Identifying the best plant water status indicator for bio-energy poplar genotypes

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Funding information
European Commission, Grant/Award Number: 657123, FP7/2007-2013 and 233366; Flemish Science Foundation

Abstract
This contribution provides better insights in the water relations and the physiological traits of four commercial poplar genotypes of different genetic background, 'Bakan', 'Oudenberg', 'Koster' and 'Grimminge'. The main continuous (nondestructive and providing continuous and automated data records) and discontinuous (destructive and not allowing automation) plant water status (PWS) indicators were monitored at a multigenotype, commercial-scale short-rotation coppice plantation in East-Flanders (Belgium), and their relationships with the principal environmental variables were assessed. All measurements were performed during the entire 2016 growing season on the third year of the third rotation in multistemmed trees. The discontinuous PWS indicators were measured on 10 separate days with a different evaporative demand and soil water content, while the continuous PWS indicators were recorded from April to November. The genotypes responded differently to environmental drivers and to soil conditions, based on the PWS indicators, featuring a different water behaviour in relation to the level of isohydricity. Poplar genotypes 'Koster' and 'Bakan' showed the typical water-conserving behaviour of isohydric species, while 'Grimminge' was more in line with the anisohydric ones. A principal component analysis showed that sap flow ($F_s$) was the most suitable PWS indicator. The $F_s$ and therefore the sap flow-based canopy transpiration ($E_c$) were tightly linked to the phenological stage of the trees as well as to vapour pressure deficit and photosynthetic photon flux density, based on relationships between $E_c$ and environmental variables. A quantitative predictive model was developed to estimate the crop water requirements for specific genotypes, by calculating transpiration per unit of ground area with a few environmental variables, monitored with easy-to-handle sensors.

KEYWORDS
environmental variables, quantitative model, short-rotation coppice, stomatal conductance, transpiration, water potential

1 | INTRODUCTION

Changes in the temporal distribution of water resources are of major importance for cultivated woody crops such as poplar (Populus spp.), one of the fastest growing cultivated tree genera. Poplars are especially sensitive to drought (Liang, Yang, Shao, & Han, 2006; Lindroth, Verwijst, & Halldin, 1992; Monclus et al., 2009; Navarro, Facciotto, Campi, & Mastrorilli, 2014) and highly depend on water availability to reach high yields.
(Allen, Hall, & Rosier, 1999; Meioresonne, Nadezhdin, Cermák, Van Slycken, & Ceulemans, 1999; Zsuffa, Giordano, Pryor, & Stettler, 1996). Thus, the productivity of poplar short-rotation coppice (SRC) cultivation largely depends on water availability and other soil characteristics, as well as climatic conditions (Crow & Houston, 2004; Dillen et al., 2007; Hillier et al., 2009; Hofmann-Schielle, Jug, Makeschin, & Rehfuess, 1999). Consequently, the study of plant water status (PWS) indicators and their response to soil and climate is highly relevant for the cultivation of SRC under seasonal droughts or floods. The PWS indicators are physiological indicators able to measure the plant response to the combined effects of soil moisture availability, evaporative demand, internal hydraulic resistance and resistance/uptake capacity of the plant/root interface, that determine either the tissue water relations (i.e. water potential, water content) or the plant response to a change in tissue water relations (i.e. stomatal closure). Such study also allows ranking commercially available poplar genotypes according to the regulation of their water balance under future climate change conditions. The PWS indicators were primarily used to schedule irrigation regimes and to predict crop response to water deficits in regions with low precipitation (e.g. Conesa et al., 2016; Intrigliolo et al., 2011; Ortuño et al., 2006). Most studies of PWS have been conducted on fruit crops using discontinuous and continuous data sets of different PWS indicators, without reaching a consensus about the most suitable indicator (Fernández, 2014; Katerji et al., 1988). Discontinuous PWS indicators are the most sensitive to water stress (McCutchan & Shackel, 1992), but these discontinuous measurements are destructive. They do not allow automation and must be made in situ (Choné, van Leeuwen, Dubourdieu, & Gaudillère, 2001; Naor, Gal, & Peres, 2006; Williams & Araujo, 2002). On the other hand, continuous PWS indicators are not destructive; they provide continuous and automated data records, but require a sophisticated implementation, a high degree of technical support and special calibration protocols for each species and edapho-climatic condition (Fernández, 2017; Jones, 2004). Among the discontinuous PWS indicators and because of their high sensitivity to water shortage, stem water potential ($\Psi_s$; McCutchan & Shackel, 1992; Naor, 2000; Navarro et al., 2014; Shackel et al., 1997) and stomatal conductance ($g_s$; Garnier & Berger, 1987; Harrison, Daniell, & Cheshire, 1989; Tan & Layne, 1991) have been widely used. The most frequently used continuous PWS indicators are: leaf temperature (e.g. Cohen et al., 1997; Leinonen & Jones, 2004; Massai, Remorini, & Casula, 2000), sap flow rate ($F_s$; e.g. Cohen, Goldhamer, Fereres, Girona, & Mata, 2001; Fernández et al., 2008; Green, Clothier, & Jardine, 2003), and a few parameters derived from micrometric stem diameter variations (SDV; e.g. Goldhamer & Fereres, 2001; Ortuño et al., 2010; Zweifel, Item, & Häslar, 2000).

A more detailed study of the relationships among specific PWS indicators in trees under conditions of non-limiting soil water availability and different environmental variables will reveal: (a) the potential of these indicators to predict the tree water status under different conditions of soil water content (SWC) and of atmospheric evaporative demand, as well as (b) which PWS indicator is best related to each environmental variable. Some variables are based on average daily measurements such as reference crop evapotranspiration ($ET_0$), SWC, air temperature ($T_{air}$), vapour pressure deficit (VPD) and solar radiation ($R_s$), while others are only available at certain times of the day such as midday temperature ($T_{mid}$) and maximum VPD (VPD$_\text{max}$). Older studies showed that discontinuous PWS indicators such as leaf relative water content (RWC$_l$) and leaf water potential ($\Psi_l$) were correlated with changes in environmental variables (SWC, $T_{air}$, VPD, $R_s$; Millar, Jensen, Bauer, & Norum, 1971; Namken, 1964; Werner, 1954). Moreover, recent studies have reported reliable relationships among continuous PWS indicators such as $F_s$ (e.g. Chen, Wang, Liu, & Wei, 2014; Rousseaux, Fig uerola, Correa-Tedesco, & Searles, 2009; Shen, Gao, Fu, & Lü, 2015) and the parameters derived from daily trunk diameter fluctuations (TDF; e.g. Conesa et al., 2016; De la Rosa, Conesa, Domingo, Torres, & Pérez-Pastor, 2013; Ortuño, Brito, García-Orellana, Conjeeiro, & Torrecillas, 2009) with different meteorological variables, especially VPD, $R_s$ and $T_{air}$. Most studies have, however, been performed on fruit trees and only very few studies report these relationships for other trees. Among those, only some studies link daily stem radius variation (SRV) with environmental variables for conifers (e.g. Gruber, Zimmermann, Wieser, & Oberhuber, 2009; Oberhuber, Hammerle, & Kofler, 2015; Tian, Zhang, Liu, & Meng, 2018) and for temperate broad-leaved species (Köcher, Horna, & Leuschner, 2012). So far, there were no studies on SRC cultivations. In view of the extensive cultivation of SRC poplars it is relevant to examine the above-mentioned relationships, and even more relevant to study genotypic differences among the commercially available poplar genotypes that are commonly used for SRC. Finally, the development of a simple predictive model that integrates the most significant environmental variables as predictive variables to determine the value of the best and most pragmatic PWS indicator would be an asset as it reveals the best environmental proxy to assess the PWS for each poplar genotype.

