Does phloem osmolality affect diurnal diameter changes of twigs but not of stems in Scots pine?

Article *in* Tree Physiology · October 2018

DOI: 10.1093/treephys/tpy121

CITATIONS
5

READS
210

4 authors, including:

Martina Lazzarin
Wageningen University & Research
1 PUBLICATION **5** CITATIONS
SEE PROFILE

Roman Zweifel
Swiss Federal Institute for Forest, Snow and Landscape Research WSL
61 PUBLICATIONS **3,081** CITATIONS
SEE PROFILE

Niels P R Anten
Wageningen University & Research
233 PUBLICATIONS **5,117** CITATIONS
SEE PROFILE

Tree Physiology 00, 1–9
doi:10.1093/treephys/tpy121

Research paper

Does phloem osmolality affect diurnal diameter changes of twigs but not of stems in Scots pine?

Martina Lazzarin[1,4], Roman Zweifel[2], Niels Anten[3] and Frank J. Sterck[1]

[1]Forest Ecology and Forest Management Group, 6700AA Wageningen, The Netherlands; [2]Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) Zürcherstrasse 111, CH-8903, Birmensdorf, Switzerland; [3]Centre for Crop Systems Analysis, 6708PB Wageningen, The Netherlands; [4]Corresponding author (martina.lazzarin@wur.nl)

Received May 18, 2018; accepted October 8, 2018; handling Editor Daniel Epron

Diel stem diameter changes measured at the stem base of temperate tree species can be mostly explained by a hydraulic system of flow and storage compartments passively driven by transpiration. Active, osmotic processes are considered to play a minor role only. Here we explore whether such osmotic processes have a stronger impact on diel changes in twig diameter than in stem diameter because twigs are closer to the leaves, the main source of newly acquired carbon. We investigated stem and twig diameter changes of wood and bark of pine trees in parallel to fluctuations of the osmolality in needles and in the bark at the stem base. We found consistent twig bark size increments concurrent with twig wood size decreases during daylight hours whereas needle osmolality was not consistently increasing even on sunny days. The size changes of bark and wood either reversed or ran in parallel from late afternoon onwards until the next morning. No such patterns were measurable at the stem base. Stem wood was hardly changing in size, whereas stem bark followed the regular pattern of a decrease during the daylight hours and an increase during the night. Osmolality at the stem base showed no particular course over 24 h. We conclude that assimilates from the needles were rapidly transported to the twigs where they increased the osmolality of the bark tissue by sugar loading, explaining the bark size increase (over-) compensating the xylem size decrease. The stem base largely followed the expectation of a passive, hydraulic system without a measurable role of osmoregulation. Diameter changes thus follow different diurnal dynamics in twigs and at the stem base.

Keywords: dual dendrometers, ecophysiology, osmolality, phloem transport, xylem and bark diameter changes.

Introduction

Stem radius changes

Daily tree stem radius changes are determined by reversible dynamics in water content, and additionally by irreversible growth processes (Zweifel et al. 2016). The water content in the stem is subject to the gradient in water potentials between roots, stem and canopy, which results in diurnal water flow dynamics and fluctuations of the internally stored water. Tree stems begin to shrink in the morning due to the transpiration-induced decrease in stem water potentials. The water stored in stems, mainly in the bark but also in the wood ray parenchyma, is gradually depleted (Pfautsch et al. 2015, 2018), but water storage recovers in the evening when transpiration is ceasing and a sufficient water uptake from the soil lowers the gradient of water potentials along the stem. Xylem and bark are anatomically and functionally coupled (Pfautsch et al. 2015) and parenchyma cells (i.e., rays) facilitate radial transport of water connecting the xylem with tissues of the inner bark. Theoretical studies hypothesized that regulation of osmotic potential originating from sugar loading and unloading in the phloem tissue affects water potential in the xylem and vice versa (Hölttä et al. 2009). Direct evidence of this phenomenon is lacking but measured stem diameter changes provide indirect support to this hypothesis. Combined dendrometer measurements of bark and xylem sizes in parallel are able to reveal the underlying dynamics. According

to the expectation from a transpiration-driven passive hydraulic system ('passive system'), the bark may lag behind the xylem in terms of its size changes but never show an opposite movement direction (Zweifel et al. 2001, Steppe et al. 2006*a*). Hydraulic models describing stem diameter variations generally divide the plant into vertical and radial compartments, along which the water potentials are calculated (Steppe et al. 2006*b*, Zweifel et al. 2001, 2007). In this type of model the crown and stem consist of two elements: the storage pool and flow path sections. The elastic living cells of crown and stem constitute the two water storage compartments connected by flow sections. Transpiration induces a gradient in water potentials from the crown to the soil, and water flows along the gradient upwards to the leaves (Zweifel and Häsler 2000, Génard 2001, Zweifel et al. 2001, Steppe et al. 2006*b*). A combination of low xylem water potentials inducing xylem shrinkage and radial water flow from the bark to the xylem depleting the bark tissues induces the stem diameter shrinkage (Zweifel et al. 2001, Steppe et al. 2006*b*).

