# Leaf water maintains daytime transpiration in young Cryptomeria japonica trees

Himeno, Sawa
Azuma, Wakana
Gyokusen, Koichiro
Ishii, Hiroaki

(Citation)
Tree Physiology,37(10):1394-1403

(Issue Date)
2017-10-01

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90005376

# Leaf water maintains daytime transpiration in young *Cryptomeria japonica* trees

SAWA HIMENO[1]

WAKANA AZUMA[2, 3]*

KOICHIRO GYOKUSEN[1]

H. ROAKI ISHII[2]

[1] Graduate School of Agriculture, Kyushu University. Fukuoka, JAPAN

[2] Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, JAPAN

[3]Present address: Field Science Education and Research Center, Kyoto University, Kyoto 606-8502, JAPAN

*Author for correspondence:

Email: wakana@kais.kyoto-u.ac.jp

Phone: +81-75-753-6428

## Abstract

Compared with stem water storage, leaf water storage is understudied although it may be important for alleviating water stress by contributing quickly and directly to transpiration demand. To quantify the relative contribution of stem versus leaf water storage to daily water deficit, we measured diurnal changes in transpiration rate, sap-flow velocity, and stem radius of 10-year-old *Cryptomeria japonica* D. Don trees. We assumed that the duration of time lags between transpiration rate and sap-flow velocity reflected stored water in the stem and leaf and that stem volume change represented water content of elastic tissue. The relationship between fresh mass and water potential of the whole tree indicated that the study trees had capacity to store, on average, 91.4 ml of water per kg$^{-1}$ fresh mass at turgor loss. Leaves, sapwood, and elastic tissue contributed around 51%, 29%, and 20% of stored water, respectively. During morning, transpiration rates were higher than sap-flow velocity suggesting depletion of stored water. During the first 2 hr after onset of transpiration, stored water contributed more than 100% of whole-tree transpiration. Depletion of leaf water ($P_{\mathrm{Leaf}}$) and sapwood water ($P_{\mathrm{Sap}}$) coincided with the onset of transpiration and became maximum around 15:00. Depletion of elastic tissue water ($P_{\mathrm{Elastic}}$) lagged behind that of $P_{\mathrm{Leaf}}$ and $P_{\mathrm{Sap}}$ by 1−2 hour indicating that replenishment of stored water occurs late in the day when low leaf water potentials resulting from day-time transpiration drive water uptake. Maximum depletion of $P_{\mathrm{Leaf}}$ was about 1−3 times and 5−10 times that of $P_{\mathrm{Sap}}$ and $P_{\mathrm{Elastic}}$, respectively. The contribution of $P_{\mathrm{Leaf}}$ to total daily transpiration was 5−8%, while those of $P_{\mathrm{Sap}}$ and $P_{\mathrm{Elastic}}$ were 3−4% and 0.7−1%, respectively. Our results suggest the importance of leaf water storage in maintaining daily transpiration in young *C. japonica* trees.

**Keywords:** hydraulic capacitance, sap flow, transpiration, water-relations

## Introduction

Foliar water storage is an important mechanism for maintaining physiological function, especially in arid environments (Chaves et al. 2002, Von Willert et al. 1990). Succulent plants survive dry periods by using water stored in thick leaves (Ogburn and Edwards, 2010, 2012, Ripley et al. 2013, Vendramini et al. 2002). In tall trees, treetop leaves experience similarly water-stressed environments with high levels of solar radiation and evapo-transpirational demand. Tall trees often experience water deficit during the day when transpiration rate in the canopy (water demand) is higher than the sap flow rate in the stem (water supply) (Meinzer et al. 2006). In tall trees, water stored in sapwood, is important for compensating this deficit (Waring and Running 1978; Meinzer et al. 2003, Phillips et al. 2003) and maintaining daytime transpiration rates (Goldstein et al. 1998). Estimates of the contribution of sapwood water storage to daily transpiration vary widely among species (e.g., Waring et al. 1979; Lostau et al. 1996; Goldstein et al. 1998; Kobayashi & Tanaka 2001) and with tree size (Phillips et al. 2003). Although much lesser in amount compared to sapwood, water may also be stored in phloem elastic tissues (Cermak et al. 2007). Collectively, stem water storage may play a significant role in buffering decreases in xylem water potentials during periods of high transpiration (Holbrook and Sinclair 1992).

Recent studies have suggested that water stored in leaves may contribute more quickly and directly to transpiration demand in trees (Zweifel et al. 2001, Ishii et al. 2014, Azuma et al. 2016). In six-year-old potted trees of *Picea abies* L., water stored in leaves contributed eight times more water to daily transpiration than that in the stem (Zweifel et al. 2001). It was recently found that tree-top leaves of *Sequoia sempervirens* (D. Don) Endl., the world's tallest species, have capacity to store nearly five times the daily transpiration demand and this may compensate for hydraulic constraints on leaf photosynthesis with increasing height by decreasing reliance on root water supply (Ishii et al. 2014). In addition to the thick parenchyma, leaves of *S. sempervirens* have transfusion tissue, which are unique to gymnosperms, surrounding the vascular bundle (Oldham et al. 2010). The amount of transfusion tissue in the leaf increases with increasing height suggesting it may function as sites for foliar water storage (Ishii et al. 2014). A similar mechanism for foliar water storage was also discovered in the tallest trees in Japan, 50-m *Cryptomeria japonica* D. Don (Azuma et al. 2016). There are a few hydraulic plant models which include water storage dynamics in crown and stem (e.g. Zweifel et al. 2007), and compared with stem water storage, foliar water storage in trees is understudied and the relative contribution of stem versus leaf water storage to daily water use in trees is not well understood.

The objective of this study was to quantify the relative contribution of stem versus foliar water storage to daytime water deficit in a young conifer tree. We made simultaneous measurements of leaf transpiration rate, sap-flow velocity, and stem volume changes (mainly induced by bark (phloem, cambium, and parenchyma) swelling and shrinkage; Zweifel et al. 2000) using 10-year-old *C. japonica* trees. We also combined these measurements with whole-tree pressure-volume analysis to show how water stored in stem and leaf are depleted and replenished during the course of a sunny day.

## Materials and Methods

The study was conducted in the nursery field of Kyushu University, Japan (33°37.9' N, 130°25.6'E, 11 m ASL). Mean annual temperature and precipitation are 16.9 °C and 1707 mm, respectively for 2005‒2014. Three 10-year-old trees of *C. japonica* (Shakain cultivar) were used in the experiment (Table 1). The trees were grown inside a scaffolding tower at 1-m spacing and branches were pruned to 50% of tree height at 9 years. The trees were watered twice per day starting from one-month prior to the experiment to maintain moist soil conditions.

### *Diurnal measurements of whole tree water use*

We made diurnal measurements on a sunny day (10 Aug., 2015). The maximum and minimum air temperature and mean relative humidity (RH) on this day, obtained from a temperature/humidity sensor (Ondotori Jr. RTR-53, T and D Inc., Japan) in the nursery field, were 33.7°C, 24.7°C, and 67.6%, respectively.

We accessed the crown of the sample trees using the scaffolding and selected three current-year shoots near the treetop for measurement of leaf transpiration rate ($T_{\mathrm{Leaf}}$, $\mu$ g$H_2O$ cm$^{-2}$ s$^{-1}$) using a leaf porometer (LI1600, Li-Cor Inc, Lincoln, NE, USA). Immediately after each transpiration measurement, we measured bulk leaf water potential ($\Psi_{\mathrm{Leaf}}$, MPa) of near-by current-year shoots using the pressure chamber (Model 600, PMS Instruments, Corvallis, OR, USA). Transpiration and water potential measurements were made every hour from 4:00 (predawn) to 6:00 of the next day.

