Transpiration at leaf and tree level in a poplar short-rotation coppice culture: seasonal and genotypic differences

A. Navarro[a], M. Portillo-Estrada, S.P.P. Vanbeveren, C. Ariza-Carricondo and R. Ceulemans

Research Center of Excellence on Plant and Ecosystems, Department of Biology, University of Antwerp, BE-2610 Wilrijk, Belgium.

Abstract

Poplar (*Populus* spp.) is one of the most commonly cultivated genera in experimental and commercial short-rotation coppice (SRC) plantations. The genus is among the fastest growing in temperate latitudes, but the success of highly productive poplar SRC plantations strongly depends on soil water availability. We examined the transpiration at the leaf and the individual tree levels of four different poplar genotypes under an SRC regime. Measurements were performed for the entire growing season of 2016, during the seventh growth year of the plantation and before the third coppice on four poplar genotypes (three individual multiple-stem trees per genotype) belonging to different species and from a different genetic background. The experimental site was a commercial scale multi-genotype SRC plantation, established in Flanders (Belgium). Measurements at the leaf level were performed on specific days of the growing season that differed in evaporative demand, temperature and incoming radiation. To determine the transpiration at the stem level, we measured single-stem sap flow using the stem heat balance (SHB) method and daily stem diameter variation measurements. The whole-tree transpiration was estimated by summing the sap flow rates from all stems. Measurements at the stem level were continuously monitored during the entire growing season. Sap flow was tightly connected to the phenological stage of the trees, thus onset of spring (leaf area development) and late autumn (leaf fall) were easily identifiable from sap flow measurements, showing differences among the four genotypes. The dynamics of transpiration at the leaf and tree level were driven by photosynthetic photon flux density (PPFD), but the sap flow intensity was controlled by vapour pressure deficit (VPD). The four poplar genotypes showed different water use strategies, based on determination of transpiration and other plant water status indicators.

Keywords: *Populus*, sap flow, bioenergy, stem heat balance method, physiological responses

INTRODUCTION

Among the available alternatives for fossil energy sources, biomass from woody species is seen as one of the possible keys to a low-carbon economy, because of its compatibility with existing agronomic practices, materials handling, and energy production systems. Among the woody species utilized for biomass production for energy purposes, poplars (*Populus* spp.) are one of the most sensitive to water availability; their productivity is closely linked to water availability in the soil (Marron et al., 2008). Future selection criteria for commercial poplar genotypes need to take the water use of bioenergy cropping systems into account, by identifying their physiological traits and transpiration responses, in combination with maximum biomass production. For most broad-leaved stands, transpiration is the major component of water use (Meiresonne et al., 1999), and the study of transpirational water loss at leaf and tree level becomes fundamental. Under natural conditions, the sap flow technique is the only approach for measuring trees transpiration. The stem heat balance (SHB) method (Sakuratani, 1981; Baker and van Bavel, 1987) is a

[a]E-mail: Alejandra.NavarroGarcia@uantwerpen.be

technique easy to use and provides data of high time resolution, although no direct, non-modeled data are available on sap flow measurements, used to obtain transpiration, in multi-stemmed SRC poplars for an entire growing season. For sustaining the extension of poplar SRC crops on lands subjected to seasonal changes of water supply provoked by the ongoing global change context, the water use responses of SRC poplar genotypes have to be studied. To achieve this goal, in this study, the water use responses of four poplar genotypes in a SRC plantation, to the environmental variables were assessed by the study of the physiological traits and the transpiration determination (water loss at leaf and tree levels) to the environmental variables.

MATERIAL AND METHODS

Study site and plant material

Measurements were made in a commercial scale multi-genotype SRC plantation, established in Lochristi, province East-Flanders, Belgium (51°06'44"N; 3°51'02"E - http://uahost.uantwerpen.be/popfull). The plantation was established on April 7-10, 2010 and consisted of 12 different poplar (*Populus*) genotypes planted in mono-genotypic blocks, on former agricultural and pasture land. A tree density of 8000 plants ha^{-1} was achieved with a double-row planting scheme. The site was neither fertilized nor irrigated. More information on the site, on the management, and on soil characteristics has been provided previously (Broeckx et al., 2012).