In contrast to patterns fitting a passive flow and storage system driven by transpiration, as described above, several studies reported wood shrinkage concurrent with bark size expansion. Such patterns were found both in conifers, such as Scots pine and European larch (Sevanto et al. 2002, 2003, Dawes et al. 2014), and also in fast growing evergreen trees, such as mangroves and Eucalyptus species (Vandegehuchte et al. 2014, Zweifel et al. 2014, Pfautsch et al. 2015, Donnellan Barraclough et al. 2018). Such a pattern of oppositely moving sizes of two hydraulically coupled tissues cannot be explained by passive mechanisms alone; an additional mechanism is needed to explain the dynamics. It has been proposed that osmotic adjustments are involved in mitigating the daily loss of water from the phloem by increasing the osmolality in the bark when transpiration is high (Mencuccini et al. 2013). It was shown from dendrometer data that the bark expansion in the morning causes a delay in the shrinkage of the stem diameter of Scots pine trees (Sevanto et al. 2003, 2002). However, experimental evidence is scarce on how much osmolality dynamics actively influence the radial size changes by altering the water exchange between xylem and bark in conifers (but see Sevanto et al. 2002, 2003, Mencuccini et al. 2017).

Sugar transport and storage

The Münch flow hypothesis has been used to explain sugar transport from the source in the leaves via the sieve elements in the phloem of the stem towards the sink in the roots (Hölttä et al. 2009, 2014). In the needles, sugar molecules produced in the mesophyll diffuse to the collection phloem of the minor veins increasing the osmotic potential, and water circulates passively from the xylem into the collection phloem. Sucrose is therefore loaded from the minor veins into the major veins, and through the transport phloem it moves basipetally to petioles, branches, stem and roots (De Schepper et al. 2013). Sugar loading in conifers mostly occurs by bulk flow and diffusion through plasmodesmata nanopores (Rademaker et al. 2017). As a consequence of sugar unloading in the sink tissues, the osmotic potential is decreasing and the turgor pressure is reduced. Sugars arriving at the sink tissue can remain as such or be transformed into various compounds and accumulated as sucrose or hexose or used for respiration and metabolic processes, e.g., growth (Taiz et al. 2015). Needle size and morphology have been recently shown to be important factors for sugar transport in conifers (Rademaker et al. 2017).

Gradient in osmolality along the tree's vertical line

According to the common assimilate pool theory, the osmotic potential was found to be constant throughout the entire stem phloem of tomato plants (Heuvelink 1995, De Swaef and Steppe 2010). The amount of soluble sugars was quantified by measuring the osmolality of different plant tissues. Paljakka et al. (2017) showed that osmolality was higher in the needles than at the stem base almost all day. Interestingly, the gradient in osmolality between needles and twigs within branches was often found to be close to zero or even reversed, leading to branch osmolality sometimes being higher than that in the needles. Magnetic resonance studies reported daily phloem downward velocities of 1.22 m h^{-1} in the stem of in poplar trees (Windt et al. 2006). Despite the fact that phloem sap velocities may be species-specific, these results support the hypothesis that sugars are able to rapidly travel from needles to the twig. However, there is no consistent evidence yet for vertical and temporal patterns in osmolality, in relation to the tree's hydraulics.

Aim of the study

We made osmolality measurements in different segments of a tree in parallel to the application of dual-dendrometers measuring xylem and bark size changes in parallel. Both methods indicate sugar loading and unloading processes and enabled us to follow the question of whether osmotic control of turgor is more distinct closer to the sources of newly synthesized sugars. We hypothesized that active mechanisms, i.e., changes in osmolality, are more strongly affecting twig sizes close to the crown periphery then at the stem base. Further, we expected to measure increased osmolality in needles during morning hours as long as there was no stomatal closure limiting high assimilation rates.

Materials and methods

Experimental design

This study was carried out in a mature Scots pine forest part of the Pfynwald irrigation experiment (Schuerch and Vuataz 2000, Timofeeva et al. 2017, von Arx et al. 2017). Tree age was on average 100 years, tree height was on average 10.8 m, stand

density was 730 stems ha^{-1} with an average basal area of 27.3 $m^2 ha^{-1}$. In total, eight Scots pine trees were used for plant material collection, and one for monitoring diel stem movements of stem and a twig. The stem diameter at breast height was 21.34 cm. The diameter of the twig measured with a dual point dendrometer was 2 cm. We measured stem diameter changes on xylem and over bark in parallel at the stem base and in branches with high precision point dendrometers (see Figure 1). From these data we obtained radial wood and bark size changes. Additionally, needle and stem bark were sampled at intervals of 2 h in cycles of 24 h for three separate days to show parallel diel courses. The data from these two independent methods were interpreted in terms of periods of loading and unloading of sugars. Additionally, soil water potential was monitored at three soil depths (10, 20 and 80 cm).

Stem radius fluctuation measurements

Dual point dendrometers (ZN12-T-2WP (stem base) and ZN12-O-2WP (twig), Natkon, Oetwil am See, Switzerland) were used to measure radius fluctuations in stems at breast height and on twigs in the crown at ~10 m above ground. The carbon fibre frames of the dendrometers were T-shaped at the stem base and O-shaped for the twigs. The electronic parts were identical for both dendrometer types. The T-shaped frame was fixed to the stem by three stainless steel threaded rods anchored in the heartwood (Zweifel et al. 2007). The O-shaped frame was fixed to the twig with three stainless steel rods pressing against the wood from three different sides of the twig. The dual point dendrometer consists of two sensors: one positioned in the outermost dead layer of the bark that monitored the total radial stem size fluctuations (called 'over inner-bark measurement' or for short 'over bark measurement') and one that tracked the changes on the wood (Figure 1). In order to place one sensor head on the xylem, a piece of bark of about 1 × 1 cm^2 was cut out and removed. The damaged tissue was covered with a thin film of silicon to reduce desiccation. The second sensor was placed over inner-bark about 5 cm away. The resolution of the dendrometers was 0.1 µm and the recording frequency was 10 min. Data were stored in a data logger (DecentNode, DecentLab, Dübendorf, Switzerland) and sent into a central database in near-real time. The daily fluctuations of the bark were then calculated as difference between the total radial change measured over (inner-) bark and the change measured directly on the xylem.