The objectives of this study were to: (a) identify which PWS indicator best described the water status of four poplar genotypes under SRC, and (b) develop a simple model to predict the best poplar PWS indicator and assess the crop water requirements, using common environmental variables that can be monitored with easy-to-handle sensors.

All abbreviations and acronyms have been listed and defined in Table 1.
**Table 1** List of abbreviations and acronyms with their definition and units

| Acronym or symbol | Description | Units |
|-------------------|-------------|-------|
| $A_{s-avg}$       | Average sapwood area at 0.22 m | m² |
| $A_{ij}$          | Sapwood area at 0.22 m above the ground for the $i$th stem number of the $j$th operative stem diameter range | m² |
| AIC               | Akaike information criterion |       |
| $d$               | Stem diameter measured at 0.22 m | mm |
| DBH               | Stem diameter at breast height, measured at 1.30 m | mm |
| DW                | Dry weight | g |
| $E_c$             | Daily transpiration rate per unit of ground area | mm H₂O/day |
| ET₀              | Reference crop evapotranspiration | mm H₂O |
| $F_{ij}$          | Sap flow rate per unit of sapwood area for the $i$th stem number of the $j$th operative stem diameter range | g m⁻² hr⁻¹ |
| $F_s$             | Sap flow rate | g/hr or kg/day |
| FW                | Fresh weight | g |
| $g_s$             | Stomatal conductance | mmol m⁻² s⁻¹ |
| PCA               | Principal component analysis |       |
| PPFD              | Photosynthetic photon flux density |       |
| PPFDₘₚₓ            | Daily maximum | µmol m⁻² s⁻¹ |
| PPFDₘ₅              | Mean daytime |       |
| PPFDₘ₆              | Midday |       |
| PWS               | Plant water status |       |
| $R_g$             | Global radiation | W/m² |
| RHₘ₅              | Relative humidity |       |
| RHₘ₅ₓ            | Daily maximum | % |
| RHₘ₅₆              | Mean daytime |       |
| RHₘ₆              | Midday |       |
| RMI               | Royal Meteorological Institute of Belgium |       |
| $R_s$             | Solar radiation | W/m² |
| RWCₓ              | Leaf relative water content | % |
| SA                | Ground surface area per tree | m² |
| SDV               | Stem diameter variations | mm |
| SHB               | Stem heat balance |       |
| SRC               | Short-rotation coppice |       |
| SRV               | Stem radius variation | mm |
| SSRV              | Standardized stem radial variations | mm |
| SWC               | Soil water content | m³/m³ |
| $T_{air}$         | Air temperature |       |
| $T_{max}$         | Daily maximum | °C |
| $T_{m₅₆}$         | Mean daytime |       |
| $T_{m₆}$          | Midday |       |
| TDF               | Trunk diameter fluctuations | mm |
| TDR               | Time domain reflectometer |       |
| $T_{soil}$        | Soil temperature | °C |
| TW                | Turgid weight | g |

(Continues)
MATERIALS AND METHODS

2.1 Study site and plant material

All measurements were carried out in an existing commercial-scale SRC plantation in Lochristi, province East-Flanders, Belgium (51°06′44ʺN, 3°51′02ʺE, 6.25 m a.s.l.; http://uahost.uantwerpen.be/popfull). The long-term average (1981–2010) annual temperature at the site is 10.6°C and the average annual precipitation is 860 mm, evenly distributed over the year (Journée, 2014; Journée, Delvaux, & Bertrand, 2015). The soil has a loamy sand texture (11% clay, 2% silt and 87% sand for the 0–30 cm soil depth, and 12% clay, 4% silt and 84% sand for the 30–60 cm depth) with deeper clay-enriched sand layers (≈75 cm; Berhongaray, Cotrufo, Janssens, & Ceulemans, 2019). The soil is classified as an Anthrosol according to the World Reference Base for Soil Resources (Dondeyne et al., 2015; Verlinden, Broeckx, Van Den Bulcke, Van Acker, & Ceulemans, 2013). Between 7 and 10 April 2010, large replicated monogenotypic blocks were established with hardwood cuttings of 12 selected and commercially available poplar (Populus) genotypes (see table 2 in Broeckx, Verlinden, & Ceulemans, 2012). The planting density was 8,000 plants/ha and the total planted area was 9 ha (Figure 1). The planting design consisted of double rows with alternating distances of 0.75 and 1.50 m between the rows and 1.1 m within the row. The site was neither fertilized, nor irrigated. The plantation was managed as an SRC regime and coppiced in February 2012 and in February 2014. This study took place during the third year of the third rotation that is during the 2016 growing season (from April to November). More details on the site, the management and soil characteristics were previously provided (Broeckx et al., 2012; Verlinden et al., 2013).

An extendable meteorological mast was positioned in the northeastern part of the plantation (Figure 1) at the beginning of June 2010, providing continuous microclimate and environmental data (Zona et al., 2013). The PWS measurements were confined to a subset of four genotypes (three individual trees per genotype), all close to the mast (<50 m; Figure 1). The selected genotypes were characterized by different parentages: 'Bakan' (Populus trichocarpa T. & G. × P. maximowiczii A. Henry), 'Oudenberg' (P. deltoides Bartr. ex Marsh. × P. nigra L.), 'Koster' (P. deltoides Bartr. ex Marsh. × P. nigra L.) and 'Grimminge' (P. deltoides Bartr. ex Marsh. × (P. trichocarpa T. & G. × P. deltoides Bartr. ex Marsh.). The four genotypes were selected because of their different genetic background, their contrasting growth pattern (e.g. number of shoots per stool, shoot diameter) and because they are hybrids of the most commonly cultivated poplar species (i.e. P. trichocarpa; Euramerican = P. deltoides × P. nigra; and P. maximowiczii). More details on the origin, family and gender of the genotypes were previously provided by Broeckx et al. (2012). All measurements were made on multistemmed trees as the plantation had already been coppiced twice. The average height of the canopy was 6.2 m in the middle of the 2016 growing season.