We measured sap-flow velocity using Granier-type sap-flow sensors (SFS2 Type M sensor, Climatec, Tokyo, Japan) installed on the stem of each tree at 1-m height above ground. To measure changes in stem volume, we installed digital dendrometers (Model ID-C112P, Mitutoyo, Tokyo, Japan) at five heights on the stem of each tree to measure diurnal changes in stem radius (ΔR, mm). Sap-flow velocity and stem radius were recorded every 30s and 5 min, respectively using a data logger. The distance between points of measurement were nearly equal for the three trees, so we could not analyze differences in time-delay in relation to distance.

On the next sunny day (14 Aug., 2015), all three trees were cut while suspended from the scaffolding and the cut base was immediately submerged and recut in a large bucket filled with water. The bucket was covered with plastic to prevent surface evaporation. To estimate whole-tree transpiration rate ($T_{\mathrm{Tree}}$, $\mu \mathrm{gH_2O\ cm^{-2}\ s^{-1}}$), the transpired water was replenished every 30 min (suspended cut tree method, Takizawa et al. 1996). The total volume of replenished water from 4:00 to 4:00 of the next day was assumed to represent cumulative daily transpiration ($\Sigma T_{\mathrm{Tree}}$, ml day$^{-1}$). Diurnal changes of sap-flow rates were measured using the same methods as above.

*Whole-tree and leaf pressure-volume relationship*

The relationship between bulk leaf water potential and water content of the stem and leaves was estimated in a whole-tree pressure-volume (WTPV) experiment. After allowing the suspended trees to rehydrate overnight, we moved the trees to a dark shed adjacent to the nursery. The trees were laid on a digital scale (SE-60KBM, A&D Company Ltd., Tokyo, Japan) and allowed to dry while their weight was measured the nearest 10 g. We made repeated measurements of whole-tree water potential ($\Psi_{\mathrm{Tree}}$, MPa) using a pressure chamber (Model 600, PMS Instruments, Corvallis, OR, USA) by sampling 10-cm-long shoots from upper, middle, and lower crown. We also made measurements on an additional branch next to the lower-crown sample, which was covered with a black plastic bag. Upon obtaining values similar to the uncovered shoots, we assumed that water potential was uniform within the tree (Ritcher 1973) and used the mean of the four shoots in our pressure-volume analysis. Measurements were taken every two hours on the first, second and third day and once per day there after until turgor loss point was reached in 10 days. To measure changes in stem volume during the WTPV experiment, stem radius was measured using digital dendrometers as described above.

Concurrently with WTPV, leaf pressure-volume (LFPV) relationships were obtained using the bench drying approach to the pressure-volume technique (Tyree and Hammel 1972; Schulte and Hinckley 1985) for three foliated shoots sampled from each tree comprising second- and current-year internodes. Bulk leaf water potential ($\Psi_{\mathrm{Leaf}}$, MPa) and fresh weight were measured repeatedly by pressure chamber.

After the WTPV experiment, each tree was separated into leaf, branch, and stem and these were oven dried to constant weight to obtain dry masses (g, Table 1). Similarly, leaf dry masses (g) were obtained after the

LFPV experiment. To compare hydraulic properties between whole-tree and leaves, water potential at turgor loss point ($\Psi_{\mathrm{w,tlp}}$, MPa), relative water content at turgor loss point ($RWC_{\mathrm{tllp}}$), saturated osmotic potential ($\Psi_{\mathrm{o,\ sat}}$, MPa), and bulk modulus of elasticity ($\varepsilon$ = (Δturgor pressur/ΔRWC) $RWC_{\mathrm{tlp}}$, MPa; Warren-Wilson 1967) were obtained from the WTPV curve and LFPV curves of three shoot samples for each study tree..

*Data analysis*

*Whole-tree water use*

Sap-flow velocity ($U$, $\mathrm{m^3\ m^{-2}}$) was estimated using the following equation (Granier, 1987):

$$U = 0.119K^{1.231} \qquad [1]$$

Where $K$ is calculated from the temperature difference between the two probes at time $i$ ($\Delta t_{\mathrm{i}}$, ℃) and maximum ($\Delta t_{\mathrm{max}}$. ℃).

$$K = (\Delta t_{\mathrm{max}} - \Delta t_{\mathrm{i}})/\ \Delta t_{\mathrm{i}} \qquad [2]$$

We calculated cumulative daily sap-flow rate ($U_{\mathrm{Day}}$, $\mathrm{m^3\ m^{-2}}$) for each tree on 14 Aug and obtained the correction coefficient ($a$) for converting cumulative daily sap-flow rate to cumulative daily transpiration ($\Sigma T_{\mathrm{Tree}}$. = $a\ U_{\mathrm{Day}}$, ml $\mathrm{day^{-1}}$). This correction coefficient was used to estimate $\Sigma T_{\mathrm{Tree}}$ of each tree from sap-flow measurements on 10 Aug. Similarly, we assumed that the relationship between whole-tree transpiration rate and leaf transpiration rates is constant during the course of the day and used the relationship between cumulative daily transpiration in whole-tree and cumulative daily transpiration of leaf , ($\Sigma T_{\mathrm{Tree}} = b\ \Sigma T_{\mathrm{Leaf}}$, ml $\mathrm{day^{-1}}$) to obtain hourly estimates of whole-tree transpiration rate from measurements of leaf transpiration rates ($T_{\mathrm{Tree}} = b\ T_{\mathrm{Leaf}}$, $\mu\ \mathrm{gH_2O\ cm^{-2}\ s^{-1}}$).

*Whole-tree water storage*

During the WTPV experiment, whole-tree mass decreased with decreasing $\Psi_{\mathrm{Tree}}$ reflecting depletion of whole-tree stored water ($M_{\mathrm{T}}$, ml). We estimated the breakdown of storage depletion among leaves and stem (sapwood and elastic tissue) as described below. Heartwood had not developed in the study trees.

Stem volume was calculated from stem radius measurements assuming a truncated cylindrical cone (Huber method). Water content of elastic tissue ($M_{\mathrm{Elastic,\ S}}$, ml) was calculated as the difference between measured ($V_{\mathrm{i}}$, ml) and maximum ($V_{\mathrm{Max}}$, ml) stem volume (Zweifel et al. 2000).

$$M_{\mathrm{Elastic,\ S}} = V_{\mathrm{Max}} - V_{\mathrm{i}} \qquad [3]$$

Since branches also have elastic tissue, first we calculated branch volume from diameter and length of branches measured in 2014 and then converted this to water content of elastic tissue ($M_{\text{Elastic, B2014}}$, ml kg$^{-1}$) using the ratio between volume and radius change of the stem, assuming stem and branches are similarly elastic. For each study tree, water content of elastic branch tissue ($M_{\text{Elastic, B}}$, ml kg$^{-1}$) was calculated using the ratio between stem diameter at breast height (DBH) in 2014 and 2015, assuming similar diameter growth rate of stem and branches. The sum of $M_{\text{Elastic, S}}$ and $M_{\text{Elastic, B}}$ was considered as the total water content of elastic tissue ($M_{\text{Elastic}}$, ml kg$^{-1}$)

$$M_{\text{Elastic}} = M_{\text{Elastic, S}} + M_{\text{Elastic, B}} \qquad [4]$$

Measurements of leaf water content obtained from LFPV experiment were multiplied by the ratio between leaf dry mass of the whole-tree and LFPV shoot sample to estimate changes in whole-tree leaf water content with $\Psi_{\text{Tree}}$. ($M_{\text{Leaf}}$, ml), assuming similar relationships between $\Psi_{\text{Tree}}$ and $M_{\text{T}}$ as $\Psi_{\text{Leaf}}$ and $M_{\text{Leaf}}$.