Plant material collection for osmolality analysis

Plant material collection was done at the beginning of the growing season. Osmolality was measured at the stem base and in the needles. Daily sugar and water cycles were assessed on the 25 May 2016, 2 June 2016 and 6 June 2016. The day-to-day variation in osmolality and water content was investigated collecting needles and bark samples 12 times per day between

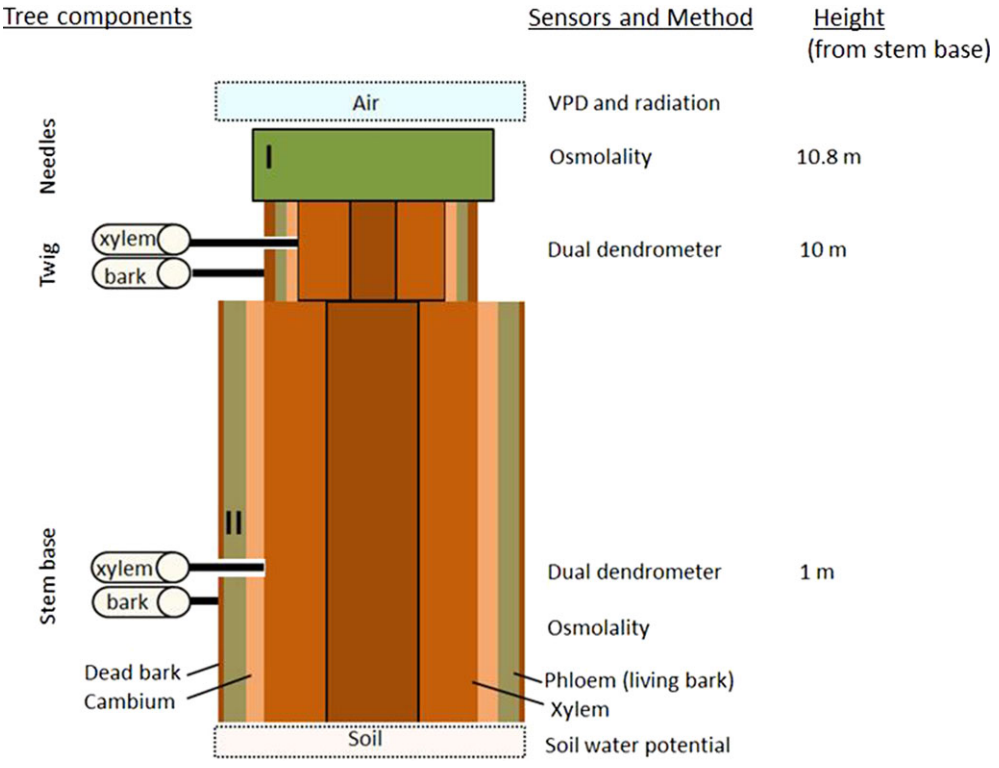

Figure 1. Illustration of sampling positions and methods. Dual point dendrometers monitoring xylem radius and over bark radius changes in a twig and at the stem base. Osmolality was measured in needles (I) and at the stem base (II).

06:00 a.m. and 4:00 a.m. on the next day. Needles were sampled from a sun-exposed branch accessible from the top of a scaffold. Four mature needles were removed from the first shoot, starting from the basal part in order to limit eventual disturbances of the phloem transport. Bark microcores were collected with an increment puncher (Trephor, Costruzioni Meccaniche Carabin C, Valle di Cadore, Belluno, Italy (Rossi et al. 2006)) at two opposite sides of the stem base (at breast height). Two phloem cores were collected per sampling. Samples were immediately frozen in liquid nitrogen in the field in order to facilitate cell membranes breakdown and release of sugars (Lintunen et al. 2016). Samples were then stored in dry ice for the duration of the campaign. Once in the laboratory, they were stored at −20 °C.

Osmolality measurements

Frozen samples of needles and bark of the stem base were kept at room temperature for 15 min to thaw. Needle fresh weight (FW) was measured and then the samples were dried at 80 °C in an oven for 24 h to obtain the dry weight (DW). The outer bark of the stem base was scraped away with a razor blade. The inner bark (excluding cambium) was separated from the xylem on the basis of the hardness and colour differences observed with the use of a binocular and weighed for FW determination (Lintunen et al. 2016). The obtained sample length (after removing outer bark, cambium and xylem) was ~2 mm. Osmolality measurements were done with a vapour pressure osmometer (5600 Wescor, Vapro, ELITechGroup, Logan, UT, USA) that was calibrated daily. This instrument has a resolution of 0.00031 °C (which equals one mmol kg^{-1}). The extraction of sugars was carried out by adding distilled water to the plant material, after drying and grinding (Arend and Fromm 2007). The constant water volume added was 0.5 ml for needles and 0.05 ml for the bark. The samples were put in into a vortex for 1 min and then centrifuged for 10 min at 11000*g* (MiniSpin Plus centrifuge, Eppendorf, Hamburg, Germany). The supernatant was transferred with a micropipette into an osmometer plate and the osmolality of extracted solution, (n/VES), was determined. For the bark each vial was analysed three times, for the needles five. The amount of solutes (n) and water content (WC) in the needles and inner bark tissue was determined from the following relations:

$$\mathrm{WC\,(kg\,kg^{-1}\ dry\ \ weight)} = (\mathrm{FW} - \mathrm{DW})/\mathrm{DW} \quad (1)$$

$$n\ \mathrm{(mol\,kg^{-1}\ dry\ weight)} = \mathrm{Osmolality} * \mathrm{WC} \quad (2)$$