Leaf and tree level measurements were confined to a subset of four genotypes (three individual trees per genotype) characterized by different parentages and chosen to cover a wide genetic background: 'Bakan' (parentage *Populus trichocarpa* T. & G. × *P. maximowiczii* A. Henry), 'Oudenberg' (parentage *P. deltoides* Bartr. ex Marsh. × *P. nigra* L.), 'Koster' (parentage *P. deltoides* Bartr. ex Marsh. × *P. nigra* L.), and 'Grimminge' (parentage *P. deltoides* Bartr. ex Marsh × (*P. trichocarpa* T. & G. × *P. deltoides* Bartr. ex Marsh.). More details on the origin, the selection and the gender of these species were previously published (Broeckx et al., 2012). Measurements were performed during the entire growing season of 2016 (from April to November), i.e., during the seventh growth year of the plantation and before the third coppice of the plantation (February 2017). All measurements were made on multiple-stem trees after they had been coppiced twice.

Air temperature (T_{air}; °C) and relative atmospheric humidity (RH_{air}; %) were continuously recorded at an extendable mast at 6.4 m above the ground surface using Vaisala probes (model HMP 45C, Vaisala, Helsinki, Finland); these data were used to calculate vapour pressure deficit (VPD; kPa). Incoming photosynthetic photon flux density (PPFD, 400-700 nm; mmol m^{-2} s^{-1}) was recorded at the same height using a quantum sensor (model LI-190, Li-COR, Lincoln, NE, USA). Precipitation was recorded using a tipping bucket rain gauge (model 3665 R, Spectrum Technologies Inc., Plainfield, IL, USA). Two data loggers (model CR5000 and CR1000, Campbell Scientific, Logan, Utah, USA) recorded 30 min averages for each environmental variable.

Leaf level measurements

Water relations and gas exchange were measured every 15-20 days during the growing season, from early May until the end of September 2016, resulting in a total of 10 days characterized by different PPFD and VPD conditions. We selected five days (one per month) that covered a range of meteorological conditions. Diurnal courses of leaf stomatal conductance to water vapour (g_s; mmol m^{-2} s^{-1}) were measured with a steady-state diffusion porometer (Delta-T AP4, Delta-T Devices Ltd., Burwell, Cambridge, UK). Three mature leaves of the same age were chosen at random from the upper canopy; g_s measurements were taken from sunrise to sunset at intervals of 2-3 h. Access to the canopy was guaranteed by ladders with a working height of 5.2 m. Leaf transpiration (E_{leaf}; mg H_2O m^{-2} s^{-1}) was determined as follows:

$$E_{\text{leaf}} = g_s \times \Delta C_{\text{wv leaf-air}} = \left(g_s \times \left[\left(C_{\text{wv sat.air}} \times \frac{RH}{100}\right)_{\text{leaf}} - \left(C_{\text{wv sat.air}} \times \frac{RH}{100}\right)_{\text{air}}\right]\right) \times 1000 \quad (1)$$

where g_s is leaf stomatal conductance to water vapour in m s^{-1}; $\Delta C_{\text{wv leaf-air}}$ is the concentration gradient in water vapour from the intercellular spaces in the leaf to the atmosphere in g m^{-3} and RH is the relative humidity (%).

