outcome of this study. Hitherto the TP could only be compared with the pressure chamber to validate its measurement. However, the comparison between the measurement of water potential at two different sites along the transpiration-induced flow path (as is the case when comparing TP installed on the stem and pressure chamber testing excised leaves) is not straightforward. Since

water flow requires water potential gradients, a water potential differential shall establish between the stem and the junction between the branch and the leaf petiole. This water potential differential is not always negligible as shown experimentally by Dainese and Tarantino (2000) and, as a result, the pressure chamber does not represent in principle a valid measurement to benchmark the measurement of the TP. This study allowed for the first time ever assessing the precision of the TP by benchmarking its measurement against an independent measurement at the same site in the transpiration-induced water flow path. Reciprocally, the TP allowed validating the HCT measurement at least in the range of water potential lower than -0.5 MPa.

Measurement precision of TP and HCT at high values of water potential

Significant discrepancies between the TP and HCT generally appeared in the range where the TP measured water potentials greater than ~ -0.5 MPa (see interval III in Fig. 6, interval I, II, and IV in Fig. 7) and the question is whether the 'faulty' measurement must be attributed to the HCT or the TP. Inspection of the measurements on the four saplings reveals that there were two exceptions, the measurement on the Pear sapling (Supplementary **Error! Reference source not found.**) and measurement of the Lemon sapling in Interval III (Fig. 7). These two sets of measurement have in common the kaolin paste that was never exposed to water potential lower than current measured value (water potential of the paste is zero at installation). Fig. 7 also shows clearly that the difference between the HCT and TP depends on the paste and not the HCT. The High and Medium HCTs used at the end of Interval II on the Lemon sapling (where significant differences appear between the TP and HCT measurement) are exactly the same HCTs used at the beginning of Interval III (where the TP and HCT measurements match remarkably). The difference between these two intervals is the lowest water potential ever experienced by the kaolin paste. In Interval II, the kaolin paste was brought to water potentials lower than -1MPa before its water potential increased again due to watering. In Interval III where a new fresh paste was applied, the paste had never experienced a water potential lower than the value currently measured. This suggests that the hydraulic history of the kaolin paste plays a role. The water retention behaviour of the kaolin initially prepared from a slurry state was investigated by Tarantino (2009) and is shown in Supplementary **Error! Reference source not found.** at JXB online. When drying the paste from its slurry state, the paste remains fully saturated until a water potential of ⦸-1 MPa (air-entry value). In this range the paste is efficient in transmitting water potential and this explains the good match of HCT and TP measurement on the Pear sapling (Supplementary **Error! Reference source not found.**) and measurement of the Lemon sapling in Interval III (Fig. 7). If the paste is dried out, i.e. it experiences water potentials lower than the air-entry value (-1 MPa), the paste de-saturates. This does not prevent the transmission of water potential as shown in Interval III of Fig. 7 where TP and HCT measurements match fairly well. Upon rewetting, the kaolin never recovers full saturation due to the air cavities remaining occluded in the pore space. Remarkably, the air-occlusion value of -0.5 MPa matches the xylem water potential where discrepancies were observed between TP

and HCT. It can therefore be concluded that if the paste first experiences water potential lower than its air entry (i.e. the paste de-saturates) and the water potential then increases again to values higher than -0.5 MPa, air cavities remain occluded in the paste and this hampers the proper transmission of the water potential.

The measurement of the HCT in the range from 0 to -0.5 MPa is therefore probably not reliable. At the same time, concerns also arise about the TP measurement in the range from 0 to -0.5 MPa. The measurement of the HCT at the beginning of the tests on Cherry and Oak saplings (Fig. 5 and Fig. 6) should not be affected by the fresh paste applied to the HCT and the discrepancy may be due to the TP rather than the HCT. Furthermore, the positive values returned by the TP at the end of the test on the Oak sapling (shown as 'zero' in Fig. 6) also seem to suggest that the TP might be not very accurate in this range. However, no clear conclusions can be drawn, and further investigation is required to address this issue.

Comparison of TP and HCT against pressure chamber

The TP and HCT can be further investigated by benchmarking their measurements against the pressure chamber measurements.