Measured osmolality values were corrected for the dilution factor as follows:

$$\mathrm{Water\ lost\ by\ fresh\ weight\ \ (kg)} = \mathrm{DW} * \mathrm{WC} \quad (3)$$

$$\mathrm{Dilution\ factor} = \ \mathrm{Added\ volume/Water\ lost\ by\ fresh\ weight} \quad (4)$$

$$\mathrm{Corrected\ osmolality\,(mol\,kg^{-1})} = \mathrm{Osmolality} * \mathrm{Dilution\ factor} \quad (5)$$

Determination of periods of wood and bark size changes in opposite directions from dendrometer data

In a hydraulic system without osmotic effects, wood and bark size changes run generally in parallel. The bark tissue may show a delayed response to changing xylem water potentials due to the additional flow resistance between wood and bark (through the cambium). Furthermore, the response of the two tissues may differ in amplitude due to different elasticity, but they should not show opposite directions of size changes. We therefore analysed radial changes of wood and bark in terms of periods when the two tissues showed opposite size changes indicating osmoregulation. (i) Decreasing xylem size concurring with increasing bark size indicates sugar loading in the bark, whereas (ii) an increasing xylem size concurring with decreasing bark size indicates sugar unloading in the bark. In order to detect opposite size changes of wood and bark, we calculated the diameter changes of bark and wood separately over a moving window of 100 min for the whole time series. The slope signs (positive for increase or negative for decrease) were compared between bark and wood and were labelled +1 when the bark showed a positive slope and the wood a negative slope, −1 for the reverse pattern, and 0 when bark and xylem showed equal slope signs.

Statistical analysis

For the statistical analysis of the dendrometer data, histograms with the frequency of labels '−1', '0' and '+1' against hours were used for the period covering the two weeks of this study (from 25 May to 7 June). For the osmolality values, normality was tested with the Shapiro test and equal variance with the Levene's test. Since the assumptions of normality and equal variance were not met, non-parametric tests, were used to access differences in osmolality across days and tissues (needle and stem base).

Results

Environmental conditions

Environmental conditions were monitored over the entire measurement campaign. Days 1 and 3 were mostly sunny, whereas Day 2 was heavily clouded with some rain. The soil water conditions (upper 20 cm) were wet for the first two days and slightly drier on the third day.

Diameter changes in twigs and at the stem base

Twig diameter changes showed a consistent pattern for all days (Figure 2), however with different amplitudes depending on environmental conditions (the sunnier, the more distinct): wood diameter was shrinking with increasing sunlight (and vapour pressure deficit (VPD)) during morning hours, whereas the bark diameter was expanding during those hours. This pattern appeared on both