Measurements of midday stem water potential (Ψ_x; MPa) and leaf relative water content (RWC_l; %) were performed between 10:30 and 12:30 h (solar time) on three trees per genotype. For the determination of Ψ_x, one sunlit leaf $tree^{-1}$ of similar age and position in the canopy was covered with both a plastic sheet and aluminum foil for at least 2 h before the measurement. These leaves were cut and enclosed in a plastic bag, and were immediately placed in a pressure chamber (ARIMAD-2, A.R.I., Ramat Hagolan, Israel) according to Scholander et al. (1965). RWC_l was measured for three leaves $tree^{-1}$, providing an average RWC_l, according to the equation (Barrs and Weatherley, 1962):

$$RWC_l = \frac{FW-DW}{TW-DW} \times 100 \quad (2)$$

where FW, DW, and TW are the fresh, dry and turgid weights (g), respectively, of the whole leaf. Leaves were weighed immediately after collection to determine the fresh weight (FW). The cut end of each leaf was placed in distilled water and kept in dim light at 4°C for 24-48 h till the turgid weight (TW) was reached and recorded. Dry weight (DW) was measured after leaves were dried for 24-48 h at 70°C.

Tree level measurements

Rates of sap flow (F_s; g h^{-1}) of individual stems were measured using the SHB technique (Sakuratani, 1981; Baker and van Bavel, 1987). This technique is relatively easy to use, provides data of high time resolution and has been successfully applied in poplar SRC plantations (Hall et al., 1998; Allen et al., 1999; Tricker et al., 2009; Bloemen et al., 2017). F_s was continuously monitored on two stems per tree and three trees per genotype throughout the entire growing season (April 1 to November 12, 2016), using 24 SHB sap flow sensors (Dynamax Inc., Houston, Texas, USA) of a wide range of stem diameters (SGEX16, SGEX19, SGEX25 and SGB35). Individual stems in trees were selected to be representative of the entire range of stem diameters (from 6 to 50 mm) measured during an extensive inventory performed in February 2016. The sensors were mounted at a height of 1.50 m above the soil surface and installed following the manufacturer's instructions (https://youtu.be/s7Zz5aNApLI).

F_s was calculated from the raw data using the equations of Baker and van Bavel (1987). As the poplars were fast growing, the increase in stem area during the growing season was considered for the energy balance equations, using the increase in stem diameter recorded by automatic point dendrometers (model ZN11-O-WP, Natkon, Switzerland). The dendrometers were installed with a ring-shaped carbon fibre frame at a height of 1.20 m, just below the sap flow sensor. Data from the sap flow sensors and the dendrometers were collected at 30 s intervals with a data logger (model CR1000, Campbell Scientific, UK) and 15-min averages were recorded.

To obtain the transpiration rate per unit of ground area (E_c; mm day^{-1}) of each poplar genotype, the transpiration of the whole tree had to be known. This whole-tree transpiration was estimated by summing the F_s contributions from all stems. The stems of each tree of each genotype were classified in terms of the operative stem diameter range of every sap flow sensor (Table 1). Sap flow rate per unit of sapwood area (F_{ij}; g m^{-2} h^{-1}) for the i^{th} stem of the j^{th} range was obtained by dividing F_{sij} rate (g h^{-1}) by the sapwood area at 0.22 m (A_{sij}, m^2). The sum of all F_{ij} was multiplied by the ratio of the average sapwood area at 0.22 m for all stems equipped with dendrometers ($A_{s\text{-}avg}$; m^2) to the ground surface area per tree (SA; m^2) as follows:

$$E_{\mathrm{c-sapwood}} = \sum_{j=1}^{4} \sum_{i=1}^{n} \left(\frac{F_{sij}}{A_{sij}} \right) \times \frac{A_{\mathrm{s-avg}}}{SA} \quad (3)$$

where *j* is the operative stem diameter range, *i* is the stem number and *n* is number of stems per operative stem diameter range.

Table 1. Parameters used for scaling F_s to E_c during the 2016 growing season, according to the stem-based approach [total number of stems (SN), operative stem diameter range of each sap flow sensor (from 1 to 5), allometric equation (AE) d=D/a where D = stem diameter at 1.20 m, d = stem diameter at 0.22 m and a = constant, and ground surface area per tree (SA)] for four poplar genotypes.