The good agreement between the HCT and TP at water potentials (as recorded by the TP) lower than ~ -0.5 MPa also emerges from Fig. 8A, C, D, F, and I. The measurement by the HCT and TP generally returns values of water potential higher than the pressure chamber, which is consistent with the direction of transpiration-induced sap flow. The discrepancy tends to reduce at lower values of water potential which is also intuitive. The transpiration is likely to enter a water-limited condition in this range, i.e. stomata partially close to reduce transpiration and this generates smaller water potential differential between the leaf and the stem. Overall, these figures show that the measurement by the pressure chamber on excised leaves can be significantly lower than the measurement of water potential at the stem (via TP and HCT) and it should therefore be used with care to validate TP or HCT measurements. It should also be noted that the water potential measured by the pressure chamber is higher than both HCT and TC after submersion of the soil in the Oak sapling test (Fig. 8D, Interval II_submersion). This might be associated with the leaves entering a state of anaerobiosis but a discussion of the processes leading to this reversed water potential differential between leaves and stem is out of the scope of this paper. However, Fig. 8D (Interval II_submersion) again confirms that the pressure chamber measurement may not always be considered as a reference to validate either TP or HCT measurements.

In the low water potential range (Fig. 8B and E, Intervals I and III, and Fig. 8G, Interval II), the pressure chamber does not seem to support either the TP or HTC measurement. Again, further studies should be carried out to investigate the precision of the measurement by HCT and TP at high water potentials.

Conclusions

This paper has cross-validated two different experimental techniques for continuous non-destructive measurement of xylem water potential, the High-Capacity Tensiometer and the TP.

The HCT and the TP were found to respond remarkably in phase to changes in boundary conditions, in particular to watering, despite very different working principles. It was concluded that response time of both instruments is adequate to capture hourly variations of xylem water potential and this is a major outcome of this study achieved thanks to the real-time comparison of these two instruments.

The HCT and the TP returned very similar xylem water potential readings for water potential values $< \sim$ -0.5 MPa (differences were typically lower than 0.10-0.15 MPa). Again, as the working principle of these two instruments is very different, these measurements made it possible to demonstrate that the HCT and the TP show satisfactory accuracy in this range of xylem water potential. Hitherto the TP could only be compared with the pressure chamber to validate its measurement. However, the water potential at the leaf (junction between leaf petiole and branch) can be significantly different from the water potential at the stem as demonstrated in this paper and as expected theoretically (transpiration-induced water flow requires a non-zero water potential differential between stem and leaves). As a result, this study has provided, for the first time, a robust assessment of the TP by benchmarking its measurement against an independent measurement at the same site in the transpiration-induced water flow path.

At water potential higher than $\sim$ -0.5 MPa, the measurement of the HCT may be affected by the clay paste used to make contact between the HCT porous ceramic filter and the xylem. If the clay paste is subjected to water potential lower than its air-entry values ($\sim$-1 MPa), it is subjected to desaturation. If rewetting is associated with water potentials higher than its air-occlusion value ($\sim$ -0.5 MPa), air cavities may remain entrapped in the paste hampering the transmission of water potential from the xylem to the HCT. Entrapped air cavities only play a role if the clay paste is first subjected to drying and then rewetting (due to hydraulic hysteresis). This problem did not appear if the clay paste is subjected to a current water potential that is the lowest ever experienced (monotonic drying path). This is a current limitation of the HCT that can be overcome by selecting a clay paste with enhanced air-entry value. For example, the London clay reconstituted from slurry tested by Marinho (1994) shows an air-entry value of the order of 10 MPa and this would have remained saturated under the water potentials investigated in this work. At water potential higher than $\sim$ -0.5 MPa, the measurement by TP also presents some inconsistencies, which would however require further studies to be investigated.

Finally, this cross-validation has been carried out in the laboratory at 20°C (HCT and TP were also calibrated at the same temperature). In the field, temperature can vary significantly and the performance of these two instruments can vary significantly. To investigate temperature effects, the TP and the HCT were calibrated in the laboratory at different temperature. It was shown that the effect of temperature on HCT measurement is negligible (error < 0.03 MPa) whereas it becomes significant for the TP, which showed error up to 0.66 MPa when temperature varied from 20 to 40°C.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author, (Alessandro Tarantino), upon request.