Sapwood water content ($M_{\text{Sap}}$, ml) was calculated by subtracting $M_{\text{Elastic}}$ and $M_{\text{Leaf}}$ from $M_{\text{T}}$.

$$M_{\text{Sap}} = M_{\text{T}} - (M_{\text{Elastic}} + M_{\text{Leaf}}) \qquad [5]$$

*Diurnal change in water storage*

We used the relationship between $\Psi_{\text{Tree}}$ and $M_{\text{Leaf}}$ and $M_{\text{Sap}}$ described above to estimate diurnal changes in water storage of leaf and sapwood ($P_{\text{Leaf}}$ and $P_{\text{Sap}}$) on Aug 10 from measurements of $\Psi_{\text{Leaf}}$. Here, we assumed that depletion of $P_{\text{Leaf}}$ and $P_{\text{Sap}}$ was 0 when $\Psi_{\text{Leaf}}$ is highest ($\Psi_{\text{pd}}$ in Figure 1) and that water depletion increased with decreasing $\Psi_{\text{Leaf}}$ ($\Psi_{\text{mid}}$ in Figure 1 represents maximum water depletion). Water storage of elastic tissue ($P_{\text{Elastic}}$) was calculated from stem radius measurements on Aug 10 by same calculations as for $P_{\text{Elastic}}$ of whole-tree water storage. The hourly rate of change in $P_{\text{Leaf}}$, $P_{\text{Sap}}$, and $P_{\text{Elastic}}$ (,$\Delta P_{\text{Leaf}}$, $\Delta P_{\text{Sap}}$, and $\Delta P_{\text{Elastic}}$, ml hr$^{-1}$) were calculated and their sum was assumed to be the hourly rate of change in whole-tree water content ($\Delta P_{\text{Tree}} = \Delta P_{\text{Leaf}} + \Delta P_{\text{Sap}} + \Delta P_{\text{Elastic}}$, ml hr$^{-1}$). And the sum of the hourly negative changes (water storage depletion) from 4:00 to 4:00 of the next day represented cumulative daily water content of leaf, sapwood, elastic tissue, and whole-tree to $T_{\text{Tree}}$ ($\Sigma P_{\text{Leaf}}$, $\Sigma P_{\text{Sap}}$, $\Sigma P_{\text{Elastic}}$, and $\Sigma P_{\text{Tree}}$, ml day$^{-1}$).

*Water storage estimation of leaf and stem elastic tissue of larger trees*

To estimate contribution of leaf versus stem water in larger trees, we calculated stored water from published data (Iehara et al. 2003, Tange et al. 1987, Watanabe and Mogi 2007) on leaf mass and stem volume of mature *C.*

*japonica* stands (DBH =19−62 cm, 45−126 years old, n=22, see Supplementary Table 1). Water storage estimation of leaf were calculated by multiplying daily maximum change of leaf water content per leaf dry mass in this study by leaf mass data of larger trees, assuming same diurnal patterns of leaf water potential as in this study. Stem volume was calculated as in this study using estimated of stem radius ($r_h$) at height (h) obtained from relative stem curve ($r_h$ =2.01909*$X_h$- 1.95113*$X_h$^2+ 0.85429*$X_h$^3, where $X_h$ is relative height (h / tree hight), Shizuoka Prefectural Forestry Technology Center 1999). As bark thickness increases very little in trees over 20cm DBH (Davis and Hofstetter 2014), stem radius change was assumed to be constant (150 μm) based on data from a large *C. japonica* tree (DBH = 43.5 cm, height = 18.8 m, Maruyama et al. 1986) for calculating water storage estimation of stem elastic tissue.

## Results

### *Diurnal patterns*

Figure 2a shows the diurnal change in temperature, calculated VPD (vapor pressure deficit), and PPFD (photosynthetic photon flux density) on 10 Aug. Transpiration rates ($T_{\mathrm{Leaf}}$) of the three trees began to increase around 7:00, reached peak values between 13:00−16:00, decreased thereafter, and ceased around 21:00 (Figure 2b). Leaf water potential ($\Psi_{\mathrm{Leaf}}$) was highest at 7:00 and lowest at 15:00, after which, it began to recover (increase, Figure 2c). $\Psi_{\mathrm{Leaf}}$ continued to recover after transpiration ceased at 21:00. Stem radius change (ΔR) was smallest at 9:00 and largest at 16:00, after which it became smaller (Figure 2d). Maximum and minimum values of ΔR occurred 1–2 hr after those of $\Psi_{\mathrm{Leaf}}$. Hysteresis was observed in the diurnal course of $T_{\mathrm{Leaf}}$ in relation to $\Psi_{\mathrm{Leaf}}$, such that $\Psi_{\mathrm{Leaf}}$ was higher in the morning when $T_{\mathrm{Leaf}}$ increased compared to the afternoon when $T_{\mathrm{Leaf}}$ decreased (Figure 3).

### *Static change of stored water*

Figure 4 shows the whole-tree and leaf pressure-volume curves for Tree 2 as an example of three study trees. Water potential at turgor loss point ($\Psi_{\mathrm{w,tlp}}$), relative water content at turgor loss point ($\mathrm{RWC_{tlp}}$), saturated osmotic potential ($\Psi_{\mathrm{o,\ sat}}$), and bulk modulus of elasticity (ε), which were obtained from WTPV curve of each tree, were higher than the average values obtained from the LFPV curves (Table 2).

Based on WTPV experiment, we calculated storage depletion (cumulative loss of stored water per kg fresh weight) of the whole tree, leaves, and stem (sapwood and elastic tissue) (Figure 5). Whole–tree storage depletion averaged 91.4 ml $kg^{-1}$ at whole-tree turgor loss point in the threes trees. Of this, stored water in the leaves, sapwood, and elastic tissue contributed around 51%, 29%, and 20%, respectively.

*Diurnal depletion and replenishment of stored water*

We estimated the diurnal pattern of depletion and replenishment of stored water in leaf and stem on 10, Aug. by combining results of WTPV experiment and diurnal measurements. Whole-tree water loss due to transpiration ($T_{\mathrm{Tree}}$) started around 7:00, was greatest between 12:00−15:00, and ceased around 21:00 (Figure 6a). Coinciding with transpiration, depletion of leaf water ($P_{\mathrm{Leaf}}$) began around 7:00. $P_{\mathrm{Leaf}}$ was lowest around 15:00, but recovered rapidly thereafter (Figure 6b). $P_{\mathrm{Leaf}}$ continued to be replenished after sunset. Depletion of sapwood water ($P_{\mathrm{Sap}}$) began around 7:00 and was lowest at 15:00, but recovered rapidly thereafter. $P_{\mathrm{Sap}}$ continued to be replenished after sunset (Figure 6c). Depletion of elastic tissue water ($P_{\mathrm{Elastic}}$) lagged behind that of leaf water by 1 to 2 h, and began around 9:00. $P_{\mathrm{Elastic}}$ was lowest at 16:00, but was replenished during the night (Figure 6d). Maximum depletion of $P_{\mathrm{Leaf}}$ was about 1−3 times that of depletion of $P_{\mathrm{Sap}}$ and 5−10 times that of $P_{\mathrm{Elastic}}$ (note difference of Y-axis ranges for Figs 6b, c, and d). Between 7:00−9:00, stored water ($\Delta P_{\mathrm{Tree}}$) contributed an average of 101% of whole-tree water loss (Table 3, Figure 6a). Replenishment of stored water coincided with the decrease in whole-tree water loss, as a result stored water contribution became negative after 15:00 (Table 3, Figure 6a). These results indicated that replenishment of stored water occurs in late afternoon when low leaf water potentials resulting from day-time transpiration drive water uptake. Then, cumulative daily water content in whole-tree ($\sum P_{\mathrm{Tree}}$) contributed around 10% of daily total water loss from the three trees (Table 4). For the segmental breakdown of $\sum P_{\mathrm{Tree}}$, cumulative daily water content of leaf ($\sum P_{\mathrm{Leaf}}$) contributed 5.3−7.8% of daily total water loss from the three trees, while cumulative daily water content of sapwood ($\sum P_{\mathrm{Sap}}$) contributed 2.9−4.4% and cumulative daily water content of elastic tissue ($\sum P_{\mathrm{Elastic}}$) contributed 0.7−1.0% (Table 4).

*Water storage estimation of leaf and stem of large trees*

In larger trees, water storage estimation of leaf and stem elastic tissue were estimated to increase with DBH (Figure 7, $R^2$=0.66, $p$<0.001 and $R^2$=0.98, $p$<0.001, respectively). Water storage estimation of leaf was, on average 250% (maximum 530%) of elastic tissue, although this ratio was not correlated with DBH.

