Sap flux and stem radius variations in mature Cedrus libani trees during the growing season

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1. Introduction
Mediterranean ecosystems are expected to face an increase in temperature, with higher frequencies of drought and a decrease in spring precipitation (IPCC, 2013), which will likely affect tree growth (Ciais et al., 2005; Boisvenue and Running, 2006). Sarris et al. (2007) observed that growth of Pinus brutia Ten. individuals on Samos already showed reduced annual increments caused by a decline in precipitation. Carbon and water fluxes of trees are strongly influenced by environmental conditions (Betsch et al., 2011). Studies using high-resolution observations of growth dynamics and sap flux in combination with environmental parameters help to decipher trees’ responses to short-term and long-term changes in environmental conditions and how these might affect tree vitality and forest dynamics (Rossi et al., 2006; Horna et al., 2011; Michelot et al., 2012). Dendrometers are practical devices that allow continuous measurements of stem radius variations and seasonal tree growth (Herzog et al., 1995). Xylem sap flux can be measured using heat dissipation sensors (constant heating method) after Granier (1985). Both methods were either used together or separately in several studies, some covering the Mediterranean region (Martínez-Vilalta et al., 2003; Čermák et al., 2007; Linares et al., 2009; Swidrak et al., 2013; Brito et al., 2017). Vieira et al. (2013) showed for Pinus pinaster growing in a Mediterranean climate that daily variations in stem radius depend on transpiration and are therefore related to temperature and the tree's water status. Sánchez-Costa et al. (2015) studied sap flow and stem diameter variations in four Mediterranean trees that showed similar responses to drought.

Studies investigating either intraannual stem growth or sap flux dynamics of conifers from the montane Mediterranean region are still rare (Camarero et al., 1998; Güney et al., 2017), although these valuable ecosystems might be negatively affected by changing climate conditions in the future. One important representative of the montane Mediterranean region is Cedrus libani A.Rich (Lebanon cedar), which has its largest distribution area in Turkey, covering around 600,000 ha (Boydak, 2003). Pure stands are mainly to be found in the Taurus Mountains (especially in the western Taurus Mountains) at altitudes...
between 800 and 2100 m under montane Mediterranean climate conditions (Evcimen, 1963; Boydak and Çalıkoğlu, 2008). *C. libani* forests are primarily found on Mesozoic limestone formations. Their taproot systems can extend rather deeply by using cracks in limestone blocks, which contain fine soils with high water-holding capacity (Nuri and Uysal, 2009). Especially in Turkey, *C. libani* is an important species for historical, cultural, and ecological reasons, but it is also important in terms of forest management (Boydak, 2003; Yaman, 2007; Brooks et al., 2008; Kavgacı et al., 2010). Its drought and frost tolerance as well as its high adaptability have led to many reforestation and afforestation projects (Fady et al., 2003; Ducci et al., 2007; Boydak and Çalıkoğlu, 2008), and to an increasing interest in the use of *C. libani* as a potential substitute for native European tree species in a changing climate (Huber and Storz, 2014; Messinger et al., 2015). Although *C. libani* has been the object of a large number of studies, e.g., dendrochronological studies (Akkemik, 2003; Touchan et al., 2007), ecophysiological studies are still rare, especially under natural conditions (Güney et al., 2016).

The main objectives of this study were therefore to investigate the course of stem radius variation (from April to September) and sap flux density (from June to August) of mature *C. libani* individuals during one growing season and to assess the climatic sensitivity of maximum daily stem radius shrinkage and daily mean sap flux density to environmental variables measured at the study site in the southwestern Taurus Mountains of Turkey.

### 2. Materials and methods

#### 2.1. Study area

The study was conducted at the Elmali Cedar Research Forest (Antalya, Turkey) during 2009. The area is characterized by cold winters with frost and hot summers with a drought period usually lasting from June to September (Atalay, 1987). Annual precipitation amounts to 640 mm and mean annual air temperature is 7.5 °C. Recorded maximum and minimum air temperatures are 34 °C and –31 °C, respectively (https://www.mgm.gov.tr/). The study site was established in a pure and mature *C. libani* stand at 1665 m a.s.l. (36°35′09″N, 30°01′14″E). Stand inclination was approximately 40° with NW exposure. The soil is clay loam to clay with a pH (H₂O) of 7.63–8.03 (Basaran, 2008).