Author Contribution

R.D., L.L., S.D., T.F. and A.T.: Conceptualization; R.D., B.C.F.L.L and G.T.: Data Curation; R.D., B.C.F.L.L, G.T. and A.T.: Formal Analysis; T.F. and A.T.: Funding Acquisition; R.D., B.C.F.L.L, and G.T.: Investigation; R.D., B.C.F.L.L, G.T., L.L., S.D., T.F. and A.T.: Methodology; T.F. and A.T.: Project Administration; T.F. and A.T.: Resources; R.D. and A.T.: Software; T.F. and A.T.: Supervision; R.D., B.C.F.L.L, G.T., L.L., S.D., T.F. and A.T.: Validation; R.D., B.C.F.L.L, G.T. and A.T.: Visualization; R.D., B.C.F.L.L, G.T., S.D., and A.T.: Writing – Original draft; R.D., B.C.F.L.L, G.T., L.L., S.D., T.F. and A.T.: Writing: review and editing.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

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FIGURE LEGENDS

Fig. 1. Water phase diagram. (a) Stable states. (b) Metastable states

Fig. 2. High-capacity Tensiometer (after by Tarantino and Mongiovì, 2002)

Fig. 3. Effect of temperature on HCT measurement: (a) Imposed water potential ▯=0.2 MPa. (b) Imposed water potential ▯=2.2 MPa; Effect of temperature on Thermocouple Psychrometer measurement: (c) Imposed water potential ▯≈-0.4 MPa (0.1m NaCl solution). (d) Imposed water potential ▯≈-4.5 MPa (0.988 m NaCl solution)

Fig. 4. Instruments position on the (a) cherry sapling, (b) oak sapling, and (c) lemon sapling

Fig. 5. Measurement of xylem water pressure via High Capacity Tensiometer (HCT), Thermocouple Psychrometer (TP), and the Pressure Chamber (PC) on non-transpiring leaves on the Cherry sapling (grey diamonds represent the average value and the error band the standard deviation of the Pressure Chamber measurements). The vertical grey bands indicate watering. The horizontal grey line marks the value of -0.5 MPa xylem water pressure.

Fig. 6. Measurement of xylem water potential measured by HCT, Thermocouple Psychrometer (TP), and Pressure Chamber on non-transpiring leaves on the Oak sapling (grey diamonds represent the average value and the error band the standard deviation of the Pressure Chamber measurements). The grey area indicates the submersion of the soil. The horizontal grey line marks the value of -0.5 MPa while the vertical dotted line separates the interval of xylem water pressure measurement by TP above (I-III) or below (II) -0.5 MPa.

Fig. 7. Measurement of xylem water pressure via High Capacity Tensiometer (HCT), Thermocouple Psychrometer (TP), and Pressure Chamber (PC) on non-transpiring leaves on the Lemon sapling (grey diamonds represent the average value and the error band the standard deviation of the Pressure Chamber measurements). The vertical light grey bands indicate watering, while the vertical dark grey bands represent the time in which HCT's were re-installed.

Fig. 8. Comparison of xylem water potential by the Pressure Chamber (horizontal axis) versus the Thermocouple Psychrometer TP (open circles) and the HCT (solid diamonds) measured on the: (a) Cherry sapling, (b) oak sapling - Interval I, (c) oak sapling - Interval II drought, (d) oak sapling - Interval II submersion, (e) oak sapling - Interval III, (f) lemon sapling – Interval I, (g) lemon sapling – Interval I, (h) lemon sapling – Interval I, and (i) lemon sapling – Interval I.
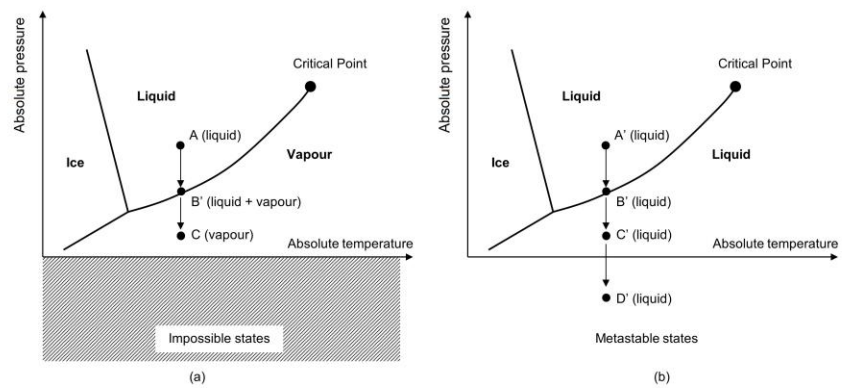
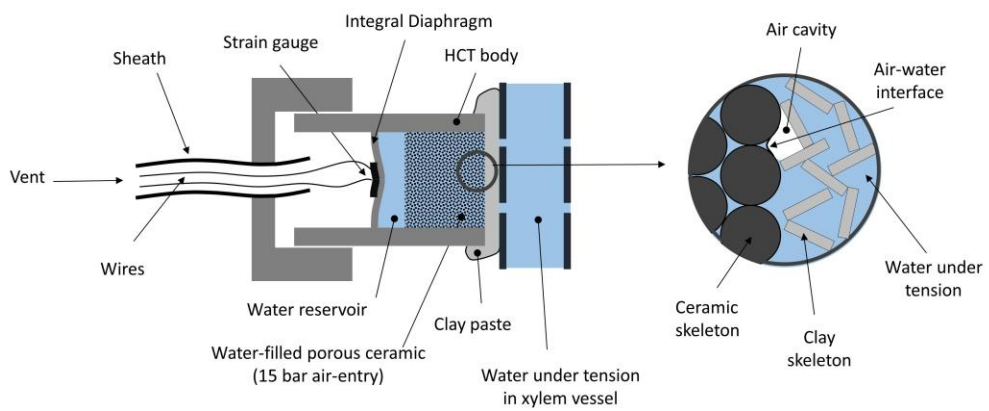Fig. 1. Water phase diagram. (a) Stable states. (b) Metastable states