## Discussion

In the three study trees, depletion and replenishment of stored water corresponded to the diurnal pattern of transpiration. Hysteresis in the relationship between leaf transpiration rate and bulk leaf water potential indicated that leaf water potential does not increase promptly in response to decreasing transpiration rate in the afternoon. We inferred that delayed recovery of leaf water potential in the afternoon drives replenishment of stored water and that water potential of the transpiration stream recovers gradually as stored water is replenished. We found that stored water contributed 100% of outflow for approximately 2 hour after transpiration began in the morning, indicating that stored water plays an important role in maintaining transpiration rates in early morning before sap-flow rates begin to increase. Thus, replenishment of stored water in the afternoon is important to prepare for transpiration demand of the following morning. The contribution of stored water to total daily transpiration was about 10%, which agrees with previous results obtained for six-year-old *P. abies* saplings and neotropical canopy trees (Zweifel et al. 2001, Meinzer et al. 2004). However, Zweifel et al. (2001) found that stored-water contribution varied from 10% on a sunny day to 65% on an overcast day, suggesting that it varies depending on total daily transpiration.

We found that, as xylem water potential decreased, leaf water was depleted in similar or slightly greater amounts than stem water. Past studies have suggested that stored water in sapwood plays an important role in maintaining hydraulic status of trees (Waring et al. 1979, Phillips et al. 2003, Cermak et al. 2007). However, Jarvis (1975) speculated that if water is withdrawn from woody tissue, cavitation rather than stem shrinkage would occur because woody tissue is inelastic. It has recently been suggested that there is significant radial water movement between the bark and the xylem tissue via symplastic water transport in ray parenchyma, which is facilitated by aquaporin (Stepee et al. 2012, Pfautsch et al. 2015). Zweifel et al. (2000) found that water stored in sapwood is not utilized at xylem pressure potentials greater than −2.3 MPa, and that water stored in bark (cambium, phloem, and parenchyma) contribute more to total daily transpiration in young *P. abies*. In *Pinus densiflora* Sieb. et Zucc., 90% of diurnal stem radius change was attributable to bark, while the xylem contributed only 10% (Maruyama et al. 1986). Therefore, our measurements of stem radius change are likely to reflect changes in bark water content. Bark water content contributes only 6 and 14% of stem water content in young *P. abies* and old *Pseudotsuga menziesii* (Mirb.) Franco trees, respectively (Zweifel et al. 2001, Cermak et al. 2007), suggesting it may be a small pool for stored water. In agreement with our estimates, the contribution of bark water to total daily transpiration is estimated to be less than 2% in *Fagus sylvatica* L. (Betsch et al. 2011) and 5% in mature *P. abies* (Zweifel and Hasler 2001).

On the other hand, studies on *Larix* spp. and *P. abies* have shown that the major part of internally stored water is withdrawn from the foliage (e.g., Schulze et al. 1985, Edwards et al.1986). More recent studies on water storage mechanisms indicate that leaf water contributes directly to leaf transpiration, while a large pressure difference is needed to draw stem water into the transpiration stream (Zweifel et al. 2001, Edwards et al. 1986). The hydraulic segmentation hypothesis (Zimmerman 1983, Tyree and Ewers 1991, Sack and Holbrook 2006) postulates that because of the terminal status in the transpiration stream, leaves may act as control valves for species with high stem hydraulic conductivity, or a low safety margin against water stress (Pivovaroff et al. 2014). For such species, maintenance of leaf water status is important for keeping stomata open and avoiding carbon starvation during drought (McDowell 2011, Sevanto et al. 2014). Our study showed that hydraulic properties measured at whole-tree and leaf levels are different. At the leaf level, osmoregulatory capacity was higher, while turgor loss point, relative water content at turgor loss, and bulk modulus of elasticity were lower compared to measurements at the whole-tree level. This suggested that leaves regulate water flow and define the hydraulic safety margin of the whole tree (Pivovaroff et al. 2014). In 10-year-old trees of *Populus deltoides* Bartr., water stored in one-year-old twigs contributes to alleviating daytime water deficit (Lo Gullo and Salleo 1992). Our study showed that, in young *C. japonica* trees, leaf water responds quickly and directly to daytime transpiration demand to maintain whole-tree hydraulic conductance. Even in short-stemmed woody plants, there may be a considerable delay between water loss from leaves and water uptake by roots (Cermak et al. 2007). Conifers have high hydraulic resistance to water flow, resulting in considerable delays in water uptake (Matsumoto et al. 1992). Therefore, it is likely that leaf water plays an important role for avoiding water stress and maintaining leaf water status in *C. japonica* trees.

In large trees, water stored in sapwood may amount to as much as 50% of daily transpiration demand (Holbrook and Sinclair 1992, Kobayashi and Tanaka 2001, Waring et al. 1979), and is considered important for compensating hydraulic limitation of physiological function (Meinzer et al. 2003, Phillips et al. 2003) and maintaining daytime transpiration rates (Goldstein et al. 1998). Our calculations suggest water depletion of leaf and stem elastic tissue increase with DBH, assuming same diurnal patterns of leaf water potential and stem radius changes in larger trees. Although our results suggest water storage estimation of leaf is higher than that of elastic tissue, stem water storage is likely to be greater for large trees if including sapwood water storage is considered, because increasing reliance on sapwood water storage corresponds to increasing sapwood volume relative to leaf area with increasing tree size (Phillips et al. 2003). Also, for larger trees, heartwood would become much more important for water storage. We postulate that, stem water may be a long-term reserve for use under severe water stress, while leaf water supports daily and immediate transpiration demand.

Our methods for measuring whole-tree pressure-volume relationships and diurnal patterns of depletion/replenishment of stored water are widely applicable to trees of various species. The only constraint is that the study trees would need to be small enough to be placed on a scale for continuous measurement of fresh weight. Leaf and stem water contents for larger trees can be estimated based on leaf mass and stem radius under the same assumptions as in this study. In the WTPV experiment, we assumed that stem xylem pressure potential (XPP) was equal to leaf water potential, and calculated sapwood water content as the difference between total and leaf plus elastic water contents. In standing trees, however, stem XPP shows less diurnal variation and lags behind leaf water potential (McCutchan and Shackel 1992). In our study, the diurnal variation in sapwood water content may be over-estimated because our calculations are based on the diurnal pattern of leaf water potential. Thus, it is possible that water stored in leaf and elastic stem tissue may contribute more to whole-tree transpiration than our estimates.

## Conclusion

In this study, we quantified the relative contribution of foliar water storage versus stem to daytime water deficit in three 10-year-old *C. japonica* trees. Whole-tree pressure-volume experiment showed that leaves contributed around half of whole-tree stored water (Figure 5). In diurnal measurements of whole tree water use, maximum depletion of leaf water ($P_{\mathrm{Leaf}}$) was higher than that of sapwood and elastic tissue (mainly bark) water ($P_{\mathrm{Sap}}$ and $P_{\mathrm{Elastic}}$) (Figure 6). Then, cumulative daily water content in whole-tree ($\sum P_{\mathrm{Tree}}$) contributed around 10% of daily total water loss ($\sum T_{\mathrm{Tree}}$), of which 5.3−7.8% was contributed by leaves ($\sum P_{\mathrm{Leaf}}$) (Table 4). Despite the small number of trees sampled, these results suggested the importance of leaf water storage in maintaining daily transpiration in young conifer trees.

## Acknowledgements

We thank Mr. T. Hosaka,S Kokushi ,H Murakami ,G Yamada, and Y Hirose, Laboratory of Silviculture, Kyushu University, for help of field measurement and thank Dr. K. Sakuta, for helpful advice on this study. This research was funded by JSPS Research Fellowship (#02502390) to WA.