2.2 Environmental variables

Environmental variables were continuously recorded at the site on the extendable mast: $T_{\text{air}}$ and relative humidity (RH$_{\text{air}}$), were recorded at 7.7 m above the ground using Vaisala probes (HMP 45C, Vaisala); these data were used to calculate the VPD. The incoming photosynthetic photon flux density (PPFD, 400–700 nm) was measured at the same height using a quantum sensor (LI-190, Li-COR). The global radiation ($R_g$) was measured with a pyranometer (CM3 component of CNR4 radiometer, Kipp & Zonen). Precipitation was recorded using a tipping bucket rain gauge (3665 R, Spectrum Technologies Inc.). The SWC was measured at five different spatial locations, each one with a vertical profile of five different depths (5, 10, 20, 50 and 100 cm from the soil surface), using time domain reflectometer (TDR) probes (CS616, Campbell Scientific) inserted horizontally. The values of SWC at depths of 5, 10 and 20 cm were averaged and represented as SWC at 0–20 cm depth. The soil water table depth (WTD) was measured in the proximity of each SWC profile with a pressure transducer (CS451, Campbell Scientific Inc.) installed in PVC pipes vertically inserted into the ground to a depth.
of approximately 2 m. All measurements were logged continuously through the combination of a data logger (CR1000, Campbell Scientific Inc.) and multiplexers (AM 16/32B, Campbell Scientific Inc.). For each variable, the values were sampled once every minute and averaged every 30 min. If an instrument occasionally failed, the missing environmental variable (\(T_{air}, RH, R_g, PPFD\) or precipitation) was gap-filled using data from nearby meteorological stations operated by the Royal Meteorological Institute of Belgium (RMI) at 8 and 10 km from the research site, respectively in Zelzate (51°10′53″N, 3°48′19″E) and Melle (50°58′51″N, 3°49′03″E).

2.3 | PWS indicators

The discontinuous indicators RWC\(_t\), \(\Psi_l\), \(\Psi_x\) and \(g_s\) were measured during measurement campaigns every 15–20 days between early May and end September 2016. This resulted in a total of 10 separate measurement days, on three trees per genotype.

The midday RWC\(_t\), \(\Psi_l\) and \(\Psi_x\) were measured between 10:30 and 12:30 hr (solar time) using fully developed leaves of a similar age and position in the canopy (eight-leaves down from the apex). For the determination of \(\Psi_x\), the leaves were covered with both a plastic bag and an aluminium foil for at least 2 hr before the measurement. Bagging prevented leaf transpiration, allowing the leaf water potential to equal the stem water potential (Begg & Turner, 1970). The leaves sampled for \(\Psi_l\) and \(\Psi_x\) were cut and immediately placed in a pressure chamber (ARIMAD-2, A.R.I. Kfar Charuv) in the field following Scholander, Hammel, Bradstreet, and Hemmingsen (1965). The pressure in the pressure chamber was raised using nitrogen gas at a rate of 0.03 MPa/s. The RWC\(_t\) was measured for three leaves per tree providing an average RWC\(_t\), according to the equation (Barrs & Weatherley, 1962):

\[
RWC_t = \frac{FW - DW}{TW - DW} \times 100, \tag{1}
\]

where FW, DW and TW are the fresh, dry and turgid weights, respectively, of the whole leaf. These weights were measured with a precision scale (model KB1200-2N, Kern & Sohn
GmbH; accuracy of 0.01 g). Leaves were weighed immediately after collection to determine the FW. The cut end of each leaf was placed in distilled water and kept in dim light at 4°C for 24–48 hr until the TW was reached and recorded. The DW was measured after air-drying the leaves at 70°C for 48 hr.

Midday $g_s$ was measured with a steady-state diffusion porometer (Delta-T AP4, Delta-T Devices Ltd). Three mature leaves of a similar age were randomly chosen from the upper canopy level (Heilman, Hinckley, Roberts, & Ceulemans, 1996; Hinckley et al., 1994). Access to the canopy was guaranteed by ladders with a working height of 5.2 m.

The $F_j$ and SRV of individual stems were continuously monitored on two stems per tree and three trees per genotype throughout the entire growing season (9 April–12 November 2016). Individual stems on the trees were selected to be representative of the entire range of stem diameters measured at 0.22 m (d) and at 1.30 m (diameter at breast height [DBH]) of height during an extensive inventory performed in February 2016. The $F_j$ was measured using the stem heat balance (SHB) technique (Baker & van Bavel, 1987; Sakuratani, 1981), as already successfully applied in previous studies on SRC (Allen et al., 1999; Bloemen et al., 2017; Hall, Allen, Rosier, & Hopkins, 1998; Tricker et al., 2009).

A total of 24 SHB sap flow sensors were used (SGEX16, SGEX19, SGEX25 and SGB35, Dynamax Inc.) to operate in a wide range of stem diameters. The sensors were installed at a height of 1.50 m above the ground, always below the first branch of each stem and installed following the manufacturer’s instructions (Dynagage sap flow sensors manual, Dynamax Inc.). Additionally, the sensors were thermally insulated from the environment with the weather shields supplied by the manufacturer and several layers of insulating aluminium foil wrapped around the sensor. Finally, conical funnels of appropriate diameters were placed upside down around the stems above each sensor, thus preventing water from entering the sensors during rain events (for installation of the sap flow sensors, see https://youtu.be/s7Zz5aNApLI and https://www.uantwerpen.be/en/rg/pleco/research/research-projects/marie-curie/physio-pop/).

The $F_j$ was calculated from the raw data according to the standard procedure for SHB sensors as previously described (Baker & van Bavel, 1987; Sakuratani, 1981, 1984; Steinberg, van Bavel, & McFarland, 1990). The low temperature cut-off filter and the high flow filter were applied as described in the manufacturer’s manual (Dynagage sap flow sensors manual, Dynamax Inc.).

To obtain the canopy transpiration and, therefore, the daily transpiration rate per unit of ground area ($E_c$) of each genotype, the transpiration of the entire tree was quantified. This whole-tree transpiration was estimated by summing the $F_j$ contributions from all stems as previously described in detail (Navarro, Portillo-Estrada, Arriga, Vanbeveren, & Ceulemans, 2018). As poplars are fast-growing trees the increase in stem surface area during the growing season was taken into account in the energy balance equations by including the increase in stem diameter recorded by automatic point dendrometers (ZN11-O-WP, Natkon). The dendrometers were installed with a ring-shaped carbon fibre frame at a height of 1.30 m, just below the sap flow sensors. A data logger (CR1000, Campbell Scientific) collected the data from the sap flow sensors and the dendrometers at 30 s intervals, averaged every 30 min. The sap flow rate per unit of sapwood area ($F_{sj}$) for the $i$th stem number of the $j$th operational stem diameter range (stem diameter range of every sap flow sensor used, from 1 to 5, Table S1) was obtained by dividing $F_{sj}$ by the sapwood area at 0.22 m above the ground ($A_{sj}$). The sum of all $F_{sj}$ was multiplied by the ratio of the average sapwood area at 0.22 m ($A_{s-avg}$) for all stems equipped with dendrometers to the ground surface area per tree (SA). Finally the result was transformed to kg m$^{-2}$ hr$^{-1}$ and therefore the hourly values of each day were summed to obtain $E_c$ in mm of water column per day, as follows:

$$E_c = \sum_{h=1}^{24} \left( \sum_{j=1}^{4} \sum_{i=1}^{n} \left( \frac{F_{sj}}{A_{sj}} \right) \times \frac{A_{s-avg}}{SA} \times 10^{-3} \text{kg/g} \right), \quad (2)$$

where $j$ is the operational stem diameter range, $i$ is the stem number and $n$ is the number of stems per operational stem diameter range. Both $A_s$ and $A_{s-avg}$ were estimated using the stem DBH from the automatic point dendrometers (ZN11-O-WP, Natkon) and converting them to stem diameter via allometric equations ($d = \text{DBH}/a$; see Table S1 for further details). The SA was estimated for each genotype based on the tree density and on the mortality of trees in each monogenotypic block of the site (Table S1).