#### 2.2. Stem radius variations

Stem radius changes were measured for nine mature *C. libani* individuals using high-precision point dendrometers (linear displacement potentiometers, accuracy <10 µm; MMR 10_11 R5K, MEGATRON Elektronik AG & Co., Munich, Germany). We chose trees of different sizes that were representative for the study site to ensure natural variability for our measurements. All trees were vital and had no visible damage or infestation (for characteristics of the study trees, see Table 1). Dendrometers were installed at breast height (1.30 m) on the slope-parallel sides of the stem. The contact heads of the dendrometers were placed at the cortex surface after removing as much bark as possible without damaging living tissue (Zweifel et al., 2006). To shield the dendrometers from direct sunlight.
and rainfall, they were covered with aluminum foil and Styrofoam covers. Dendrometer signals were recorded hourly and stored using an automatic Delta-T DL2e data logger from April until September 2009. Maximum daily shrinkage (MDS) of the stem radius was extracted from raw dendrometer data by calculating the difference of the measured daily maximum and daily minimum stem radii (Deslauriers et al., 2007; Oberhuber et al., 2015). MDS is an indirect measure of tree water status, as it provides information about the proportion of water taken up at night and the loss of that water from elastic tissues during the day (Zweifel and Häslar, 2001; Giovannelli et al., 2007).

2.3. Xylem sap flux

Xylem sap flux was measured for four of the nine C. libani individuals chosen for the stem radius variation monitoring mentioned in Section 2.2 (Table 1). Measurements were done using constant thermal dissipation sensors (constant heating method) that were manufactured in our laboratories according to Granier (1985). Two probes, 20 mm long and 2 mm wide, were inserted at a distance of 15–20 cm from each other into the first 2 cm of outer sapwood at about 1.30 m height. The upper probe was constantly heated with 200 mW, while the lower one remained unheated to measure the reference temperature of the stem. To avoid radiant heating and convective heat loss, the sensors were inserted into the north-facing side of the stem and were covered with Styrofoam. The temperature difference (∆T) between the heated and reference probes was recorded (with copper-constantan thermocouples) every 10 min and stored as 60-min averages by the data logger. Temperature differences were converted to sap flux densities \( J_s, \) g m\(^{-2}\) s\(^{-1}\), flow of xylem water per area of hydroactive xylem) based on the following empirical calibration equation after Granier (1985, 1987):

\[
J_s = 119 \left( \frac{\Delta T_{max}-\Delta T}{\Delta T} \right)^{1.231},
\]

where \( \Delta T_{max} \) is the maximum temperature difference during times of zero sap flow, \( \Delta T \) is the actual temperature difference, and 119 and 1.231 are constants determined empirically over a large range of plant species (Granier, 1985). Measurements of \( J_s \) were started on 11 June and lasted until 14 August 2009. Daily mean \( J_s \) was calculated for each tree, and then the daily values of the four trees were averaged for statistical analysis.

2.4. Climate measurements

Air temperature (Tair) and relative air humidity (RH) were measured with a SKYE sensor (SKH-2011 0196-12070, 10 mV = 1% RH, 10 kΩ = 25 °C). The device was installed in the center of the study site approximately 2 m above the forest ground. Incoming photosynthetic active radiation (PhAR) was measured with an LI-190 Quantum Sensor (LI-COR Biosciences, Lincoln, NE, USA), which was attached about 1 m above the SKYE sensor. Soil temperature was measured at 20-cm soil depth using an NTC thermistor (S 861 10 kΩ, Siemens Matsushita Components, Munich, Germany). Soil water content was measured with a Theta Equitensiometer ML-2 (EQ ML2 9-23, Delta-T Devices, Cambridge, UK) from 6 June onwards, and precipitation (Pp) was measured using an ARG-100 rain gauge (Campbell Scientific, Shepshed, UK). All devices were connected to the logger, which took measurements every 10 min, saving the average of these measurements per hour in its internal memory. Site-specific vapor pressure deficit (VPD) was calculated from Tair, RH, and ambient air pressure.