Parameters		Genotype			
		Bakan	Grimminge	Koster	Oudenberg
SN		4	5	10	7
Operative stem diameter range	1-SGB35 (>31 mm)	1	1	0	1
	2-SGEX25 (23-31 mm)	1	1	2	1
	3-SGEX19 (18.5-23 mm)	0	1	1	1
	4-SGEX16 (14-18.5 mm)	1	1	2	1
	5-no SF sensor[1] (<14 mm)	1	1	5	3
AE	a	0.82	0.80	0.77	0.86
	r^2	0.99	0.98	0.98	0.98
SA (m^2)		1.49	1.70	1.53	1.41

[1]Stems within the operative stem diameter range 5 were not accounted for calculating the whole tree transpiration.

$E_{\mathrm{c-sapwood}}$ (mm day^{-1}) corresponded to the sapwood area scaled E_c. Both A_s and $A_{\mathrm{s-avg}}$, were estimated using the dendrometer data at 1.20 m (D) and converting these to the diameter at 0.22 m (d) using allometric equations (AE) of Table 1. SA was estimated for each genotype based on tree density (1.24 m^2) and mortality of trees (12, 27, 17 and 19% for 'Oudenberg', 'Grimminge', 'Bakan' and 'Koster', respectively) in each mono-genotypic block at the site (Table 1).

Changes in stem water status were also characterized by an index derived from micrometric stem diameter fluctuations (TDF) (Goldhamer and Fereres, 2001), i.e., the maximum daily shrinkage (MDS = MXTD – MNTD), were MXTD and MNTD are the maximum and minimum daily trunk diameter, respectively.

Statistical analysis

For the water relation parameters (Ψ_x RWC_l and MDS), a one-way ANOVA tested for differences between the genotypes for each measurement day (10 days). Differences of E_{leaf} among genotypes were tested separately for each measurement date (5 days) hour by hour in a one-way ANOVA. When the differences were significant among genotypes ($P \leq 0.05$), Tukey HSD's test was used to do pairwise comparisons. Data of E_{stem} were tested with one-way repeated measures ANOVA on Ranks test at a significance level of $P<0.001$. A subsequent Tukey HSD's pairwise comparison ($P \leq 0.05$) was used to isolate different groups.

These statistical analyses were performed using the statistical software Statgraphics Plus 5.1 (StatPoint Technologies Inc., Warrenton, Virginia, USA) and SigmaPlot 13.0 (Systat Inc., San Jose, California, USA).

RESULTS AND DISCUSSION

The diurnal courses of E_{leaf} and E_{stem} of the four genotypes reflected typical Belgium weather conditions (of temperature, PPFD and VPD; Figure 1). Dynamics of E_{leaf} and E_{stem} were driven by PPFD, although this was more evident for $E_{stem,}$ which was measured every 30 min than for E_{leaf}, which was measured every 2-3 h (Figure 1). The maximum values of transpiration were reached during summer time (Figure 1c, e) coinciding with the maximum values of VPD. This was probably due to a lower leaf development (Tricker et al., 2009). Highest transpiration (leaf and stem) was observed during summer months (max. VPD 1.3-1.0 kPa), and lowest values were measured in June following the low values of PPFD and VPD. Genotype 'Grimminge' had the highest values of E_{leaf} and E_{stem}, while 'Bakan' and 'Koster' had the lowest values (Figure 1f-o). At the beginning of May the only genotype with transpiration was 'Bakan', since this was the first to develop new leaves at the onset of spring (Figure 1f, k). The stomatal behaviour of 'Koster' and 'Bakan' showed a reduced transpiration after midday, regardless of PPFD and VPD trends.