Stem diameter fluctuations were monitored throughout the growing season using the automatic point dendrometers. Mean daily SRV was calculated by averaging all daily stem diameter measurements (48 values per day), and by dividing this value by two, thus providing linear measurements of the radius. The SRV was then determined by calculating the difference between mean values of two consecutive days (‘daily mean approach’, Deslauriers, Anfodillo, Rossi, & Carraro, 2007), which represented a combination of water- and growth-induced radius expansion (e.g. Daudet, Ameglio, Cochard, Archilla, & Lacoindre, 2005; Herzog, Hässler, & Thum, 1995; Steppe, De Pauw, Lemeur, & Vanrolleghem, 2006). To separate daily patterns of water movement from irreversible expansion growth, the Gompertz function was applied. The function describes the long-term development of the radial increment over the entire growing season (e.g. Rossi, Deslauriers, & Morin, 2003). Standardized stem radial variations (SSRV) were calculated by subtracting the
long-term Gompertz-modelled daily growth trend from the SRV data. The SSRV represents reversible shrinkage and swelling of tissues outside the cambium, which contributes the most to the stem water storage capacity (De Swaef, De Schepper, Vandegheuvel, & Steppe, 2015; Oberhuber, 2017). Positive SSRV values indicate water replenishment of the expandable tissues outside the cambium when tree water status is high (stem swelling); negative SSRV values indicate that water stored in expandable tissues outside the cambium is translocated to the xylem (stem shrinkage).

### 2.4 Reference equations

The relationships between the PWS indicators with a number of selected environmental variables, from early June to end of September (RWC, Ψ, gs, and SSRV) and from middle May to middle November (Fs and Ec), were assessed by first-, second-, third-order equations and exponential functions (see Tables S2–S4). The environmental variables used were daily maximum (max), mean daytime (with daytime defined as periods when PPFD > 5 µmol m⁻² s⁻¹; md) and midday (between 11:00 and 14:00 hr solar time; md) Ta, RHa, PPFD, and daily mean SWC and Tsoil.

### 2.5 Model to predict canopy evapotranspiration (Ec) from environmental variables

Principal component analysis (PCA) was performed to identify the relationships among PWS indicators for the poplar genotypes. The PWS indicators that showed a higher relationship with the PCA axes (components) were selected due to their correlation with the variance represented by the axes. When few indicators were correlated to each other, only the ecologically more meaningful were chosen for the model and the rest discarded for the next step, to avoid variance inflation. PWS indicators with continuous data sets were prioritized to give more power to the model.

Several environmental parameters related to PWS were used to generate a linear model to predict canopy evapotranspiration (Ec) using ‘genotype’ as a fixed factor (see Table S5). The large set of environmental parameters used in the reference equations was evaluated to narrow down the amount of variables for further modelling exercise. The criteria used were (a) their physiological and ecological meaning, and (b) their higher correlation levels with Fs, Ec and SSRV as compared with other options to compute daily values (i.e. daily maximum mean, daytime, midday; Table S4).

The models predicted the relationships of each variable with the different poplar genotypes, which presumably had a different water behaviour. The model generation was based on the lowest AIC (Akaike information criterion) value, via step-by-step removal of the environmental variables with the highest p-value starting from the beyond-optimal model, until the lowest AIC was reached (Zuur, Ieno, Walker, Saveliev, & Smith, 2009), taking into account a difference in AIC of at least two to allow variable removal. The model generation was done in IBM SPSS Statistics v21 (IBM Corp.).

### 2.6 Statistical analysis

The differences of RWC, Ψ, gs and SSRV among genotypes were analysed for each measurement data through a repeated measures ANOVA test (at p ≤ .05 level) followed by a Tukey HSD pairwise comparison test using Statgraphics Plus 5.1 (StatPoint Technologies Inc.) and SigmaPlot 14.0 (Systat Inc.). The relationships between PWS indicators and environmental variables were explored by linear and non-linear regressions using SigmaPlot 14.0. PCA was performed to identify the relationship among PWS indicators for the poplar genotypes studied using SigmaPlot 14.0. In the beginning, all PWS indicators (continuous and discontinuous measured throughout the growing season) were used. Thereafter, a second PCA, only using the continuous PWS indicators, was generated to describe the best PWS indicator and the relationships among them.

### 3 RESULTS

#### 3.1 Dynamics of PWS indicators

The Fs values of the four genotypes followed the trends of VPD and PPFD during the growing season, especially when both environmental parameters decreased. In ‘Bakan’ and ‘Oudenberg’ the onset of transpiration started mid-April 2016 (Figure 2c), but in ‘Bakan’ values > 1 kg/day were already recorded from 1 May onwards. In ‘Oudenberg’ and ‘Grimminge’ this happened 2 weeks later. The transpiration (Fs) in ‘Grimminge’ and ‘Koster’ started 20 days later than in the other two genotypes (5 May). The maximum daily Fs values were reached on 6 June for ‘Koster’ (1.1 kg/day) and on 10 July for ‘Oudenberg’ (2.7 kg/day) coinciding with high values of daily PPFDmax. ‘Grimminge’ and ‘Bakan’ reached maxima (2.8 kg/day) on August (17 and 24, respectively), and values > 2.0 kg/day were reached for ‘Grimminge’ during most of the days between 15 August and 15 September. These values concurred with high values of temperature and VPD, with low precipitation and with a decrease in SWC (Figure 2a–c). Negative values of SSRV (stem shrinkage), although always > −0.03 mm, were achieved most of the days by ‘Grimminge’ (Figure 2d),
while 'Koster' and especially 'Bakan' showed positive values or stem swelling (<0.04 mm).

The low atmospheric evapotranspirative demand (max. VPD$_{max}$ of 1.9 kPa) and the high rainfall (total precipitation of 946 mm) during the 2016 growing season (Figure 2a,b) explained that all genotypes had adequate water supply and no water stress situations during the entire growing season, as shown by the high values of RWC$_l$ (>85%; Figure 2e),
g_s (>300 mmol m\(^{-2}\) s\(^{-1}\) from June onwards; Figure 2e), \(\Psi_x\) (−0.2 to −0.9 MPa; Figure 2f) and \(\Psi_l\) (>−1.5 MPa; Figure 2f). The RWCl did not largely vary throughout the season or among the poplar genotypes (88%−98%; Figure 2e). The highest g_s values were reached on 18 July and 23 August (1,070 and 1,575 mmol m\(^{-2}\) s\(^{-1}\) on average, respectively) concurrently with the highest temperature and VPD. The lowest values, regardless of the transpiration onset (in May), were on 1 June and 1 August (435 and 346 mmol m\(^{-2}\) s\(^{-1}\) on average, respectively) concurrently with the lowest PPFD and VPD (Figure 2a,e). The \(\Psi_x\) but especially the \(\Psi_l\) decreased in all genotypes during summer (from middle July to middle September) with the decrease of SWC and the increase of temperatures (Figure 2a,b,f). Although water shortage situations were not recorded, the four poplar genotypes responded differently to the climatic and soil conditions during the growing season, as indicated by their different water status responses. 'Koster' and 'Bakan' showed the lowest values of g_s and the highest of \(\Psi_x\), while 'Oudenberg' and 'Grimminge' had the highest g_s and lowest \(\Psi_x\) values. The lowest values of \(\Psi_l\) were reached in 'Koster' and 'Oudenberg', but values of \(\Psi_l\) and \(\Psi_x\) did not change much with changing levels of SWC, T or VPD, while in 'Grimminge', the \(\Psi_l\) values decreased as SWC decreased or VPD/T increased over the course of the growing season (Figure 2a,b,f).