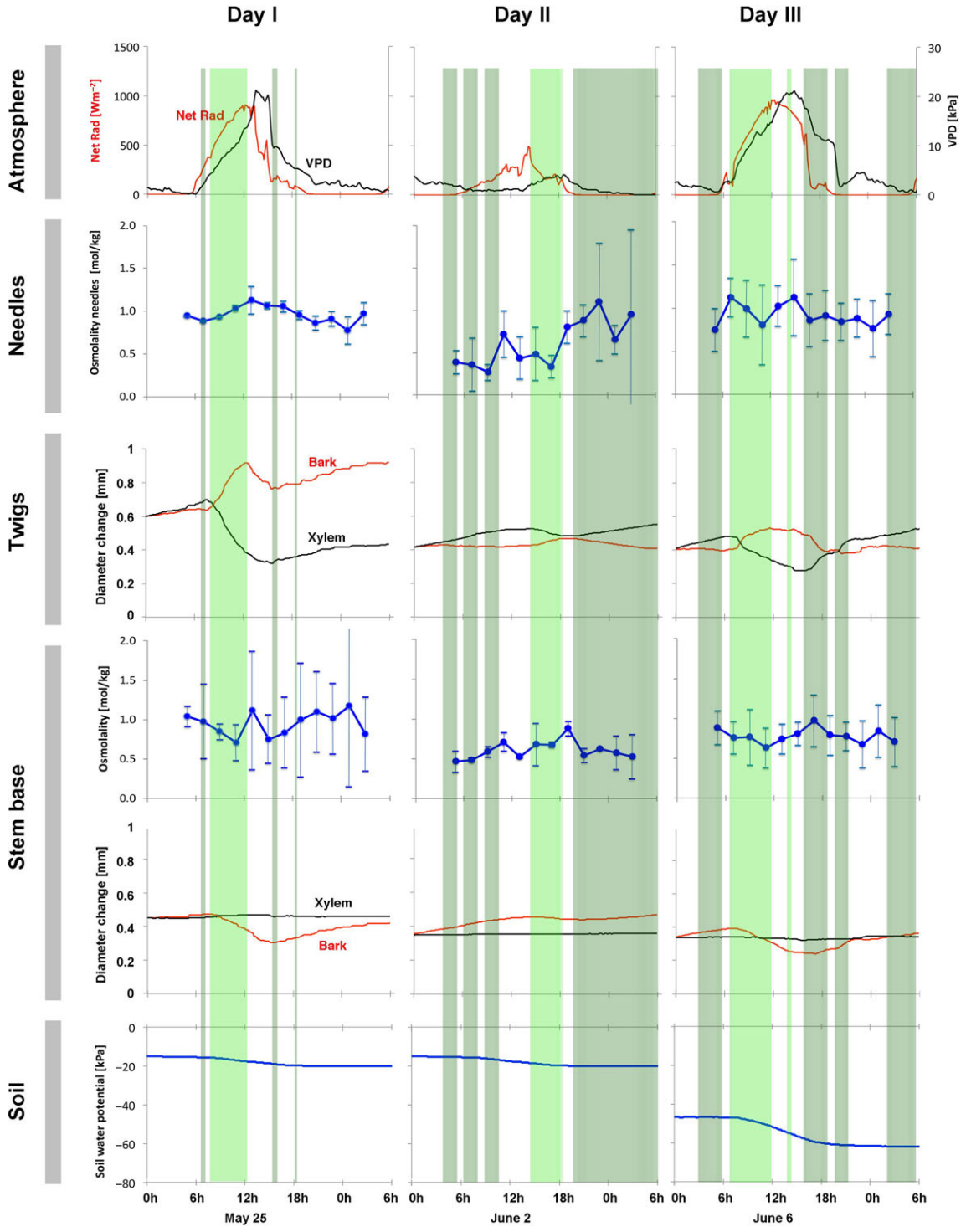

Figure 2. Measurements of the microclimate, of pine needles, of twigs, of the stem at breast height, and of the soil at three days in the wood growth phase of 2016. Measurements are shown according to their vertical location in the tree. Beginning from the top: vapour pressure deficit of the air (VPD, black line, data resolution 10 min) and net radiation (Net Rad, blue line, data resolution 10 min); osmolality of pine needles (data resolution 2 h, mean and standard deviation); diameter changes of wood (black line, data resolution 10 min) and bark of a twig (red line, data resolution 10 min) close to the crown top and at the stem base; osmolality of the bark at the stem base (data resolution 2 h, mean and standard deviation); and average soil water potentials to a depth of 80 cm (kPa). Periods of shrinking wood concurrent with expanding bark at the twig level are shown with light green areas. Periods of expanding wood concurrent with a shrinking bark at the twig level are shown with dark green areas.

sunny days and on the wet day with some delay in the afternoon (light green areas highlighted in Figure 2). Before and after this period of wood shrinkage concurrent with bark expansion, there appeared several phases where this pattern was reversed: the wood was expanding, whereas the bark was shrinking (dark green areas in Figure 2).

These two patterns of opposing bark and wood size changes (light and dark green areas in Figure 2) appeared very consistently not only for the three days of osmolality measurements but throughout the entire period from 25 May 25 to 6 June (Figure 3). The pattern of shrinking wood and expanding bark started with the early daylight hours, was most frequent around noon and ceased towards the evening. The opposite pattern of expanding wood with shrinking bark most often occurred during night time with the highest frequency in the first half of the night. Times with parallel running wood and bark size changes appeared most frequent in the second half of the night.

The dendrometer readings at the stem base showed very small wood size changes, close to the reasonably resolvable range with the equipment used. Thus, total stem diameter changes were mainly determined by bark size variations and hardly by wood size changes. The pattern of stem shrinkage and expansion followed the expected timing of a passively driven hydraulic system: the stem size decreased in the morning and expanded in late afternoon and at night.

Osmolality of needles and of the bark at the stem base

Needle osmolality (between 0.136 and 1.750 mol kg^{-1}) significantly differed from stem bark osmolality (0.246–1.903 mol kg^{-1}, Mann Whitney *U* test, $W = 3616$, $P = 0.018$). Stem bark osmolality values also differed between individuals (Kruskal–Wallis rank sum test, $\chi^2 = 71.28$, df = 7, $P < 0.05$). The expected increase in needle osmolality during morning hours was not consistent (Figure 2). Daily courses of osmolality differed considerably between the three days: on the wet Day 2, the osmolality records of needles (average: 0.613 ± 0.397 mol kg^{-1}) and stem bark (average: 0.605 ± 0.154 mol kg^{-1}) were lower than on the two dry days (Day 1: needle average 0.959 ± 0.117 mol kg^{-1} and stem bark average 0.948 ± 0.413 mol kg^{-1}; Day 3: needle average 0.917 ± 0.291 mol kg^{-1} and stem bark average 0.778 ± 0.243 mol kg^{-1}) (Kruskal–Wallis rank sum test, $\chi^2 = 35.76$, df = 2, $P < 0.05$).