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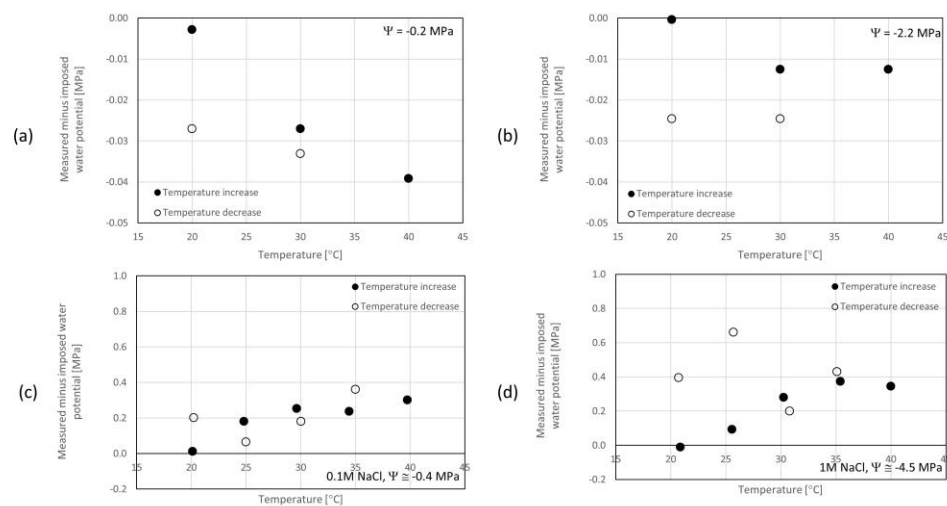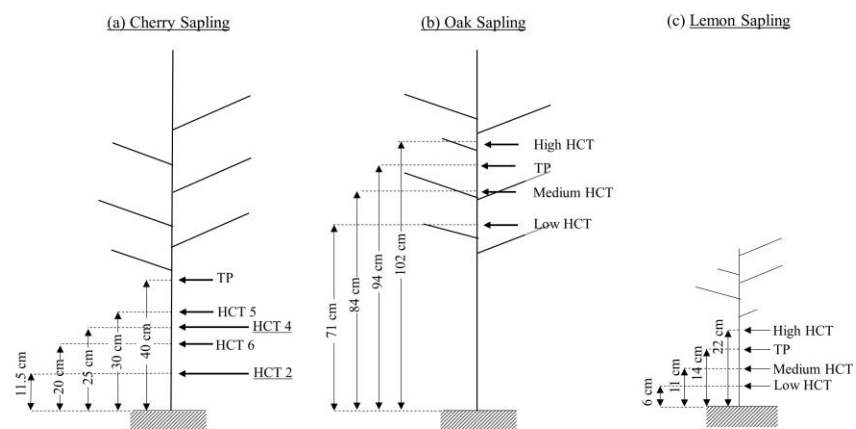
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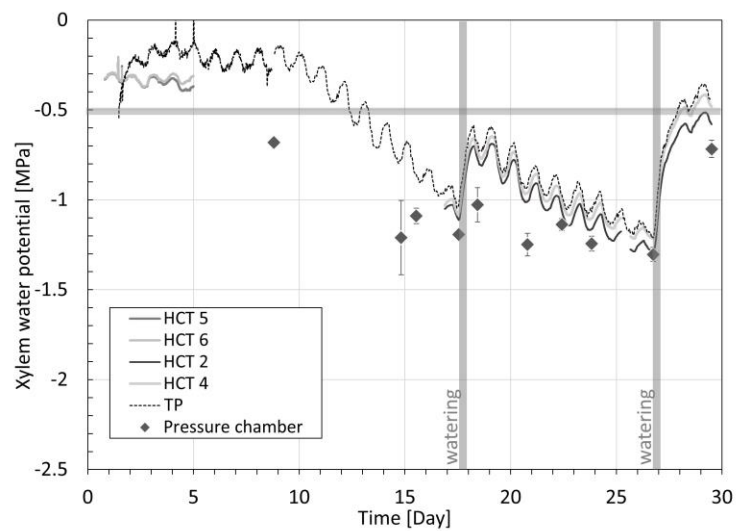Fig. 4. Instruments position on the (a) cherry sapling, (b) oak sapling, and (c) lemon sapling