### 3.2 Relationships between PWS indicators and environmental variables

The best-fit linear regressions of RWCl versus the environmental variables were only evident in genotype 'Koster'. The regressions were negative for PPFD, \(T_{\text{air}}\), VPD and \(T_{\text{soil}}\) and positive for RH (\(R^2 = .52\) on average; Table 2). The indicators \(\Psi_l\), \(\Psi_x\) and g_s, were best fitted with the environmental variables measured at midday or with the maximum values of these (Table 2; Figure 3). The relationships of \(\Psi_l\) with the environmental variables illustrated that \(\Psi_l\) was correlated with most of the environmental variables in 'Oudenberg' and especially in 'Koster' (negative with \(T_{\text{air}},\) VPD and \(T_{\text{soil}}\), and positive with RH). The \(\Psi_l\) of 'Grimminge', conversely to 'Koster', was not significantly correlated with any environmental variable, except with the SWC (\(R^2 = .88\)). Best-fit linear regressions of \(\Psi_x\) were found with the midday and with the maximum values of climatic variables (PPFD, \(T_{\text{air}}\), RH and VPD). The g_s showed a high dependency on maximum T and VPD in 'Bakan' (\(R^2 = .97\)) and midday T and VPD in 'Oudenberg' (\(R^2 = .94\); Table 2).

The continuous PWS indicators, SSRV, \(F_s\) and \(E_c\), were better correlated with environmental variables measured on a whole-day basis, that is, mean daytime (Table 3; Figure 3). The best PWS indicators for SRC poplar were those related with transpiration, \(F_s\) and therefore \(E_c\), that strongly correlated with VPD\(_{\text{mdt}}\) and PPFD\(_{\text{mdt}}\) showing 'Bakan' the highest \(R^2\) (between .72 and .82) and 'Oudenberg' the lowest (between .52 and .66; Table 3). Although SSRV was significantly correlated with most environmental variables, especially in 'Oudenberg' and 'Grimminge', it showed a very weak \(R^2\) overall (\(R^2 < .25\)) and was the less sensitive PWS indicator (Table 3). The best-fit equation was achieved between SSRV and SWC in 'Grimminge' (\(R^2 = .28\)). The weak correlations revealed that throughout the growing season, SSRV was not affected by environmental conditions, and thus, the stem water reserves were not used to sustain high leaf transpiration when T and VPD increased and SWC decreased.

The highly significant relationships between PWS indicators and environmental variables—nine in total—showed that overall, VPD was the climatic variable that best correlated with the PWS indicators (Tables 2 and 3; Figure 3c,e,f,h). The most significant linear regressions between \(\Psi_l\) versus SWC data were obtained in 'Oudenberg' (\(R^2 = .83, p = .002\)) and 'Grimminge' (\(R^2 = .88, p = .001\)), where a decrease in the SWC was accompanied by a decrease of \(\Psi_l\) (Figure 3a). In 'Koster' \(\Psi_l\) values remained low and stable despite changes of soil moisture (Figure 3a). The performance of \(\Psi_x\) throughout the genotypes was similar with \(T_{\text{air max}}\) and with VPD\(_{\text{max}}\) (Figure 3b,c), although in 'Bakan' the correlations were not significant (\(R^2 = .40\) and \(.44\), respectively for \(T_{\text{air max}}\) and VPD\(_{\text{max}}\)). In 'Grimminge' and 'Oudenberg' an increase of \(T_{\text{air}}\) or VPD produced a larger decrease of \(\Psi_x\) (average slopes of −0.042 and −0.318 for \(T_{\text{air max}}\) and VPD\(_{\text{max}}\), respectively) than in 'Koster' and 'Bakan' (average slopes of −0.025 and −0.203 for \(T_{\text{air max}}\) and VPD\(_{\text{max}}\), respectively). In all genotypes g_s was significantly (\(p > .02\)) correlated to \(T_{\text{air max}}\) and VPD\(_{\text{max}}\) (Figure 3d,e), achieving 'Grimminge' and 'Oudenberg' the higher values of g_s with the highest values of \(T_{\text{air max}}\) and VPD\(_{\text{max}}\), and 'Koster' the lower ones.

Day-to-day variations of \(F_s\) and \(E_c\) were controlled by VPD and PPFD. \(F_s\) and \(E_c\) were correlated sigmoidally with VPD (Figure 3f,h) and linearly with PPFD (Figure 3g,i). The plateau of \(F_s\) and \(E_c\) at high VPD (>1.0 kPa) differed considerably among genotypes, with \(F_s\) values in 'Grimminge' being fourfold than those in 'Koster'(2.21 kg/day vs. 0.61 kg/day; Figure 3f) and \(E_c\) values double (5.82 mm/day vs. 3.40 mm/day; Figure 3h). Within our observation range of PPFD (50 to 1,000 mmol m\(^{-2}\) s\(^{-1}\)), \(F_s\) and \(E_c\) responded linearly to PPFD.

### 3.3 PCA and model results

The PCA taking into account all PWS indicators overall explained 65.4% of the total variance (Figure 4a). The first component (F1; X-axis), accounted for 42.9% of the total
TABLE 2  Coefficient of determination of best fit first- and second-order equations between the discontinuous plant water status (PWS) indicators: leaf relative water content (RWC), midday stem ($\Psi_l$) and leaf ($\Psi_l$) water potentials, and midday stomatal conductance ($g_s$) and the environmental variables: air temperature ($T_{air}$), relative humidity (RH), vapour pressure deficit (VPD), incoming photosynthetic photon flux density (PPFD), soil water content (SWC) and soil temperature ($T_{soil}$). $R_{WCl}$, $\Psi_l$ and $\Psi_l$ fit with all the environmental variables by first-order linear equations ($y = a + bx$). $g_s$ fit with PPFD, $T_{air}$, VPD and $T_{soil}$ by second-order quadratic equations ($y = a + bx + cx^2$) and fit with RH by inverse second-order equations ($y = a + bx + cx^2$). Values are shown for four poplar genotypes: Oudenberg (O), Grimminge (G), Bakan (B) and Koster (K).