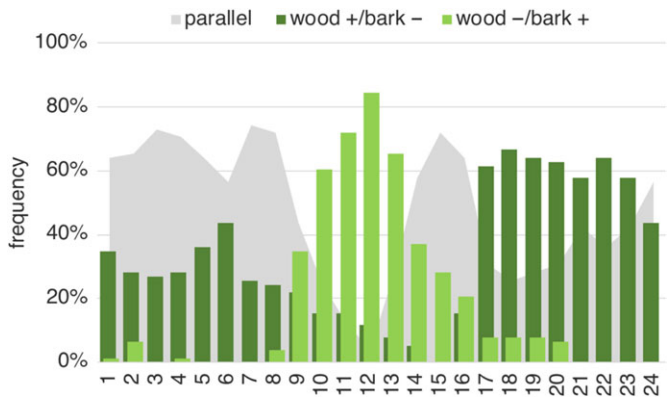

Figure 3. Frequency analysis of wood and bark size changes of twigs from 25 May to 7 June 2016. The pattern of shrinking wood concurrent with expanding bark (wood +/bark –) is shown with the light green bars (colour-coding like in Figure 2). The pattern of expanding wood concurrent with shrinking bark (wood –/bark +) is shown with the dark green bars, and the pattern of parallel running size changes of wood and bark are shown with the grey areas (parallel). Data source: hourly resolved dual point dendrometer measurements.

Soil water

Soil water potentials gradually decreased on the two sunny days by 0.031 MPa on Day 1 and 0.035 MPa on Day 3. Soil water potentials were generally higher and decreased less (0.022 MPa) on the wet Day 2 (−0.022 MPa). Overall, soil water potentials were in a wet range on all three days suggesting that negative impacts of soil water supply on gas exchange were low.

Discussion

Different patterns of stem radius changes of twigs and stem

Size changes of wood and bark showed distinctly different patterns between twigs and stems (Figure 2). We hypothesized that such differences result from osmoregulation changing the related patterns in twigs, since twigs are closer to the sources of sugar production. We indeed observed that wood and bark size fluctuations at the stem base are largely explicable with a hydraulic system (flow and storage dynamics) with water flow passively driven by transpiration and the xylem water potentials along the flow path (Zweifel et al. 2001, 2007, Steppe et al. 2006*b*). In contrast, the fluctuations in twigs were not explicable with passive hydraulic mechanisms only, since wood shrinkage concurrently appeared with bark size expansion and vice versa (Figure 2). In a hydraulic system, such behaviour implies an additional active process that alters the size fluctuations expected from passive mechanisms (Sevanto et al. 2002, 2003, Mencuccini et al. 2013, Dawes et al. 2014, Vandegehuchte et al. 2014, Zweifel et al. 2014, Pfautsch et al. 2015). In fact, our approach to identify periods of opposite diameter changes of wood and bark (green areas in Figures 2 and 3) from dual-dendrometer measurements was able to track periods of sugar loading (light green areas in Figure 2) and unloading in the twig's bark (dark green areas in Figure 2) as already suggested previously (Génard 2001, Hölttä et al. 2009, De Schepper and Steppe 2010, De Swaef et al. 2012, Steppe et al. 2015*b*). During periods of sugar loading, the twig bark actively acts as a sink for carbohydrates, whereas during periods of sugar unloading the bark turns into a sugar source. Both processes induce a diameter change of the bark confirming a strong role of osmotic adjustment of twig water relations.

Osmoregulation in twigs

There are several carbon-related processes, such as leaf photosynthesis, sink activities for growth, stem tissue photosynthesis and respiration (Steppe et al. 2015*b*) potentially altering osmolality in trees' tissues and consequently stem size changes (Sevanto et al. 2002, 2003, Daudet et al. 2004; De Schepper and Steppe 2010). Mencuccini et al. (2013) compared bark size changes against model predictions (assuming linear variation of osmotic potential with water potential) and they found that for measurements at the upper position of their trees, the bark size reached an osmoregulation-induced peak at around midday while the bark at the lower position reached a minimum at the same time, very much in line with our findings (Figure 2).

Needle osmolality ranging between 0.14 and 1.75 mol kg^{-1} was found to be similar in magnitude to previously reported work (Paljakka et al. 2017). Furthermore, as also described previously, we did not find a consistent diurnal pattern of needle osmolality dynamics over a day. During the morning hours, when twig wood size was decreasing and twig bark size was increasing, the needle osmolality was not clearly increasing despite the sunny conditions on two of the three days investigated (Figure 2). We therefore conclude that the assimilates from the needles were rapidly transported into the phloem of the twigs, where they changed the osmolality of the bark and were thus not measurable in the needles. The increasing twig bark size indicates an active process of sugar loading in the phloem leading to an increased turgor expanding the bark tissue. This explanation fits both the measured relatively low osmolality in the needle despite the high photosynthetic activity (assuming no stomatal closure) and the osmotic-induced bark size increase at this time of the morning. Such a rapid sugar transport from leaves to the twigs may also keep mesophyll carbohydrate levels in the needle phloem low, reducing a negative feedback on the assimilation process (Ainsworth and Bush 2011).

Another idea we propose is the one of actively increased turgor pressure in twigs in order to avoid a feedback signal from negative water potentials closing the stomata (Zweifel et al. 2006, 2007). Osmotically raised turgor pressure, due to accumulation of recently fixed assimilates in the twig bark, could thus sustain assimilation high during a high transpiration period in the sunny morning hours. It further implies that such a mechanism induces a steep water potential gradient between the needles (very low water potentials) and the twig (moderate water potentials) with potential effects on the entire water flow and storage system of a tree.