All genotypes had adequate water supply and little water stress during the entire growing season, as shown by the high values of Ψ_x (-0.2 to -0.9 MPa, Figure 2b) and RWC_l (>85%, Figure 2c), and low MDS (<80 µm, Figure 2d). This could be explained by the low evapotranspirative demand (max. VPD of 1.8 kPa) and the high rainfall during the growing season 2016 (Figure 2a). Genotype 'Koster' had higher values of Ψ_x and lower MDS while the opposite happened for 'Grimminge', especially during the summer months (July and August, Figure 2b, d). These differences among genotypes were less evident for the RWC_l values (Figure 2c). The pattern of stem diameter variation in response to the replenishment of water storage has been previously observed for poplars under controlled (Giovannelli et al., 2007) and natural conditions (Bloemen et al., 2017). At our site the highest MDS was observed for the genotype with the highest transpiration ('Grimminge'), showing that this genotype used an important fraction of its water storage to satisfy its transpiration demand.

The $E_{c\text{-}sapwood}$ values of the four genotypes followed the trends of VPD and PPFD during the growing season, especially when the values of these environmental parameters decreased. In 'Bakan' and 'Oudenberg' the onset of transpiration started April 9 (Figure 3b, d), but in 'Bakan' values higher than 1 mm day^{-1} were already recorded from April 20 onward, while in 'Oudenberg' this happened 15 days later. The transpiration in 'Grimminge' and 'Koster' started one month later than in the other two genotypes (May 5). In terms of daily transpiration values, 'Bakan' and 'Koster' showed the lower values (avg. = 1.7 mm day^{-1}, max. = 5.4 mm day^{-1} and total = 340 mm) while 'Grimminge' recorded the highest (avg. = 3.1 mm day^{-1}, max. = 8.2 mm day^{-1} and total = 620 mm), i.e., being almost double that those obtained in 'Bakan' and 'Koster' (Figures 3b-e). The average daily $E_{c\text{-}sapwood}$ values for our site were within the range of 1-8 mm day^{-1} reported for poplar stands of different genotypes, stand age and geographic locations in temperate climate zones (Bloemen et al., 2017).

The SHB technique (Baker and van Bavel, 1987) has been successfully used in previous studies on SRC trees (Hall et al., 1998; Allen et al., 1999; Tricker et al., 2009; Bloemen et al., 2017). At our site this technique yielded data of high time resolution with rather few gaps. The difference with the previously mentioned studies was the recording period (max. seven weeks in the previous studies versus six months in the present contribution), which involved a number of practical problems. As SRC poplars are fast-growing, the SHB sap flow sensors of several stems had to be changed throughout the growing season from a smaller to a larger sensor. Furthermore, the occurrence of adventitious roots and mold on the stem portion where the sensor was placed required removing sensors often for cleaning and reinstallation afterwards.