## References

Azuma W, Ishii HR, Kuroda K, Kuroda K (2016) Function and structure of leaves contributing to increasing water storage with height in the tallest *Cryptomeria japonica* trees of Japan. Trees 30: 141-152

Betsch P, Bonal D, Breda N, Montpied P, Peiffer M, Tuzet A, Granier A (2011) Drought effects on water relations in beech: The contribution of exchangeable water reservoirs. Agr For Meteorol 151: 531-543

Cermak J, Kucera J, Bauerle WL, Phillips N, Hinckley TM (2007) Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree Physiol 27:181-198

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML (2002) How plants cope with water stress in the field: photosynthesis and growth. Ann Bot 89: 907-916

Davis, T. S., & Hofstetter, R. W. (2014). Allometry of phloem thickness and resin flow and their relation to tree chemotype in a southwestern ponderosa pine forest. For Science 60: 270-274

Edwards WRN, Jarvis PG, Landsberg JJ, Talbot H (1986) A dynamic model for studying flow of water in single trees. Tree Physiol 1: 309-324

Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Celis A (1998) Stem water storage and diurnal patterns of water use in tropical forest canopy trees. Plant, Cell Environ 21: 397-406

Granier A (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol 3: 309-319

Holbrook NM (1995) Stem water storage. In: Gartner BL (ed) Plant stems: physiology and functional morphology. Academic Press, San Diego, pp151-174

Holbrook NM, Sinclair TR (1992) Water balance in the arborescent palm, Sabal palmetto, II. Transpiration and stem water storage. Plant, Cell Environ 15: 401-409

Iehara T, Hosoda K, Fukuda M, Ishibashi S, Nishizono T, Kondoh H, Tanaka K, Kotani E, Noda I, Amano M (2003) Study on transparent and verifiable method of evaluating carbon sinks. Global environmental conservation research expenses: annual research report set in 2002: 105-144 〔In Japanese〕

Ishii HR, Azuma W, Kuroda K, Sillett SC (2014) Pushing the limits to tree height: could foliar water storage compensate for hydraulic constraints in *Sequoia sempervirens*? Funct Ecol 28: 1087–1093

Jarvis PG (1975) Water transfer in plants. In: De Vries DA, Afgan NH (eds) Heat and mass transfer in the biosphere. Part 1: Transfer processes in the plant environments. John Wiley, New York, pp 369-394

Kobayashi Y, Tanaka T (2001) Water flow and hydraulic characteristics of Japanese red pine and oak trees. Hydrol Proc 15: 1731-1750

Lo Gullo MA, Salleo S (1992) Water storage in the wood and xylem cavitation in 1-year-old twigs of *Populus deltoides* Bartr. Plant, Cell Environ 15: 431-438

Lostau D., Berbiger P., Roumagnac P., Arruda-Pacheco C., David J.S., Ferreira M.I., Pereira J.S. & Tavares R. (1996) Transpiration of a 64-year-old maritime pine stand in Portugal I. Seasonal course of water flux through maritime pine. Oecologia 107, 33–42.

McCutchan H, Shackel KA (1992) Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L.cv. French ). J Amer Soc Hort Sci 117:607-611

Maruyama K, Nakazawa M, Fukumoto Y, Matsuda U (1986) Effect of debarking on the daily radial fluctuation of trunks of some tree species. Bull.Niigata Univ For. 19: 105-115

Matsumoto Y, Maruyama Y, Morikawa Y 1992. Some aspects of water relations on large *Cryptomeria japonica* D. Don trees and climatic changes on the Kanto plains in Japan in relation to forest decline. Jpn J For Environ 34: 2–1 [In Japanese]

McDowell NG (2011) Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiol 155:1051-1059

Meinzer FC, Brooks JR, Domec J-C, Gartner BL, Warren JM, Woodruff DR, Bible K, Shaw DC (2006) Dynamics of water transport and storage in conifers studied with deuterium and heat tracing techniques. Plant, Cell Environ. 29: 105-114

Meinzer FC, James SA and Goldstein G (2004): Dynamics of transpiration, sap flow and use of stored water in tropical forest canopy trees. Tree Physiol 24: 901–909

Meinzer FC, James SA, Goldstein G, Woodruff DR (2003) Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. Plant, Cell Environ 26: 1147-1155

Milne R (1989) Diurnal water storage in the stems of *Picea sitchensis* (Bong.) Carr. Plant, Cell and Environ 12: 63 -72

Ogburn RM, Edwards EJ (2010) The ecological water-use strategies of succulent plants. Adv Bot Res 55: 179-225

Ogburn RM, Edwards EJ (2012) Quantifying succulence: a rapid, physiologically meaningful metric of plant water storage. Plant, Cell Environ 35: 1533-1542

Oldham AR, Sillett SC, Tomescu AMF, Koch GW (2010) The hydrostatic gradient, not light availability, drives height-related variation in *Sequoia sempervirens* (Cupressaceae) leaf anatomy. Am. J. Bot. 97: 1-12

Pfautsch S, Renard J, Tjoelker MG, Salih A (2015) Phloem as capacitor: radial transfer of water into xylem of tree stems occurs via symplastic transport in ray parenchyma. Plant Physiol 167: 963-971

Phillips NG, Ryan MG, Bond BJ, McDowell NG, Hinckley TM, Cermak J (2003) Reliance on stored water increases with tree size in three species in the Pacific Northwest. Tree Physiol 23: 237-245

Pivovaroff AL, Sack L, Santiago LS (2014) Coordination of stem and leaf hydraulic conductance in southern California shrubs: a test of the hydraulic segmentation hypothesis. New Phytol 203: 842-850

Richter H (1973) Frictional potential losses and total water potential in plants: a reevaluation. J. of Exp. Bot 24: 983 - 994

Ripley BS, Abraham T, Klak C, Cramer MD (2013) How succulent leaves of Aizoaceae avoid mesophyll conductance limitations of photosynthesis and survive drought. J Exp Bot 64: 5485-5496

Running SW (1980) Relating plant capacitance to the water relations of Pinus contorta. Forest Ecol Manage 2: 237-252

Sack L, Holbrook NM (2006) Leaf hydraulics. 57: 361-381

Schulze ED, Cermak J, Matyssek R, Penka M, Zimmermann R, Vasicek F, Gries W, Kucera J (1985) Canopy transpiration and water fluxes in the xylem of the trunk of *Larix* and *Picea* trees - a comparison of xylem flow, porometer and cuvette measurements. Oecologia 66:475-483

Schulte PJ, Hinckley TM (1985) A comparison of pressure-volume curve data analysis techniques. J Exp Bot 36: 1590-1602

Sevanto S, McDowell NG, Dickman LT, Pangle R, Pockman WT (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant, Cell Environ 37:153-161

Shizuoka Prefectural Forestry Technology Center (1999) Estimates of log volume without cutting- Trunk diameter table-. New forestry technology 348: 1-18 ［In Japanese］

Stratton L, Goldstein G, Meinzer FC (2000) Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. Plant, Cell Environ 23: 99-106

Steppe K, Cochard H, Lacointe A, Améglio T (2012) Could rapid diameter changes be facilitated by a variable hydraulic conductance? Plant, Cell Environ 35: 150-157

Tange T, Yamanaka I, Suzuki M (1987) Growth and biomass of an old manmade *Cryptomeria japonica* stand. Bull Tokyo Univ For 25: 243-259 [In Japanese]

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol 119: 345–360

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J Exp Bot 23: 267-282

Vendramini F, Diaz S, Gurvich DE, Wilson PJ, Thompson K, Hodgson JG (2002) Leaf traits as indicators of resource-use strategy in floras with succulent species. New Phytol 154: 147-157

Von Willert DJ, Eller BM, Werger MJA, Brinckann E (1990) Desert succulents and their life strategies. Vegetatio 90: 133-143

Waring RH, Running SW (1978) Sapwood water storage: its contribution to transpiration and effect upon water conductance through the stems of old‐growth Douglas‐fir. Plant, Cell Environ 1: 131-140

Waring RH, Whitehead D, Jarvis PG (1979) The contribution of stored water to transpiration in Scots pine. Plant, Cell Environ 2: 309-317

Warren-Wilson J (1967) The components of leaf water potential II. Pressure potential and water potential. Aust J Biol Sci 20: 349-357

Watanabe H, Mogi Y (2007) Growth process and biomass of 92 years old manmade *Cryptomeria japonica* stand. Gifu Pref For Res Inst Res Rep 36:7-13 [In Japanese]

Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer Verlag, N.Y.