Around noon (timing varied with environmental conditions of specific days), this mechanism seemed to stop: the dual dendrometers indicate no more sugar loading or even sugar unloading in the bark (Figures 2 and 3), eventually because the negative xylem water potentials were too low to be compensated by osmoregulation (Donnellan Barraclough et al. 2018) or because the transfer of sugars from the needles into the twigs ceased due to a reduced photosynthesis. In fact, the twig bark did not further swell, quite the contrary, it started to shrink. The timing of this proposed switch from sugar loading to unloading, however, varied largely with weather conditions. On sunny days the switch appeared to occur earlier than on rainy days (Figures 2 and 3).

Osmoregulation plays a minor role at the stem base

In line with the common assimilate pool theory, which assumes the osmotic potential to be constant throughout the entire stem phloem (Heuvelink 1995, De Swaef et al. 2012), we find similar levels of osmolality in needles and stems. However, the proposed rapid transport of sugars from the needles into the twigs suggests much higher osmolality values at the twig level than in needles and at the stem base. Unfortunately, the osmolality in the twig bark was not measured in this study. Generally, we found evidence for a strong osmoregulation at the twig but not at the stem base level (Figure 2). Given daily sugar transport rates in the order of about 1 m h^{-1} (e.g., Windt et al. 2006), the transport of sugars along the stem will take several hours from needles to stem base in our study pine trees with a tree height of 10 m (Windt et al. 2006), which might be one of the reasons for a less visible osmoregulation at the stem base. From a theoretical point of view, osmoregulation makes most sense close to the needles, where water potential changes are most pronounced. Osmoregulation is able to buffer such peaks in water potentials and might, this way, be able to increase the transport capacity of a water flow and storage system under stress and coupled to it the assimilation. The further away from the location of transpiration, the weaker are the peaks in water potentials (Zweifel et al. 2007) and the less a buffering by osmoregulation may be necessary.

Elasticity of xylem

In contrast to the minor changes of wood size at the stem base, twigs showed considerably larger changes in xylem diameter (Figure 2). It was proposed that incomplete wood differentiation or a partial lignification could be reasons for an increased elasticity of the wood tissue in twigs (Zweifel and Häsler 2000, Sevanto et al. 2002, Steppe et al. 2006*b*, Zweifel et al. 2014) since non-lignified wood tends to shrink to a greater extent under decreasing water potentials than fully lignified tissues, as e.g., observed in fast-growing *Eucalyptus globulus* (Zweifel et al. 2014). Our measurements were made in the wood growth phase, which may support this idea of not yet fully developed wood of the twig.

Conclusions

Radial stem size dynamics in wood and bark largely differ between twigs and stem. The weak trend in needle osmolality

changes and the distinct expansion of twig bark in the morning hours implies that trees rapidly move the newly synthesized sugars to the twigs. We conclude that the solutes from the needles increase the osmotic potential in the twig bark and thus induce the bark size increases concurrently with a decreasing xylem size on sunny mornings. In the stem, however, diameter movements of the bark were driven by passive hydraulic mechanisms and we did not find any support for osmoregulation. Stem radius changes close to stem base vs close to leaves are thus driven by different mechanisms.

Acknowledgments

We thank Marcus Schaub for the support with the infrastructure in the Pfynwald irrigation experiment and Arthur Gessler for his help with interpreting the carbon transport processes. We also thank Elena Haeler and Marleen Vos for their contribution to the data collection, and Leonie Schönbeck for the advice given in the statistical analysis. We appreciate the technical support received from HYDRO Exploitation SA in Sion.

Conflict of interest

None declared.

Funding

The study was supported by the Swiss Federal Institute of Forest, Snow and Landscape Research WSL with field infrastructure, covering travel costs for M.L. and a fellowship for F.S. It was further supported by the project TreeNet and the Swiss Long-term Forest Ecosystem Research Programme LWF with data and field equipment.

References

Ainsworth EA, Bush DR (2011) Carbohydrate export from the leaf – a highly regulated process and target to enhance photosynthesis and productivity. Plant Physiol 155:64–69.

Arend M, Fromm J (2007) Seasonal change in the drought response of wood cell development in poplar. Tree Physiol 27:985–992.

Daudet FA, Améglio T, Cochard H, Archilla O, Lacointe A (2004) Experimental analysis of the role of water and carbon in tree stem diameter variations. J Exp Bot 56:135–144.

Dawes MA, Zweifel R, Dawes N, Rixen C, Hagedorn F (2014) CO_2 enrichment alters diurnal stem radius fluctuations of 36-yr-old Larix decidua growing at the alpine tree line. New Phytol 202:1237–1248.

De Schepper V, Steppe K (2010) Development and verification of a water and sugar transport model using measured stem diameter variations. J Exp Bot 61:2083–2099.

De Schepper V, De Swaef T, Bauweraerts I, Steppe K (2013) Phloem transport: a review of mechanisms and controls. J Exp Bot 64: 4839–4850.

De Swaef T, Steppe K (2010) Linking stem diameter variations to sap flow, turgor and water potential in tomato. Funct Plant Biol 37: 429–438.

De Swaef T, Driever SM, Van Meulebroek L, Vanhaecke L, Marcelis LF, Steppe K (2012) Understanding the effect of carbon status on stem diameter variations. Ann Bot 111:31–46.

Donnellan Barraclough A, Zweifel R, Cusens J, Leuzinger S (2018) Daytime stem swelling and seasonal reversal in the peristaltic depletion of stored water along the stem of *Avicennia marina* (Forssk.) Vierh. Tree Physiol 38:965–978.