| Environmental variables | Discontinuous PWS indicators | $R_{WCl}$ (%) | $\Psi_l$ (MPa) | $\Psi_l$ (MPa) | $g_s$ (mmol m$^{-2}$ s$^{-1}$) |
|-------------------------|--------------------------------|---------------|---------------|---------------|-------------------|
|                         |                                | O  | G  | B  | K  | O  | G  | B  | K  | O  | G  | B  | K  | O  | G  | B  | K  | O  | G  | B  | K  |
| PPFD (µmol m$^{-2}$ s$^{-1}$) | Maximum                        | 0.01** | 0.03** | −0.04** | −0.34** | −0.15** | −0.03** | −0.14** | −0.36** | −0.28** | −0.21** | −0.29** | −0.32** | 0.21** | 0.16** | 0.13** | 0.06** |
|                         | Mean daytime                    | 0.02** | 0.04** | −0.02** | −0.52*  | −0.29** | −0.09** | −0.23** | −0.42** | −0.48** | −0.36** | 0.40**  | −0.53*  | 0.60**  | 0.54**  | 0.62**  | 0.27**  |
|                         | Midday                          | 4 * 10$^{-3}$ | 0.05** | 1 * 10$^{-4}$ | −0.59*  | −0.31** | −0.10** | −0.22** | −0.38** | −0.48** | −0.36** | −0.35** | −0.49*  | 0.59**  | 0.51**  | 0.61**  | 0.24**  |
| $T_{air}$ (°C)          | Maximum                        | 0.01** | 0.03** | −0.01** | −0.48*  | −0.51** | −0.24** | −0.41** | −0.56*  | −0.79** | −0.69*  | −0.40** | −0.64*  | 0.91**  | 0.89**  | 0.97*** | 0.83*   |
|                         | Mean daytime                    | 6 * 10$^{-6}$ | 4 * 10$^{-3}$ | 0.01** | −0.53*  | −0.49** | −0.29** | −0.46** | −0.58*  | −0.70** | 0.60*   | 0.33**  | 0.47**  | 0.91**  | 0.79**  | 0.83*   | 0.50**  |
|                         | Midday                          | 1 * 10$^{-3}$ | 0.02** | −0.02** | −0.51*  | −0.52*  | −0.27** | −0.44** | −0.62*  | −0.78** | −0.69*  | −0.61*  | 0.94**  | 0.87**  | 0.93**  | 0.71*   |
| RH (%)                  | Maximum                        | 0.60*  | 0.75** | 2 * 10$^{-3}$ | 3 * 10$^{-4}$ | 0.03** | 0.27** | 0.06** | −4 * 10$^{-3}$ | −0.01** | −2 * 10$^{-3}$ | 0.02** | −0.03** | 0.27** | 0.40** | 0.33** | 0.28** |
|                         | Mean daytime                    | 0.06** | 0.03** | 0.07**  | 0.49*   | 0.44** | 0.28** | 0.45** | 0.61*   | 0.53*  | 0.44** | 0.55*   | 0.45** | −0.42** | −0.26** | −0.27** | −0.08** |
|                         | Midday                          | 2 * 10$^{-3}$ | 0.01** | 0.03**  | 0.55*   | 0.50*  | 0.28** | 0.42** | 0.56*   | 0.62*  | 0.52*  | 0.60*   | 0.59*  | −0.57** | −0.41** | −0.50** | −0.28** |
| VPD (kPa)               | Maximum                        | 2 * 10$^{-3}$ | 0.31** | −0.04** | −0.49*  | −0.44** | −0.20** | −0.37** | −0.56*  | −0.74** | −0.62*  | −0.44** | −0.67*  | 0.92**  | 0.91**  | 0.97*** | 0.79*   |
|                         | Mean daytime                    | 2 * 10$^{-3}$ | 1 * 10$^{-3}$ | −0.03** | −0.57*  | −0.47** | −0.26** | −0.45** | −0.64*  | −0.67*  | −0.56*  | −0.41** | −0.50*  | 0.85**  | 0.71**  | 0.74*   | 0.39**  |
|                         | Midday                          | 1 * 10$^{-3}$ | 0.01** | −0.05** | −0.51*  | −0.47** | −0.22** | −0.42** | −0.64*  | −0.72** | −0.61*  | −0.47** | −0.61*  | 0.93**  | 0.83**  | 0.87**  | 0.59**  |
| SWC (m$^3$/m$^3$)       | Maximum                        | 0.21** | 0.24** | −0.01** | 0.22**  | 0.83**  | 0.88**  | 0.62**  | 0.30**  | 0.24**  | 0.61*   | 0.52*   | 0.43**  | 0.47**  | 0.39**  | 0.46**  | 0.17**  |
|                         | Mean daytime                    | 7 * 10$^{-6}$ | 4 * 10$^{-3}$ | 0.01** | −0.53*  | −0.61*  | −0.42** | −0.55*  | −0.58*  | −0.58*  | −0.47*  | −0.37** | −0.34** | 0.47**  | 0.39**  | 0.46**  | 0.17**  |

*, **, *** and ns denote significance at $p < .05$, $p < .01$, $p < .001$ and the absence of significance respectively.
The second component (F2; Y-axis), which explained 22.5% of the total variance, was positively correlated to $F_s$ and negatively to SSRV. The sensitivity of the PWS indicator is revealed by the ‘length’ of each indicator line from ‘0’ to the end of the quadrant. The long lines departing from the graph origin to the centre of gravity of each variable highlight the importance of $F_s$ and $E_c$, and therefore the less relevance of SSRV (shorter line). The projection of the accessions on the two-dimensional PCA graph evidenced a discrete intraspecific variability within poplar genotypes under study revealing a water status differentiation among genotypes. Most of the data points of ‘Koster’ were placed below the X-axis, data points of ‘Grimminge’ above the X-axis and ‘Oudenberg’ and Bakan were interspersed between the Koster and Grimminge groups (Figure 4b).

In as much as the number of available parameter values was much higher for the continuous PWS indicators, a new PCA was inferred using only these (Figure 4b). In this case, the first two components explained almost the 92% of total variation. Most of the samples were concentrated along with X-axis (F1), accounting for 58.30% of the total variance and positively correlated to $F_s$ and $E_c$. The different water behaviour of ‘Grimminge’ and ‘Koster’ was confirmed again with the new PCA results, when only the continuous indicators were plotted. F1 clearly separated the values of genotypes ‘Koster’ (negative side) and ‘Grimminge’ (positive side), suggesting that ‘Grimminge’ was more related to $F_s$ (Figure 4b).

The $F_s$ was considered the most suitable PWS indicator because it correlated significantly with the environmental variables (Table 3; Table S4), and it described the dynamics in PWS (Figures 2 and 3). The $F_s$, furthermore, explained a
**TABLE 3** Coefficient of determination of best fit equations between the continuous plant water status (PWS) indicators: daily sap flow rates ($F_s$), daily transpiration per unit of ground area ($E_c$) and standardized stem radius variation (SSRV) and the environmental variables: air temperature ($T_{air}$), relative humidity (RH), vapour pressure deficit (VPD), incoming photosynthetic photon flux density (PPFD), soil water content (SWC) and soil temperature ($T_{soil}$). $F_s$ and $E_c$ fit with PPFD, RH and SWC by first-order linear equations ($y = a + bx$), with $T_{air}$ and VPD by sigmoidal equations ($y = a/(\exp(bx+c) + 1)$), and with $T_{soil}$ by second-order quadratic equations ($y = a + bx + cx^2$). SSRV fits with $T_{air}$, SWC, and $T_{soil}$ by first-order linear equations ($y = a + bx$), with VPD and RH by second-order quadratic equations ($y = a + bx + cx^2$) and with PPFD by third-order equations ($y = a + bx + cx^2 + dx^3$). Values are shown for four poplar genotypes: Oudenberg (O), Grimminge (G), Bakan (B) and Koster (K).