2.5. Data analysis

Pearson’s correlation, simple regression, and multiple stepwise regression analyses were used to assess the climatic sensitivity of MDS and \( J_s \) (dependent variables) during the growing season. Meteorological variables (independent variables) used in the analysis were the daily sum of precipitation and daily means of air temperature, soil temperature, RH, VPD, PhAR, and soil water content. Analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) with a significance level of 0.05.

3. Results

3.1. Environmental conditions during the measurement period

During the measurement period (31 March–3 September 2009), mean Tair was 14.3 °C and mean soil temperature was 11.9 °C (Figure 1). Pp amounted to 216.8 mm, of which 68% (148.2 mm) fell during April and May. The driest months were June and July (only 15.8 mm of Pp). Mean RH was 52.2% and mean VPD was 9.4 hPa. Highest daily means of VPD (approximately 20 hPa) were reached at the end of July. PhAR reached its highest values during June (daily mean of 238 µmol m\(^{-2}\) s\(^{-1}\) on 9 June), and daily means of soil water content, measured from 6 June onwards, ranged between 25.6% and 44.0%.

3.2. Daily sap flux and stem radius variations

The main period of stem radius increase in C. libani was observed from mid-May to July (Figure 1). All four study trees showed a similar daily course of \( J_s \) but with different daily maxima. During the observation period, daily maximum values of \( J_s \) varied between 8 and 17 g m\(^{-2}\) s\(^{-1}\) for tree C8, between 20 and 33 g m\(^{-2}\) s\(^{-1}\) for C1, between 19 and 27 g m\(^{-2}\) s\(^{-1}\) for C7, and between 12 and 30 g m\(^{-2}\) s\(^{-1}\) for C4. Means of \( J_s \) (Figures 1A and 1B) showed a decreasing trend until 8 August, when it started to rain for a few days. Typical daily courses of stem radius variation and \( J_s \) are shown as an example for tree C7 on three different days (Figure 2A). Hourly values of stem radius variation and \( J_s \) were plotted against each other, resulting in a daily hysteresis effect (Figure 2B), which reflects the...
Figure 1. Course of stem radius variation and sap flux density $J_s$ of *Cedrus libani*, and meteorological variables from April to September 2009. (A) Hourly values of stem radius variations of the study trees (gray lines), mean hourly stem radius variation of all trees (black line), and mean hourly $J_s$ of the four selected trees (blue line). (B) Mean maximum daily shrinkage (MDS, black line) and mean daily $J_s$ (blue line). (C) Daily sum of precipitation (blue bars) and daily means of air temperature (red line) and soil temperature (dashed line). (D) Daily means of relative air humidity (RH, solid line) and vapor pressure deficit (VPD, dotted line). (E) Daily means of photosynthetic active radiation (PhAR, black line) and soil water content (green line). Ticks on the x-axis correspond to the beginning of each month.
pattern of water storage and retrieval. The magnitude of the daily temporal depletion of internal water storage is proportional to the size of the inner area of the hysteresis effect. From June to August, the inner area of the hysteresis effect increased accordingly with the increased amplitudes of daily stem radius variations. Although only shown for tree C7, this trend was observed for all study trees.

3.3. Maximum daily shrinkage (MDS) and sap flux density (J) related to tree size
MDS, which was calculated from dendrometer records, and J, which was calculated from sap flux measurements, were averaged for each tree for the period of observation (Table 1). Mean MDS and mean J were correlated against tree size parameters (DBH, height, PCA) and annual radial stem increment (Table 1). Simple linear regressions resulted in a significant positive relationship between mean J and DBH (Figure 3). Correlations between MDS and DBH as well as between the other parameters (data not shown) were not significant at the 0.05 level.