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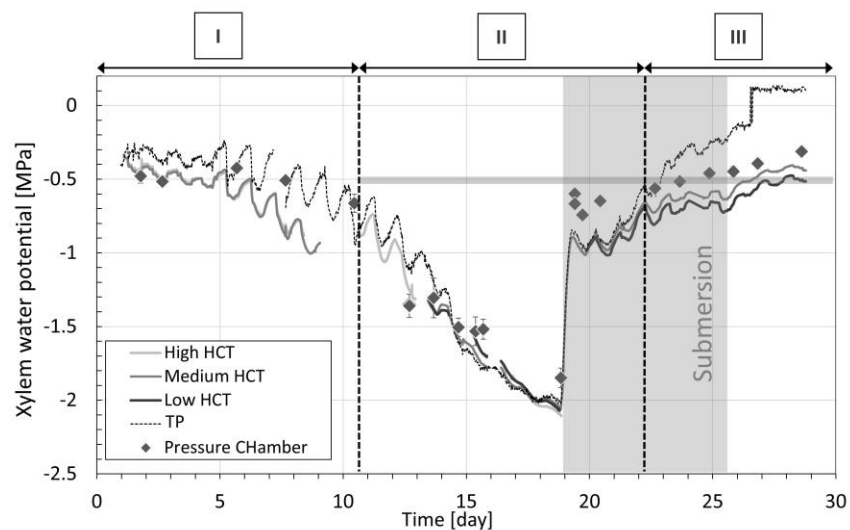
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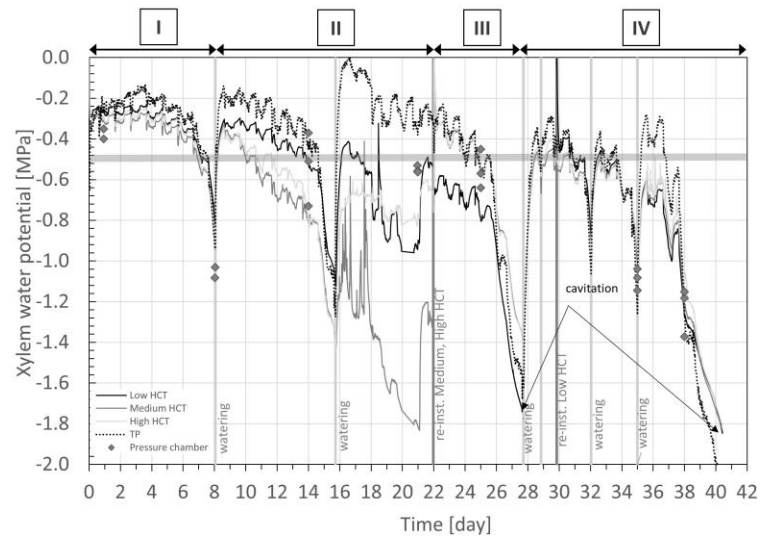
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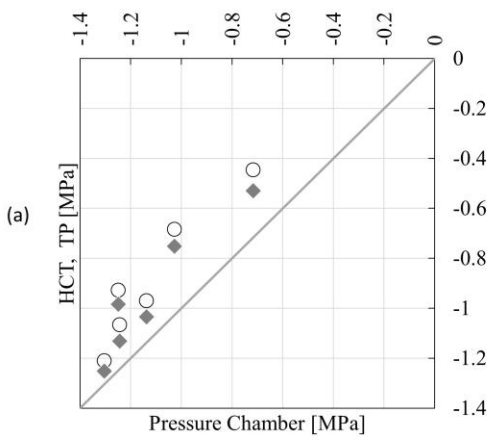
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Interval I