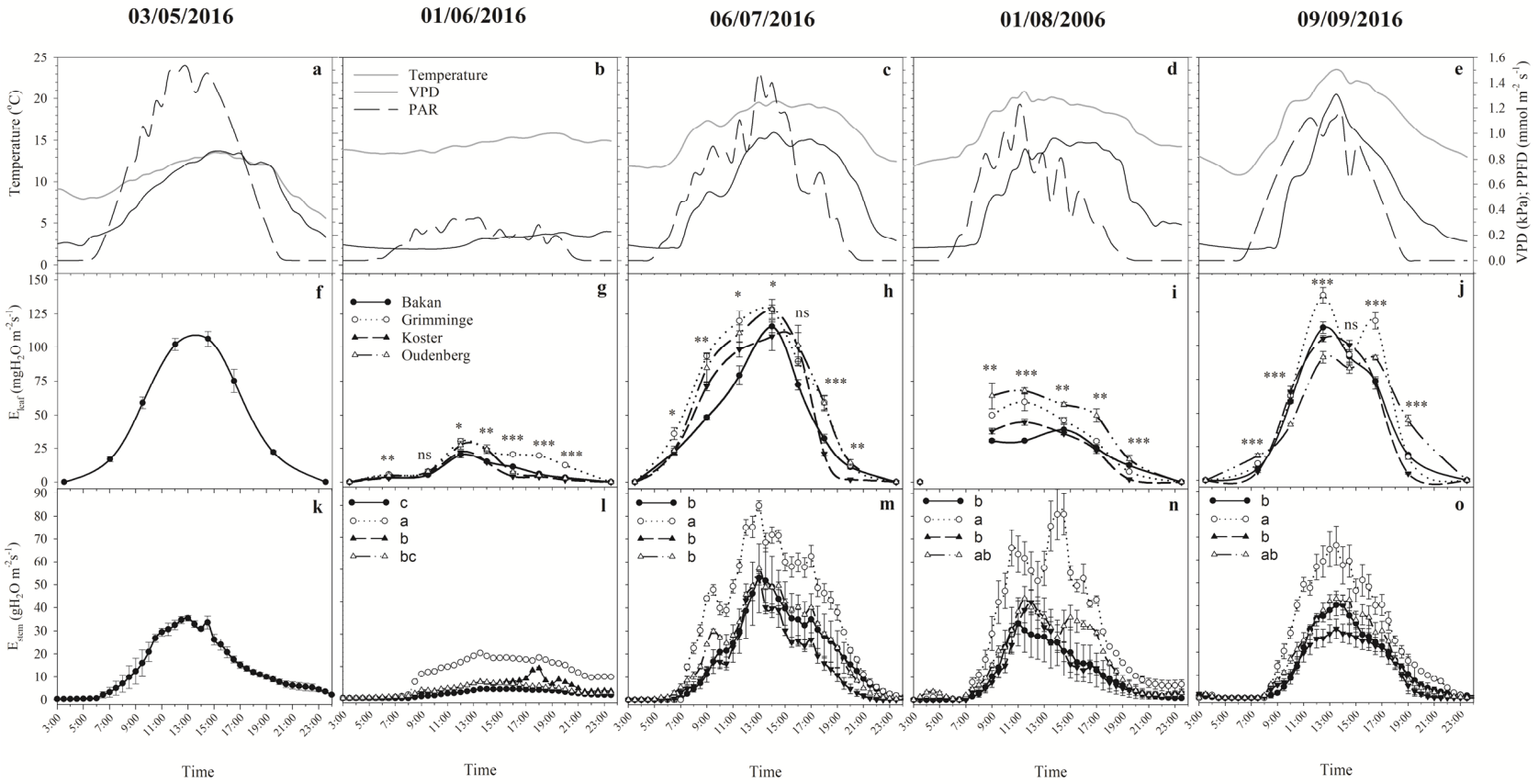

Figure 1. Diurnal courses of temperature (grey solid line), PPFD (black dash line) and VPD (black solid line) on five days in 2016 (a, b, c, d, e), and diurnal courses of leaf transpiration (E_{leaf} – f, g, h, i, j) and stem transpiration (E_{stem} – k, l, m, n, o) for poplar genotypes 'Bakan' (black circle solid line), 'Grimminge' (white circle dotted line), 'Koster' (black triangle dash line) and 'Oudenberg' (white triangle dash dotted line) registered on same days of 2016. *, **, *** denote statistical significance at the 0.05, 0.01 and 0.001 levels respectively; ns: no significant differences. Different letters denote significant differences among genotypes according to Tukey HSD's test ($P \leq 0.05$). Vertical bars represent standard errors.