3.4. Climatic sensitivity of MDS and J
Results from Pearson’s analysis and simple regression analysis (linear or quadratic) are shown for the climate variables VPD, RH, Tair, PhAR, and soil water content (Figure 4). The directions of the relationships were the same for MDS and J. MDS showed a highly significant positive correlation to VPD, followed by Tair, and was negatively correlated with RH. J showed a highly significant positive correlation with PhAR and VPD. A less significant correlation was found between Tair and J (P < 0.05). Soil water content was positively correlated only with MDS.

Multiple linear regression (MLR) models for MDS and J of C. libani explained 48% and 69% of the variation, respectively (Table 2). Collinearity diagnosis was performed before choosing the final climate variables for the models. In the final MLR model, standardized coefficients (β) showed that VPD had the greatest influence on MDS, followed by Tair and PhAR. The MLR model of J included only two meteorological variables, of which PhAR was the more important variable, followed by Pp.

4. Discussion
The natural habitat of C. libani is expected to face drier and hotter summers in the near future due to global climate change. We therefore investigated radial stem growth dynamics and sap flux of this tree species in order to improve our understanding of its ecophysiological characteristics and responses to environmental variables.

4.1. Radial stem variations and sap flux densities of C. libani during the 2009 growing season
Dendrometer measurements revealed a uniform seasonal growth pattern without the summer dormancy reported for Cupressus sempervirens (L.) (Senitza, 1989; Güney et al., 2017), a species that can be found in mixed stands with C. libani (Schütt et al., 2004). The fact that radial stem growth continued during July and August, when there was little rain and low soil water content, suggests that C. libani can control water loss efficiently and that water supply from the soil was sufficient (Güney et al., 2017). Total annual increments were slightly below the values reported for the same study trees by Güney et al. (2017), which might be caused mainly by comparably lower spring precipitation in 2009. Daily courses of radial growth, as well as swelling and shrinkage of the bark and xylem (caused by changing tree water status), were similar among the trees. MDS values calculated from dendrometer records (mean MDS ranging between 0.09 and 0.15 mm in C. libani) were comparable to values reported in other studies. Deslauriers et al. (2007) studied MDS in the conifers Larix decidua Mill., Picea abies (L.) Karst., and Pinus cembra L. growing in the Italian Alps. For all three species, average MDS was around 0.05 mm, but around August, MDS values were markedly higher, with up to 0.10 mm in P. abies and P. cembra, and 0.15 mm in L. decidua. In a 37-year-old Fagus sylvatica L. stand, maximum values of MDS reached 0.10 mm, and average values amounted to 0.05 mm (Betsch et al., 2011). Oberhuber et al. (2015) showed that MDS in P. abies reached maximum values of up to 0.15 mm. In our study, during the observation period, the mean MDS of C. libani showed a tendency to increase. This indicates higher amplitudes of shrinking and swelling of the stem due to drier conditions (see Section 4.2).