Zweifel R, Item H, Hasler R (2000) Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees 15: 50-57

Zweifel R, Item H, Hasler R (2001) Link between diurnal stem radius changes and tree water relations. Tree Physiol 21: 12-13

Zweifel R and Hasler R (2001) Dynamics of water storage in mature subalpine *Picea abies*: temporal and spatial patterns of change in stem radius. Tree Physiol 21:561-569

Zweifel R, Steppe K, Sterck FJ (2007) Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. J Exp Bot 58: 2113-2131

## Figure legends

Figure 1. Example from Tree 1 of the relationship between whole-tree water potential ($\Psi_{\mathrm{Tree}}$) and depletion of leaf and sapwood water ($M_{\mathrm{Leaf}}$ and $M_{\mathrm{Sap}}$) obtained from the whole-tree pressure-volume experiment (left). This relationship was used to estimate diurnal change in $P_{\mathrm{Leaf}}$ and $P_{\mathrm{Sap}}$ from measurements of $\Psi_{\mathrm{Leaf}}$ on a sunny day, (10 Aug., 2015, right).

Figure 2. Diurnal changes of air temperature, vapor pressure deficit (VPD), and photosynthetic photon flux density (PPFD) in the nursery field (a), diurnal patterns of leaf transpiration rate ($T_{\mathrm{Leaf}}$) (b), leaf water potential ($\Psi_{\mathrm{Leaf}}$) (c), and stem radius change (⊿R) (d), for the three study trees on a sunny day (10 Aug., 2015). Lines in (c) and (d) are same as in (b). Black arrows and dotted lines indicate the time of day when maximum and minimum values, respectively, were observed for $\Psi_{\mathrm{Leaf}}$ and ⊿R.

Figure 3. Hysteresis curves of diurnal course of leaf transpiration rate ($T_{\mathrm{Leaf}}$) in relation to bulk leaf water potential ($\Psi_{\mathrm{Leaf}}$) on a sunny day (10 Aug. 2015).

Figure 4. Example from Tree 2 of whole-tree and bulk-leaf pressure-volume curves.

Figure 5. Example from Tree 3 of the relationship between whole-tree water potential ($\Psi_{\mathrm{Tree}}$) and storage depletion (cumulative loss of stored water per 1 kg fresh mass) for whole-tree, leaf, sapwood, and elastic tissue obtained from the whole-tree pressure-volume experiment.

Figure 6. Diurnal patterns of whole-tree water loss ($T_{\mathrm{Tree}}$, thick lines) and the hourly rate of change in whole-tree water storage ($⊿P_{\mathrm{Tree}}$, narrow lines) (a), and depletion of leaf (b), sapwood (c), and elastic tissue (d) water ($P_{\mathrm{Leaf}}$, $P_{\mathrm{Sap}}$, and $P_{\mathrm{Elastic}}$, respectively) for the three study trees on 10 Aug., 2015. Legends of (b), (c), and (d) are same as (a). Black arrows and dotted lines indicate times when maximum and minimum values, respectively, were observed for $P_{\mathrm{Leaf}}$, $P_{\mathrm{Sap}}$, and $P_{\mathrm{Elastic}}$.

442 Figure 7. Water storage estimation of leaf and stem elastic tissue in larger trees in relation to DBH.

Table 1. Diameter at breast height (DBH), tree height, and fresh and dry masses of the three study trees of *Cryptomeria japonica*.

| | | Tree1 | Tree2 | Tree3 |
|---|---|---|---|---|
| DBH (cm) | | 6.1 | 7.2 | 6.6 |
| Tree height (m) | | 6.1 | 6.3 | 5.6 |
| Fresh mass (g) | | 22400 | 35300 | 25900 |
| Dry mass (g) | Leaf | 3200 | 5200 | 4000 |
| | Stem | 4100 | 6300 | 4400 |
| | Branch | 800 | 1400 | 1100 |
| | Total | 8100 | 12900 | 9500 |

Table 2. Hydraulic properties of whole-trees and leaves: water potential at turgor loss point ($\Psi_{w,tlp}$), relative water content at turgor loss point ($RWC_{tllp}$), saturated osmotic potential ($\Psi_{o,\ sat}$), and bulk modulus of elasticity ($\varepsilon$) obtained from whole-tree and leaf pressure volume curves (WTPV and LFPV, respectively). Values for leaf were the mean and standard errors of bulk-leaf measurements made on three foliated shoots per tree.

| | WTPV | | | LFPV | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Hydraulic properties | Tree1 | Tree2 | Tree3 | Tree1 | | Tree2 | | Tree3 | |
| $\Psi_{w,tlp}$ (MPa) | -1.2 | -1.2 | -1.3 | -2.0 | ±0.15 | -1.7 | ±0.10 | -2.0 | ±0.48 |
| $RWC_{tllp}$ | 0.87 | 0.84 | 0.84 | 0.73 | ±0.04 | 0.75 | ±0.02 | 0.75 | ±0.03 |
| $\Psi_{o,\ sat}$ (MPa) | -0.88 | -0.88 | -1.0 | -1.3 | ±0.12 | -1.2 | ±0.34 | -1.4 | ±0.61 |
| $\varepsilon$ (MPa) | 6.1 | 5.1 | 5.8 | 3.6 | ±0.56 | 3.6 | ±1.84 | 4.1 | ±1.15 |

Table 3. Contribution (%) of the hourly rate of change in whole-tree water content ($\Delta P_{\mathrm{Tree}}$) to whole-tree transpiration rate ($T_{\mathrm{Tree}}$) of the study trees at each time interval on 10 Aug., 2015. Negative values represent replenishment of water storage.

| | $\Delta P_{\mathrm{Tree}}/T_{\mathrm{Tree}}$ (%) | | | |
|---|---|---|---|---|
| Time | Tree1 | Tree2 | Tree3 | average |
| 7:00 to 9:00 | 142.4 | 79.9 | 80.3 | 100.9 |
| 9:00 to 11:00 | 35.4 | 37.6 | 26.4 | 33.1 |
| 11:00 to 13:00 | 15.1 | 9.4 | 13.4 | 12.6 |
| 13:00 to 15:00 | 3.8 | 5.6 | 2.3 | 3.9 |
| 15:00 to 17:00 | -14.4 | -24.4 | -41.0 | -26.6 |
| 17:00 to 19:00 | -51.3 | -61.3 | -51.6 | -54.7 |
| 19:00 to 21:00 | -75.0 | -31.9 | -189.9 | -99.0 |

Table 4. Cumulative daily transpiration ($\sum T_{\mathrm{Tree}}$) and water contents ($\sum P$ for leaf, sapwood, elastic tissue, and whole-tree) of the study trees on 10 Aug., 2015. Number in parentheses represents their contributions to water depletion (% relative to $\sum T_{\mathrm{Tree}}$).

| | tree1 | | tree2 | | tree3 | |
|---|---|---|---|---|---|---|
| Transpiration (ml day$^{-1}$) | 10841.3 | | 17014.3 | | 11624.2 | |
| Water content (ml day$^{-1}$) | | | | | | |
| Leaf | 572.8 | (5.3) | 1327.0 | (7.8) | 762.1 | (6.6) |
| Sap | 457.1 | (4.2) | 487.8 | (2.9) | 509.6 | (4.4) |
| Elastic | 74.6 | (0.7) | 130.1 | (0.8) | 112.9 | (1.0) |
| Tree | 1104.2 | (10.2) | 1734.7 | (10.4) | 1257.8 | (10.9) |