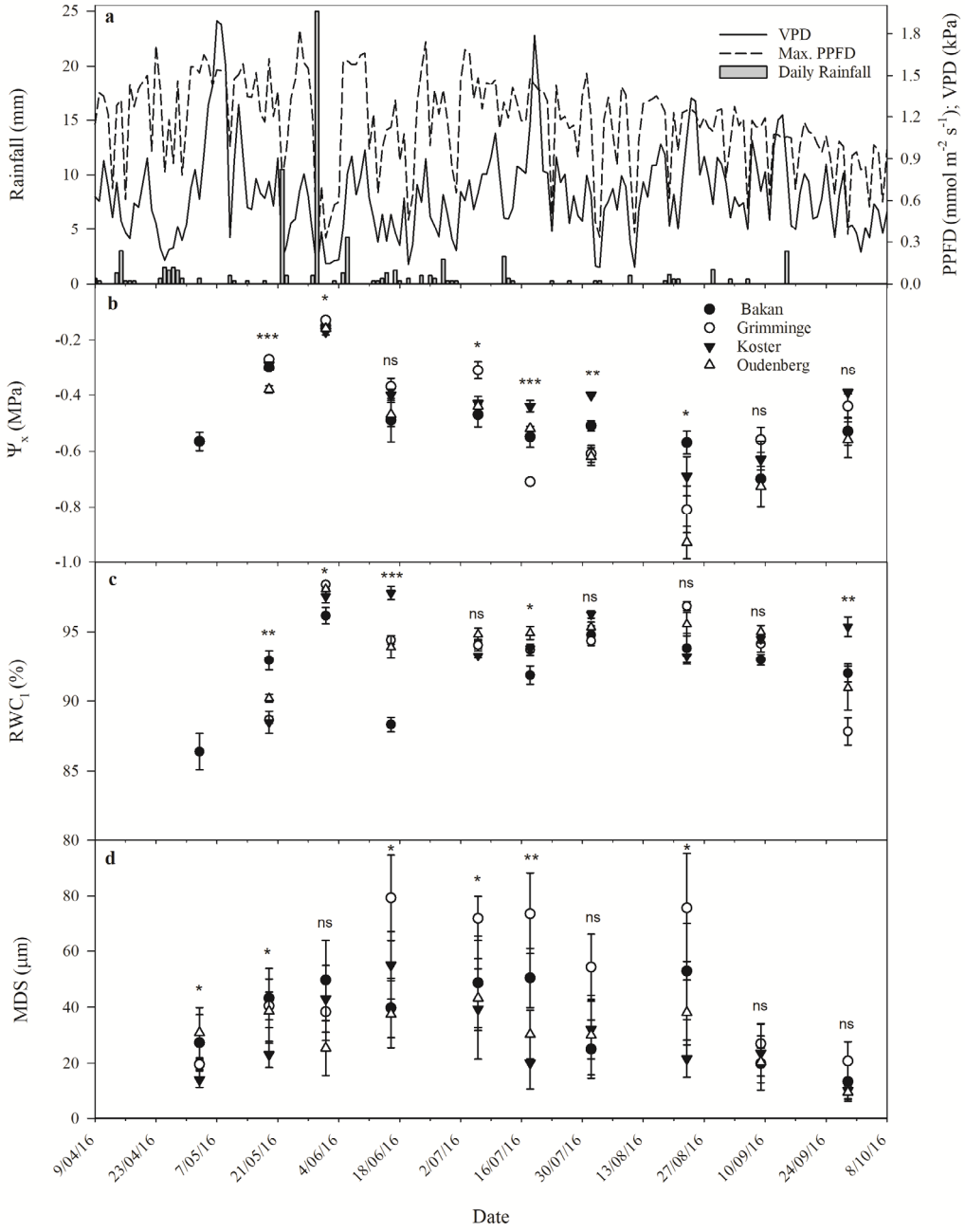

Figure 2. Daily summed rainfall (grey colum) and time course of PPFD (black dash line) and VPD (black solid line) during the 2016 growing season (a). Midday stem water potential (Ψ_x, b), leaf relative water content (RWC_l, c) and maximum daily shrinkage (MDS, d) in poplar genotypes 'Bakan' (black circle), 'Grimminge' (white circle), 'Koster' (black triangle) and 'Oudenberg' (white triangle). *, **, *** denote statistical significance at the 0.05, 0.01 and 0.001 levels respectively; ns: no significant differences. Vertical bars represent standard errors.