Comparable to stem radius variations, daily courses of J were similar among the study trees, although with different daily maxima ranging from 8.0 to 33 g m⁻² s⁻¹. Maximum values of J in C. libani are comparable to values observed for coniferous trees in other sap flow studies. At the Alpine timberline near 2000 m a.s.l. in northeastern Italy, L. decidua was reported to reach maximum J of up to 91 g m⁻² s⁻¹, while maximum J for P. abies and P. cembra was reported to reach up to 25 g m⁻² s⁻¹ and 19.5 g m⁻² s⁻¹, respectively (Anfodillo et al., 1998; Köstner et al., 1998). For tree species growing under a warm temperate oceanic climate in North America, maximum J in Pinus taeda L. ranged between 30 and 50 g m⁻² s⁻¹ and between 37 and 97 g m⁻² s⁻¹ for Pinus palustris Mill.; it was reported to reach values of up to 40 g m⁻² s⁻¹ in Pseudotsuga menziesii (Mirb.) Franco (Oren et al., 1996; Phillips et al., 2002; Ford et al., 2004). Sánchez–Costa et al. (2015) showed that maximum J in the Mediterranean conifer Pinus halepensis Mill. increases up to 50 g m⁻² s⁻¹ between March and June, and then drops to about 25 g m⁻² s⁻¹. Therefore, it is to be assumed that our C. libani individuals might have reached higher maxima of J before June, when our observations started. At our study site, daily maximum J always occurred around midday and was lowest on cloudy or rainy days. During rain events, transpiration decreases, which leads to increased tree water status and lower J. Apparently, freely
Figure 2. Stem radius variation and sap flux density ($J_s$) of the study tree C7 for three selected days during the measurement period in 2009. (A) Diurnal course of hourly measured stem radius variation (black) and $J_s$ (blue) from midnight until morning of the following day. Selected time periods were 19–20 June, 29–30 June, and 4–5 August. (B) $J_s$ plotted against stem radius variation showing a daily hysteresis effect. At around 0800 hours, $J_s$ starts to rise, reaching its highest rates during midday (1400/1500 hours), and then going back to zero at late night or early morning (after 2000 hours). Arrows indicate dynamic changes in stem radius variation; vertical shifts indicate stem water storage changes. Please note that the amount of the amplitude for stem radius variation is always 0.4 mm.
available water is quickly absorbed into conductive tissues by *C. libani*, without a complete replenishment of water storage (Küppers, 1982). The effect of tree size was only found for *J_s*, which was positively correlated to DBH. In most trees, the water-conducting area of the stem (sapwood) in which *J_s* is measured is also positively correlated to DBH (Delzon et al., 2004; Horna et al., 2011; Güney, 2018). Although *J_s* was only measured for four study trees, the results still show the relationship between *J_s* and DBH. Nevertheless, our results may be expanded by further measurements of *J_s* on a larger number of trees of varying size. MDS was not correlated to any of the size parameters. Oberhuber et al. (2015) showed that mean MDS values did not significantly differ among mature trees and saplings of *P. abies*. However, when conditions got drier, saplings developed higher daily MDS than mature trees since the former more strongly deplete their internal water storage. DBH of our study trees varied only between 26 and 48 cm, which might be the reason why there was no significant correlation to tree size.

4.2. Hysteresis effects of varying magnitude due to changing tree water status

The observed hysteresis effect shows that there is a close coupling between stem radius change and stem sap flux regarding patterns of water storage and retrieval (Tyree, 1988). When transpiration rapidly increases in the morning, most of the water used is retrieved from water stored in leaves, twigs, and stem tissue (in that order). The rate of sap flow during the day in the lower parts of a tree (stem and roots) typically lags behind that in shoots (Schulze et al., 1985). Conversely, when transpiration decreases in the late afternoon, sap flow rates in the roots and stem exceed flow rates for maintaining transpiration. By this means, tissue water reserves that are used up are refilled by root water uptake during the night. A daily hysteresis effect was also described by Schmitt et al. (2007) for woody plants of a montane rain forest in Ecuador. Varying magnitudes of hysteresis effects were related to the changing water status of the tree. Water-related shrinking and swelling of the woody and bark tissues were proportional to daily transpiration sums. In our study, observed hysteresis at the beginning was very low, indicating a good water supply for the trees, which resulted in low sap flux resistance and no significant use of tree water storage (Küppers, 1982). However, when environmental conditions became drier, soil water storage decreased, and our study trees could no longer retrieve enough soil water to maintain transpiration. The *C. libani* individuals began to draw water from internal storage, leading to increased hysteresis (increasing inner area of the hysteresis loop; Figure 2B). The presence of a hysteresis effect indicates that the study trees are well adapted to the observed summer drought and that photosynthesis and transpiration seemed to continue during summer months. A large hysteresis effect, which was not observed, would indicate a very poor water supply resulting in complete depletion of water resources from internal storage (Küppers, 1982). We conclude that *C. libani* at our study site seemed to have only moderate water stress during the observation period, without significant diurnal water supply or recharge deficiencies.