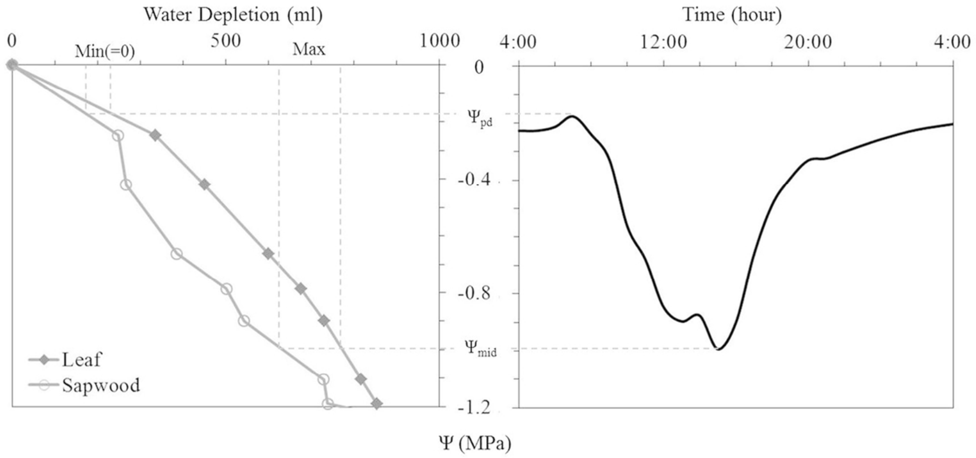

Figure 1. Example from Tree 1 of the relationship between whole-tree water potential ($\Psi_{Tree}$) and depletion of leaf and sapwood water ($M_{Leaf}$ and $M_{Sap}$) obtained from the whole-tree pressure-volume experiment (left). This relationship was used to estimate diurnal change in $P_{Leaf}$ and $P_{Sap}$ from measurements of $\Psi_{Leaf}$ on a sunny day, (10 Aug., 2015, right).

110x52mm (300 x 300 DPI)

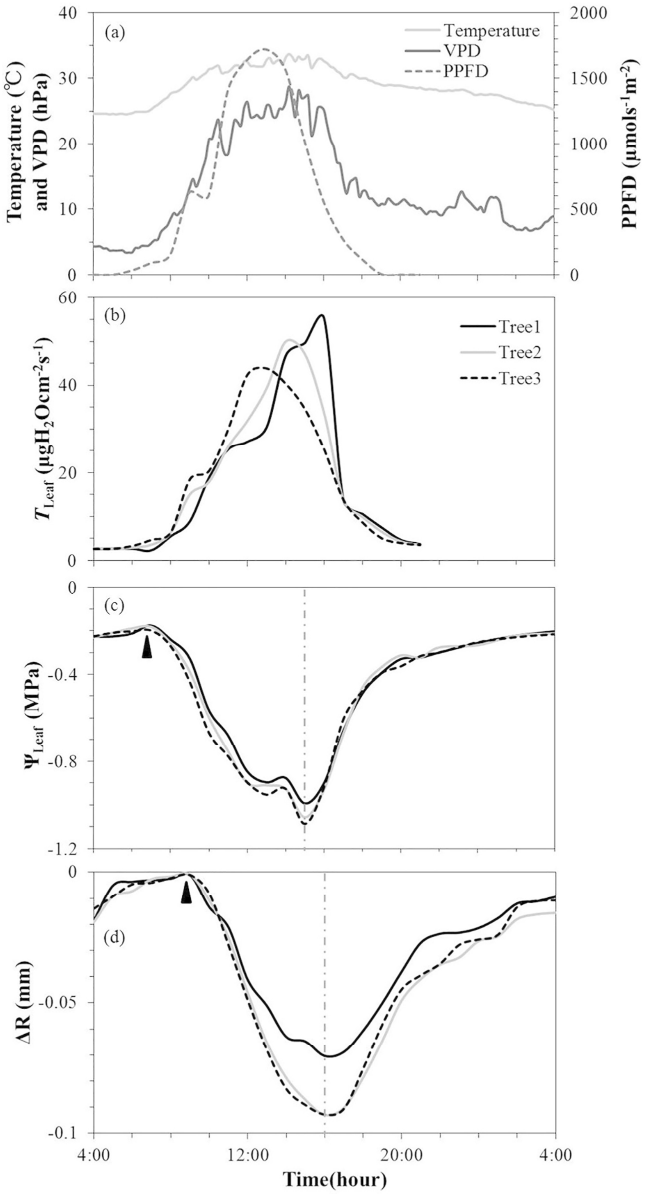

Figure 2. Diurnal changes of air temperature, vapor pressure deficit (VPD), and photosynthetic photon flux density (PPFD) in the nursery field (a), diurnal patterns of leaf transpiration rate ($T_{\mathrm{Leaf}}$) (b), leaf water potential ($\Psi_{\mathrm{Leaf}}$) (c), and stem radius change (⊿R) (d), for the three study trees on a sunny day (10 Aug., 2015). Lines in (c) and (d) are same as in (b). Black arrows and dotted lines indicate the time of day when maximum and minimum values, respectively, were observed for $\Psi_{\mathrm{Leaf}}$ and ⊿R.

211x388mm (300 x 300 DPI)

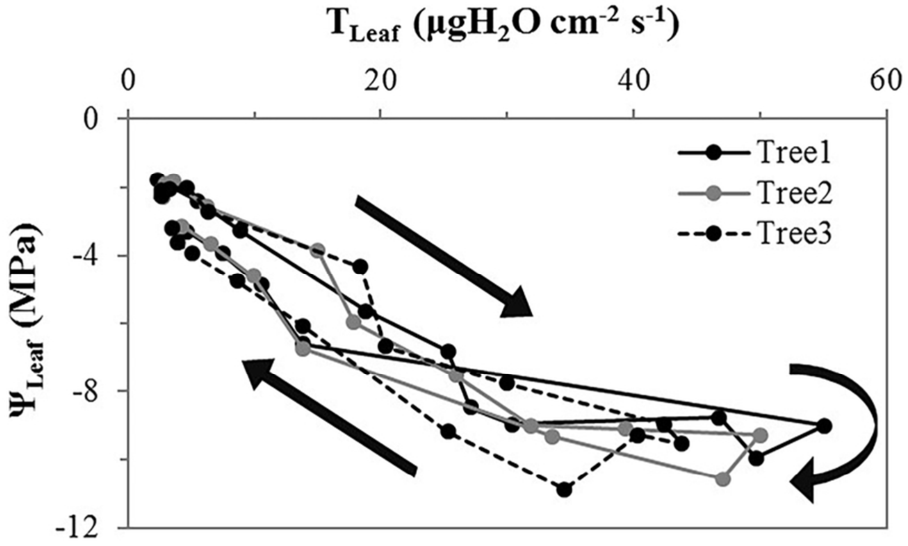

Figure 3. Hysteresis curves of diurnal course of leaf transpiration rate ($T_{Leaf}$) in relation to bulk leaf water potential ($\Psi_{Leaf}$) on a sunny day (10 Aug. 2015).

76x45mm (300 x 300 DPI)

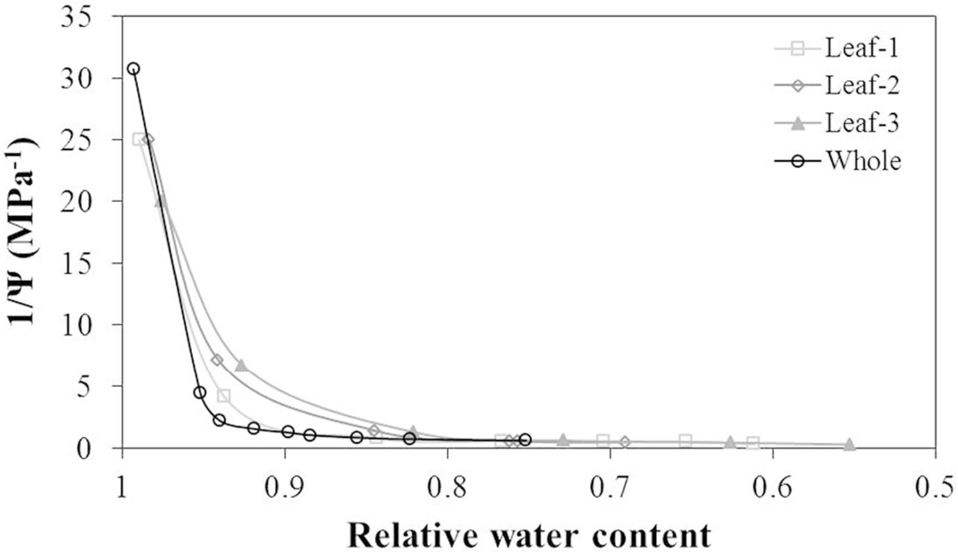

Figure 4. Example from Tree 2 of whole-tree and bulk-leaf pressure-volume curves.