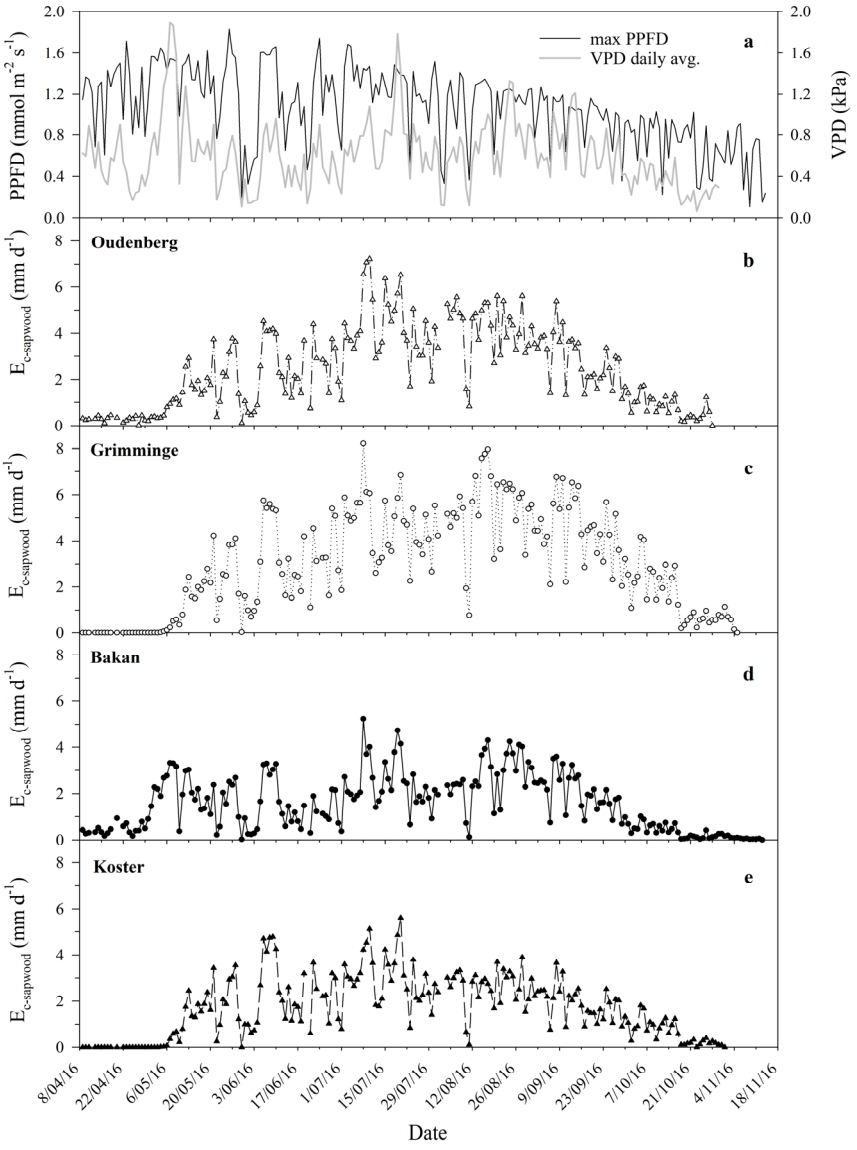

Figure 3. Daily average of VPD and maximum daily PPFD (a) and, daily transpiration per unit of ground area ($E_{c\text{-}sapwood}$) in poplar genotypes 'Oudenberg' (b), 'Grimminge' (c), 'Bakan' (d), and 'Koster' (e) during the growing season of 2016.

CONCLUSIONS

Both at leaf and tree level we observed important genotypic differences showing different water use strategies of the four genotypes. 'Bakan' and especially 'Koster' showed the lowest transpiration as compared to 'Oudenberg' and 'Grimminge'. This indicated that the two former genotypes might better tolerate environmental changes linked to projected climate changes and water shortage periods compared to the two latter types. Due to the fast growing characteristics inherent to the SRC cultivation, we cannot recommend the SHB technique as an automatic and independent system over a long period.

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