4.3. Environmental control of maximum daily shrinkage (MDS) and sap flux density *J_s*

MDS provides information about the tree’s water status (Zweifel and Häslar, 2000; Brito et al., 2017) as stem radius variation is correlated to depletion of internal water storage due to transpiration, which in turn varies with changing environmental conditions (Zweifel and Häslar, 2001). In our study, mean MDS of *C. libani* was most significantly related to VPD. In the work of Deslauriers et al. (2007), simple correlations also resulted in a most significant relationship between MDS and VPD, with values varying between 0.48 and 0.69 depending on species (*P. abies*, *P. cembra*, and *L. decidua*). In another study, MDS of *P. abies* was most closely related to air temperature and soil water content, followed by VPD (Oberhuber et al., 2015).

MDS of *Pinus canariensis* (Sweet ex Spreng.) growing in a drought-induced treeline (Canary Islands) was also significantly positively correlated with air temperature and VPD (Brito et al., 2017). Cocozza et al. (2015) showed that in *Olea europaea* L. MDS was most closely correlated with VPD. Results from our study and others are in accordance with how closely MDS is related to atmospheric conditions, especially VPD. Atmospheric conditions directly influence transpiration and draw upon
Figure 4. Correlations between (A) mean maximum daily shrinkage (MDS, n = 157) and (B) mean daily sap flux density ($J_s$, n = 65) of Cedrus libani and daily means of the meteorological variables VPD (vapor pressure deficit), RH (relative air humidity), air temperature, PhAR (photosynthetic active radiation), and soil water content. Pearson's correlation coefficient ($r$) was calculated and significant values ($P < 0.05$) are indicated with an asterisk.
water reserves. Therefore, when atmospheric conditions get hotter and drier, MDS values increase, which means that more water is drawn from the internal storage of the trees. With regard to future climate change scenarios (drier and hotter summers), higher VPD may limit transpiration, leading to a decrease in *C. libani* stem growth (Zweifel et al., 2005; Ehrenberger et al., 2012). In our study, soil water content was positively correlated to MDS but played a minor role, which might be caused by the shorter measuring period of soil water content (starting in June). In contrast to MDS, mean $J_s$ of *C. libani* showed the strongest correlation to PhAR (significant positive linear relationship), although atmospheric conditions also resulted in significant relationships to mean $J_s$ (positive quadratic relationship to VPD and negative quadratic relationship to RH). That the relationship between $J_s$ and VPD is not linear was also shown in the results of Sánchez-Costa et al. (2015) for four cooccurring Mediterranean tree species, including *P. halepensis*. Tree transpiration is low during rainy/cloudy days (low VPD) and during hot and sunny days (high VPD); therefore, the highest $J_s$ values of *C. libani* can be found during times of medium VPD (between 10 to 15 hPa). The strong correlation with PhAR is partly caused by high PhAR heating leaves faster than it heats the air, so the driving force for transpiration and $J_s$ is actually VPD$_{leaf}$ (not measured), and, to a lesser extent, VPD$_{air}$ (measured) (Larcher, 1994). Although in many forests of different ecosystems soil water content, VPD, and global radiation are known to be the main environmental drivers controlling transpiration (Meinzer et al., 2003; Horna et al., 2011), soil water content showed no significant relationship to $J_s$ in our study. This might be due to the restricted observation period of 2 months, which did not include the moister conditions during spring. Nevertheless, our results show that *C. libani* maintained a uniform pattern of $J_s$ when environmental conditions got drier (no precipitation, high VPD, low soil water content) and hysteresis effects increased towards the end of the observation period, which points to the ability for effective stomatal regulation, which limits maximum sap flow by stomatal closure (Larcher, 1994).

In conclusion, our findings regarding the environmental control of $J_s$ and MDS in *C. libani* coincide with the findings of other studies and show the close coupling of solar radiation (PhAR) and atmospheric conditions on photosynthetic activity and transpiration, and therefore tree sap flux ($J_s$). Our results are useful for improving our understanding of the ecophysiological properties of *C. libani* and its adaptation potential to changing environmental conditions. However, further studies on sap flux and water use of *C. libani* on a higher number of trees and during a longer observation period will help to improve our understanding of how this species might cope with the expected drier and hotter summers in the future.

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