| Environmental variables | Continuous PWS indicators |  |
|-------------------------|----------------------------|---|
|                         | $F_s$ (kg/day)              | $E_c$ (mm/day) | SSRV (mm) |
|                         | O   | G   | B   | K   | O   | G   | B   | K   | O   | G   | B   | K   |
| PPFD (µmol m$^{-2}$ s$^{-1}$) |   |     |     |     |     |     |     |     |     |     |     |     |     |
| Maximum                 | 0.52*** | 0.52*** | 0.63*** | 0.65*** | 0.45*** | 0.45*** | 0.56*** | 0.60*** | 0.02ns | 0.02ns | 0.04ns | 0.03ns |
| Mean daytime            | 0.55*** | 0.73*** | 0.79*** | 0.69*** | 0.59*** | 0.64*** | 0.72*** | 0.68*** | 0.21*** | 0.15*** | 0.19*** | 0.20*** |
| Midday                  | 0.51*** | 0.65*** | 0.72*** | 0.70*** | 0.49*** | 0.57*** | 0.66*** | 0.64*** | 0.17*** | 0.08* | 0.15*** | 0.16*** |
| $T_{air}$°C             |   |     |     |     |     |     |     |     |     |     |     |     |     |
| Maximum                 | 0.43*** | 0.64*** | 0.68*** | 0.47*** | 0.57*** | 0.60*** | 0.67*** | 0.53*** | 0.12*** | 0.19*** | 3 × 10$^{-3}$ns | 0.13*** |
| Mean daytime            | 0.42*** | 0.59*** | 0.63*** | 0.44*** | 0.57*** | 0.58*** | 0.65*** | 0.50*** | 0.14*** | 0.22*** | 0.04ns | 0.16*** |
| Midday                  | 0.42*** | 0.65*** | 0.69*** | 0.48*** | 0.57*** | 0.61*** | 0.68*** | 0.54*** | 0.14*** | 0.23*** | 0.01ns | 0.14*** |
| RH (%)                  |   |     |     |     |     |     |     |     |     |     |     |     |     |
| Maximum                 | −0.04* | 2 × 10$^{-4}$ns | −0.01ns | −0.06** | 4 × 10$^{-3}$ns | 2 × 10$^{-3}$ns | −0.01ns | −0.03* | 0.01ns | 0.03ns | 2 × 10$^{-3}$ns | 6 × 10$^{-3}$ns |
| Mean daytime            | −0.32*** | −0.46*** | −0.51*** | −0.47*** | −0.35*** | −0.39*** | −0.53*** | −0.41*** | 0.05* | −0.08* | 0.03ns | 0.07* |
| Midday                  | −0.30*** | −0.53*** | −0.57*** | −0.44*** | −0.37*** | −0.46*** | −0.57*** | −0.42*** | 0.04* | −0.10*** | 0.02ns | 0.04ns |
| VPD (kPa)               |   |     |     |     |     |     |     |     |     |     |     |     |     |
| Maximum                 | 0.51*** | 0.76*** | 0.79*** | 0.59*** | 0.62*** | 0.69*** | 0.78*** | 0.63*** | 0.20*** | 0.21*** | 0.04ns | 0.20*** |
| Mean daytime            | 0.53*** | 0.78*** | 0.82*** | 0.61*** | 0.66*** | 0.72*** | 0.82*** | 0.66*** | 0.21*** | 0.21*** | 0.05* | 0.24*** |
| Midday                  | 0.45*** | 0.76*** | 0.80*** | 0.57*** | 0.59*** | 0.69*** | 0.80*** | 0.62*** | 0.17*** | 0.20*** | 0.02ns | 0.18*** |
| SWC (m$^3$/m$^3$)       | 0.07** | 0.02ns | 1 × 10$^{-3}$ns | 0.08** | 4 × 10$^{-4}$ns | 0.04* | 0.01ns | 0.01** | 0.01ns | 0.28*** | 0.01ns | 7 × 10$^{-4}$ns |
| $T_{soil}$°C            | 0.33*** | 0.47*** | 0.52*** | 0.36*** | 0.46*** | 0.48*** | 0.53*** | 0.41*** | 0.05* | 0.12*** | 2 × 10$^{-4}$ns | 0.07** |

*, **, *** and ns denote significance at $p < .05$, $p < .01$, $p < .001$ and the absence of significance, respectively.
Figure 4 Principal component analysis (PCA) for (a) all seven PWS indicators (RWC, \( \Psi_l \), \( \Psi_x \), \( g_c \), \( E_c \) and SSRV) measured in poplar genotypes Oudenberg (dark red circle), Grimminge (orange down triangle), Bakan (light green square) and Koster (dark green rhombus) at a bio-energy plantation in Lochristi (Belgium). The PWS indicators measured during the 2016 growing season are: leaf relative water content (RWC; %), leaf water potential (\( \Psi_l \); MPa), xylem water potential (\( \Psi_x \); MPa), stomatal conductance (\( g_c \); mmol m\(^{-2}\) s\(^{-1}\)), standardized stem radial variation (SSRV; mm), daily sap flow (\( F_c \); kg/day) and daily transpiration per unit of ground area (\( E_c \); mm H\(_2\)O/day). The centres of gravity per genotype in (a) are: −0.35, −0.45 (Oudenberg); −0.21, 1.04 (Grimminge); 0.01, 0.20 (Bakan); and 0.55, −0.79 (Koster). In (b) are: −0.01, 0.09 (Oudenberg); 1.15, −0.56 (Grimminge), −0.16, 0.20 (Bakan); and −0.98, 0.27 (Koster).

Table 4 Fitting equations of the daily canopy transpiration per unit of ground area (\( E_c \); mm H\(_2\)O/day) for each genotype using commonly measured predictive variables. PPFD\(_{mdt}\) is the mean daytime photosynthetic photon flux density (µmol m\(^{-2}\) s\(^{-1}\)), VPD\(_{mdt}\) is the mean daytime vapour pressure deficit (kPa), \( T_{soil} \) is the mean daily soil temperature at 10 cm depth (°C), SWC is the mean daily soil water content at 20 cm depth (%). All fitting equations were significant and most of the individual variables had a \( p < .001 \) (Table S5 and S6)

Fitting equations of the simplified model

\[
E_{c, Oudenberg} = -4.166 + 4.279 \times 10^{-3} \times \text{PPFD}_{mdt} + 0.871 \times \text{VPD}_{mdt} + 0.297 \times T_{soil} + 0.036 \times \text{SWC} \\
E_{c, Grimminge} = -2.960 + 5.761 \times 10^{-3} \times \text{PPFD}_{mdt} + 0.568 \times \text{VPD}_{mdt} + 0.301 \times T_{soil} - 4.50 \times \text{SWC} \\
E_{c, Bakan} = -1.709 + 2.269 \times 10^{-3} \times \text{PPFD}_{mdt} + 1.93 \times \text{VPD}_{mdt} + 0.090 \times T_{soil} - 0.304 \times \text{SWC} \\
E_{c, Koster} = -2.867 + 3.316 \times 10^{-3} \times \text{PPFD}_{mdt} + 0.966 \times \text{VPD}_{mdt} + 0.143 \times T_{soil} + 3.017 \times \text{SWC} \\
\]