(b)

HCT, TP [MPa]

Pressure Chamber [MPa]

Interval II_drought

(c)

HCT, TP [MPa]

Pressure Chamber [MPa]

Interval II_submersion

(d)

HCT, TP [MPa]

Pressure Chamber [MPa]

Interval III
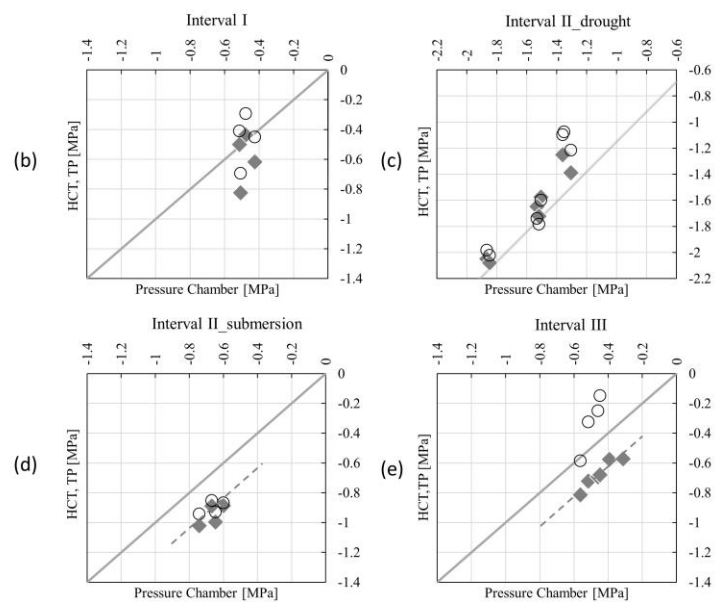
(e)

HCT,TP [MPa]

Pressure Chamber [MPa]

Fig. 8. Comparison of xylem water potential by the Pressure Chamber (horizontal axis) versus the Thermocouple Psychrometer TP (open circles) and the HCT (solid diamonds) measured on the: (a) Cherry sapling, (b) oak sapling - Interval I, (c) oak sapling - Interval II drought, (d) oak sapling - Interval II submersion, (e) oak sapling - Interval III, (f) lemon sapling – Interval I, (g) lemon sapling – Interval I, (h) lemon sapling – Interval I, and (i) lemon sapling – Interval I.

(f) Interval I

(g) Interval II

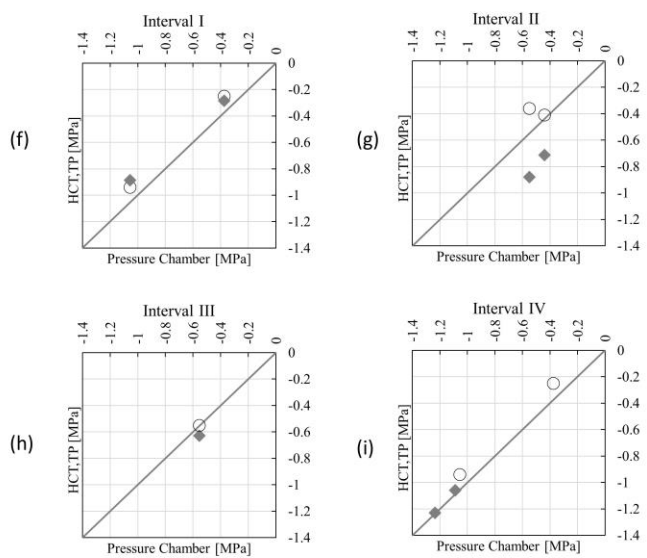**(h)** Interval III

(i) Interval IV

Fig. 8. Comparison of xylem water potential by the Pressure Chamber (horizontal axis) versus the Thermocouple Psychrometer TP (open circles) and the HCT (solid diamonds) measured on the: (a) Cherry sapling, (b) oak sapling - Interval I, (c) oak sapling - Interval II drought, (d) oak sapling - Interval II submersion, (e) oak sapling - Interval III, (f) lemon sapling – Interval I, (g) lemon sapling – Interval I, (h) lemon sapling – Interval I, and (i) lemon sapling – Interval I.