Génard M (2001) A biophysical analysis of stem and root diameter variations in woody plants. Plant Physiol 126:188–202.

Heuvelink E (1995) Dry matter production in a tomato crop: measurements and simulation. Ann Bot 75:369–379.

Hölttä T, Mencuccini M, Nikinmaa E (2009) Linking phloem function to structure: analysis with a coupled xylem-phloem transport model. J Theor Biol 259:325–337.

Hölttä T, Mencuccini M, Nikinmaa E (2014) Ecophysiological aspects of phloem transport in trees. In: Tausz M, Grulke N (eds) Trees in a changing environment: ecophysiology, adaptation, and future survival, Dordrecht, Springer, pp 25–36.

Lintunen A, Paljakka T, Jyske T et al. (2016) Osmolality and non-structural carbohydrate composition in the secondary phloem of trees across a latitudinal gradient in Europe. Front Plant Sci 7:726.

Mencuccini M, Hölttä T, Sevanto S, Nikinmaa E (2013) Concurrent measurements of change in the bark and xylem diameters of trees reveal a phloem-generated turgor signal. New Phytol 198:1143–1154.

Mencuccini M, Salmon Y, Mitchell P et al. (2017) An empirical method that separates irreversible stem radial growth from bark water content changes in trees: theory and case studies. Plant Cell Environ 40: 290–303.

Paljakka T, Jyske T, Lintunen A, Aaltonen H, Nikinmaa E, Hölttä T (2017) Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.). Plant Cell Environ 40:2160–2173.

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.

Pfautsch S, Aspinwall MJ, Drake JE, Chacon-Doria L, Langelaan RJA, Tissue DT, Tjoelker MG, Lens F (2018) Traits and trade-offs in whole-tree hydraulic architecture along the vertical axis of *Eucalyptus grandis*. Ann Bot 121:129–141.

Rademaker H, Zwieniecki MA, Bohr T, Jensen KH (2017) Sugar export limits size of conifer needles. Phys Rev E 95:042402.

Rossi S, Anfodillo T, Menardi R (2006) Trephor: a new tool for sampling microcores from tree stems. IAWA J 27:89–97.

Schuerch M, Vuataz FD (2000) Natural tracers to quantify seasonal variations of groundwater mixing in a complex alluvial aquifer (Pfynwald, Switzerland). In: Tracers and modelling in hydrogeology. Proceedings of TraM'2000, the International Conference on Tracers and Modelling in Hydrogeology held at Liège, IAHS Press, Belgium, May 2000, pp 533–538.

Sevanto S, Vesala T, Peramaki M, Nikinmaa E (2002) Time lags for xylem and stem diameter variations in a Scots pine tree. Plant Cell Environ 25:1071–1077.

Sevanto S, Mikkelsen TN, Pilegaard K, Vesala T (2003) Comparison of tree stem diameter variations in beech (*Fagus sylvatica* L.) in Soro Denmark and in Scots pine (*Pinus sylvestris* L.) in Hyytiala, Finland. Boreal Environ Res 8:457–464.

Steppe K, De Pauw DJW, Lemeur R, Vanrolleghem PA (2006*a*) A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiol 26: 257–273.

Steppe K, Saveyn A, Vermeulen K, Lemeur R (2006*b*) A comprehensive model for simulating stem diameter fluctuations and radial stem growth. Tree Physiol 26:257–273.

Steppe K, Vandegehuchte MW, Tognetti R, Mencuccini M (2015*b*) Sap flow as a key trait in the understanding of plant hydraulic functioning. Tree Physiol 35:341–345.

Taiz L, Zeiger E, Møller I, Murphy A (2015) Plant physiology and development, 6th edn. Sinauer Associates, Sunderland, MA.

Timofeeva G, Treydte K, Bugmann H, Rigling A, Schaub M, Siegwolf R, Saurer M (2017) Long-term effects of drought on tree-ring growth and carbon isotope variability in Scots pine in a dry environment. Tree Physiol 37:1028–1041.

Vandegehuchte MW, Guyot A, Hubau M et al. (2014) Long-term versus daily stem diameter variation in co-occurring mangrove species: Environmental versus ecophysiological drivers. Agric For Meteorol 192:51–58.

von Arx G, Arzac A, Fonti P, Frank D, Zweifel R, Rigling A, Galiano L, Gessler A, Olano JM (2017) Responses of sapwood ray parenchyma and non-structural carbohydrates of *Pinus sylvestris* to drought and long-term irrigation. Funct Ecol 31: 1371–1382.

Windt CW, Vergeldt FJ, De Jager PA, Van As H (2006) MRI of long-distance water transport: a comparison of the phloem and xylem flow characteristics and dynamics in poplar, castor bean, tomato and tobacco. Plant Cell Environ 29:1715–1729.

Zweifel R, Häsler 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, Häsler R (2001) Link between diurnal stem radius changes and tree water relations. Tree Physiol 21:869–877.

Zweifel R, Zeugin F, Zimmermann L, Newbery DM (2006) Intra-annual radial growth and water relations of trees – implications towards a growth mechanism. J Exp Bot 57:1445–1459.

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

Zweifel R, Drew DM, Schweingruber F, Downes GM (2014) Xylem as the main origin of stem radius changes in Eucalyptus. Funct Plant Biol 41:520–534.

Zweifel R, Haeni M, Buchmann N, Eugster W (2016) Are trees able to grow in periods of stem shrinkage? New Phytol 211:839–849.