Best fitted model (based on the lowest AIC value approach)

\[
E_{c, Oudenberg} = -4.161 + 4.286 \times 10^{-3} \times \text{PPFD}_{mdt} + 0.864 \times \text{VPD}_{mdt} + 0.297 \times T_{soil10} \\
E_{c, Grimminge} = -6.046 + 6.188 \times 10^{-3} \times \text{PPFD}_{mdt} + 1.214 \times \text{VPD}_{mdt} + 0.037 \times \text{RH}_{soil} + 0.264 \times T_{soil10} - 4.151 \times \text{SWC}_{20} \\
E_{c, Bakan} = -1.765 + 2.213 \times 10^{-3} \times \text{PPFD}_{mdt} + 1.979 \times \text{VPD}_{mdt} + 0.089 \times T_{soil10} \\
E_{c, Koster} = -0.028 + 3.458 \times 10^{-3} \times \text{PPFD}_{mdt} + 0.121 \times T_{mdt} - 0.025 \times \text{RH}_{soil} + 2.208 \times \text{SWC}_{20} \\
\]

large portion of the variance in the data in the PCA (Figure 4) and it is measured continuously, which facilitates the collection of large data sets. A linear model was developed using environmental variables to predict \( E_c \) (Table 4). The \( E_c \) was chosen due to its close relationship of \( F_c \) (average Pearson's \( r^2 \) among the genotypes = .924; \( p < .001 \)) and because it represented the evapotranspiration at canopy level, which is more reliable for future comparisons with other ecosystems. Among all environmental parameters used in the reference equations, the following variables were chosen as predictive variables: PPFD\(_{mdt}\), VPD\(_{mdt}\), \( T_{soil} \) and SWC (Table 4). The model equations revealed that 'Grimminge' and 'Koster' had a very distinct relationship with SWC, both parameter values of the equation being highly significant (\( p < .001 \); Table S5). The \( E_c \) was also modelled using the approach of the lowest AIC value (Table 4) using all environmental variables measured (\( T_{air \ mdt} \), \( R_{H \ mdt} \), PPFD\(_{mdt}\), VPD\(_{mdt}\), \( T_{soil} \) and SWC) and considered the most optimal model to describe \( E_c \). The fitting parameters and their significance are shown in Table S6. The equations resulting from the lowest AIC approach did not contain the same parameters for every genotype: SWC was only relevant for 'Grimminge' and 'Koster', VPD was not included in the equation of
"Koster", and in addition $T_{\text{soil}}$ was substituted by $T_{\text{air}}$ in the equation.

## DISCUSSION

The different responses of the PWS indicators studied, especially the discontinuous ones, illustrated different water behaviours of poplar genotypes in relation to the level of isohydricity and served to explain and distinguish the best PWS indicator in relationship with the identified iso/an-iso-hydric behaviour for expressing PWS. Recent studies showed that hybrid poplars vary widely in their stomatal sensitivity to SWC and VPD (Arango-Velez, Zwiazek, Thomas, & Tyree, 2011; Attia, Domec, Oren, Way, & Moshe1ion, 2015; Navarro et al., 2018; Tricker et al., 2009). In the present study, this technique provided $F_s$ and $E_c$ were both controlled by VPD and PPDF. The saturation of daily sums of $F_s$ and $E_c$ at a VPD higher than 1.0 kPa suggested that at low VPD, VPD was the major control on $F_s$ and $E_c$ because the gradient of water vapour between the inside of stomata and ambient air drives transpiration. At high VPD, $F_s$ and $E_c$ saturated because of decreased $g_s$, despite the favourable water vapour gradient for transpiration. The $F_s$ and $E_c$ did not saturate at high PPDF on a daily basis in line with other studies (Ewers, Gower, Bond-Lamberty, & Wang, 2005; Tang et al., 2006).

The time series of SSRV, the reversible component of changes in stem size, represents the stem shrinking and swelling caused by dynamics in water storage in elastic tissues outside the cambium. These changes are the result of changing water potential gradients within the stem (De Swaef et al., 2015; Sevanto, Hölttä, & Holbrook, 2011; Zweifel, Item, & Häslcr, 2001). The low sensitivity of SSRV to environmental conditions in the young trees of our study agrees with previous findings (Mèrian & Lebourgeois, 2011; Oberhuber, 2017; Schuster & Oberhuber, 2013) that revealed higher climatic sensitivity of SSRV in older and larger trees than in young trees or saplings. Oscillations in trunk diameter are related to changes in water content of external tissues (phloem, cambium and bark) with little contribution from the xylem (Remorini & Massai, 2003). Stem cross-sections of our young SRC poplars (see figure 2 of Navarro et al., 2018) consisted mainly of sapwood (conductive tissues), with a minor contribution to trunk water content oscillations. The favourable environmental conditions at our site did not create any water stress, supported by the absence of changes in $\Psi_s$ for the entire growing season, which are approximately linearly proportional to radial changes (Chan et al., 2016).

Projected climate changes (for Belgium: increased cloud cover, gradual disappearance of cold winters, enhanced flooding, etc.—Belgium NCC NC7/BR3, 2017) lead to enhanced risks of flooding in winter by increased winter precipitation and the occurrence of more frequent and intense heavy thunderstorms in summer. Furthermore, higher risks of a serious water shortage or droughts in spring and summer are expected in many midlatitude regions by the decrease of summer rain-fall and the increase of evaporation (Collins et al., 2013). Hence, SRC crops face an uncertain future which underlines the need for an improved understanding of tree susceptibility to water stress and real water needs during the growing season. The challenge, in the near future, for growers of SRC poplar will be to resort to irrigation, even in midlatitude regions, to have profitable yields (Di et al., 2019; González-González, Oliveira, González, Cañellas, & Sixto, 2017). The
assessments of crop water requirements ($E_t$) for specific genotypes is necessary for an efficient management of the irrigation system.

In conclusion, we identified the best PWS indicator as $F_4$ and the key environmental variables influencing transpiration, and we integrated them into a quantitative predictive model to calculate transpiration per unit of ground area. The environmental variables used in the resulting fitting equations (PPFD, VPD, $T_{\text{soil}}$ and SWC) are standard for most research and agricultural locations, which makes the model practical to apply. This might enable SRC producers to estimate transpiration with a few environmental variables that can be monitored with easy-to-handle sensors.

These conclusions are based on our present study with poplar and cannot simply be extrapolated or extended to other sites or to other species and genera, since the relationships between PWS indicators and meteorological variables as well as the empirical models that we developed, are site- and species-dependent. This is one of the limitations of this—and any other—study. Comparative studies with other important SRC species (willow, elm, eucalyptus, black locust, etc.) should confirm these observations and future research could apply the established relationships and the developed models to other site conditions, other tree genera and/or other environments.

**ACKNOWLEDGEMENTS**

This research has received funding from the European Commission’s Horizon 2020 Programme through the Marie Skłodowska-Curie Actions (MSCA) - Individual fellowships (IF) as MSCA grant agreement 657123 (PHYSIO-POP) as well as from the Seventh Framework Programme (FP7/2007-2013) through the European Research Council as ERC grant agreement 233366 (POPFULL). Further support was provided by the Flemish Science Foundation (FWO, Brussels). We gratefully acknowledge the technical assistance of Jan Segers, Cristina Ariza Carricondo, Nicola Arriga and Stefan Vanbeveren, as well as the logistic support of Kristof Mouton at the field site.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Navarro A, Portillo-Estrada M, Ceulemans R. Identifying the best plant water status indicator for bio-energy poplar genotypes. *GCB Bioenergy*. 2020;12:426–444. [https://doi.org/10.1111/gcbb.12687](https://doi.org/10.1111/gcbb.12687)