85x49mm (300 x 300 DPI)

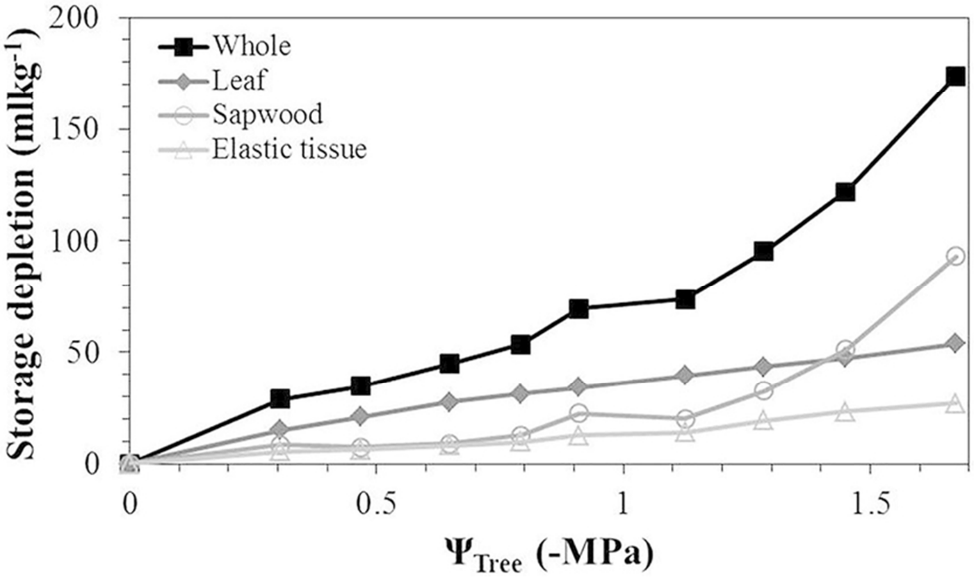

Figure 5. Example from Tree 3 of the relationship between whole-tree water potential ($\Psi_{Tree}$) and storage depletion (cumulative loss of stored water per 1 kg fresh mass) for whole-tree, leaf, sapwood, and elastic tissue obtained from the whole-tree pressure-volume experiment.

72x42mm (300 x 300 DPI)

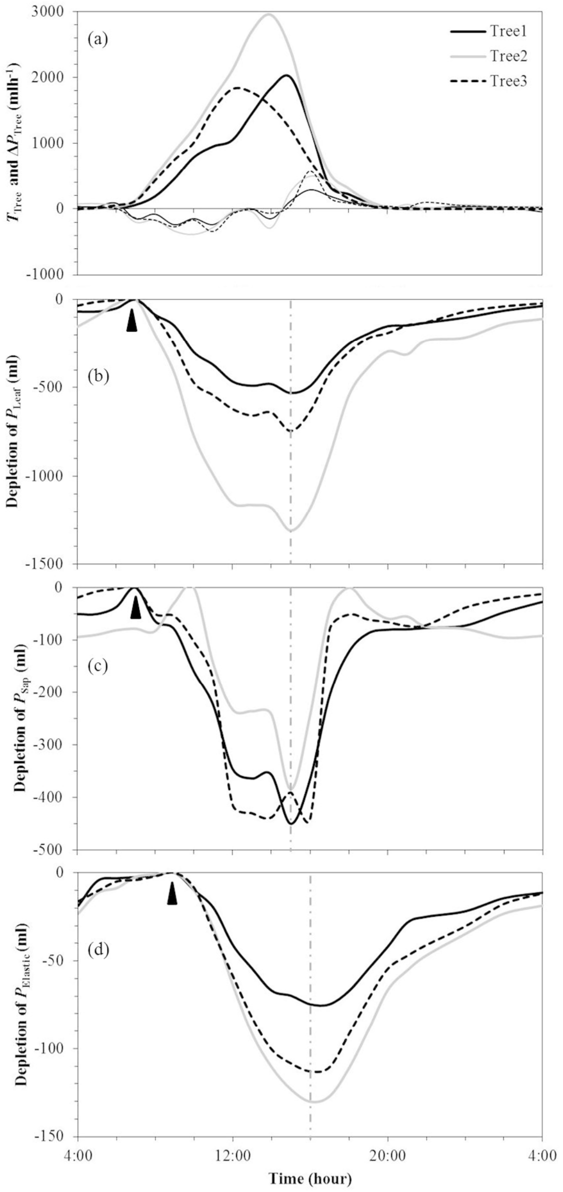

Figure 6. Diurnal patterns of whole-tree water loss ($T_{\mathrm{Tree}}$, thick lines) and the hourly rate of change in whole-tree water storage ($⊿P_{\mathrm{Tree}}$, narrow lines) (a), and depletion of leaf (b), sapwood (c), and elastic tissue (d) water ($P_{\mathrm{Leaf}}$, $P_{\mathrm{Sap}}$, and $P_{\mathrm{Elastic}}$, respectively) for the three study trees on 10 Aug., 2015. Legends of (b), (c), and (d) are same as (a). Black arrows and dotted lines indicate times when maximum and minimum values, respectively, were observed for $P_{\mathrm{Leaf}}$, $P_{\mathrm{Sap}}$, and $P_{\mathrm{Elastic}}$.

309x650mm (300 x 300 DPI)

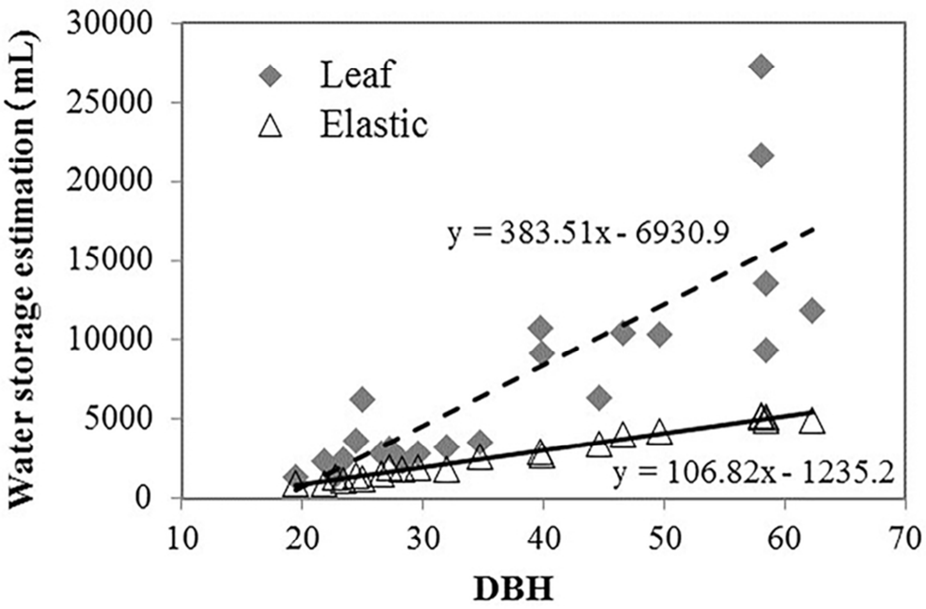

Figure 7. Water storage estimation of leaf and stem elastic tissue in larger trees in relation to DBH.

70x46mm (300 x 300 DPI)