

EVALUATION OF INDIRECT MEASUREMENT METHOD OF SEASONAL PATTERNS OF LEAF AREA INDEX IN A HIGH-DENSITY SHORT ROTATION COPPISE CULTURE OF POPLAR

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Abstract

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Leaf area index (LAI) is an important determinant of biomass production and yield of short rotation bio-energy plantation. An accurate measurement of LAI is critical for quantifying light interception and penetration within the canopy, and subsequently understanding its influence on the stand carbon and energy balance. The aim of the current study is validation of the Sunscan Plant Canopy Analyzer which serves as an indirect method for the evaluation of the seasonal patterns of LAI, relation between LAI and above ground woody dry biomass and to determine the specific leaf area in short-rotation poplar hybrid clone J-105 (*Populus nigra* × *P. maximowiczii*) in uncoppiced (1st rotation) and coppiced (2nd rotation), respectively. LAI was measured in uncoppiced and coppiced by two different methods using indirect (SunScan Plant Canopy Analyzer) and direct (litterfall collection). Sunscan Plant Canopy Analyzer was compared against litterfall collection (only way to retrieve the actual LAI). Simple regression ($R^2 = 0.82$) model was fitted to validate indirect measurement method and a very good agreement (82%) was observed in LAI values estimated from SunScan Plant Canopy Analyzer and from litterfall collection. Seasonal variability of LAI in a short rotation coppice (SRC) culture of poplar clone J-105 was evaluated over six years period (2008–2013), for uncoppiced (2008 and 2009) and coppiced (2010, 2011, 2012 and 2013) culture. The maximum canopy LAI (LAI_{max}) reached 7.3 (uncoppiced) and 9.5 (coppiced). The linear regression ($R^2 = 0.93$) for average LAI and above ground woody dry biomass was determined, and it was found that LAI acts an indicator of biomass productivity. Specific leaf area (SLA) was estimated in both uncoppiced and coppiced culture of poplar. The maximum SLA was found to be $138.9 \text{ cm}^2 \text{ g}^{-1}$ in uncoppiced and $126.9 \text{ cm}^2 \text{ g}^{-1}$ in coppiced. To conclude, the evaluated indirect LAI measurement method is portable, reliable and faster than direct LAI measurement in high density poplar short-rotation coppice culture.

Keywords: SunScan Plant Canopy Analyzer, litterfall, specific leaf area, poplar clone J-105

INTRODUCTION

Leaf area index (LAI) acts as an important input value for ecological and crop models describing canopy architecture. Canopy leaf area serves

as the dominant control over photosynthesis (primary production), radiation penetration, energy exchange, precipitation evapotranspiration, and transpiration rate in forest stands and crops

(Boreckx *et al.*, 2012). Direct measurements of canopy structure are labor intensive and tedious in small canopies (crops) and nearly impossible in large forest canopies. However, radiation transfer models show that relatively simple measurements of radiation penetration can provide accurate estimates of the canopy architecture (Grantz *et al.*, 1993; Behera *et al.*, 2010). LAI ($\text{m}^2 \text{ m}^{-2}$) is defined as the total one-sided or hemi-surface of green leaves per unit of ground surface area (Chen and Black, 1992). LAI is a key parameter in many areas of agronomy, agroforestry and plant production through its influence on light interception, plant growth, weed control, water use and soil erosion (Soltani and Galeshi, 2002). LAI is a component of plant growth analysis, which is a critical factor in understanding and the function of various plant management practices. These include leaves that form an active interface for energy and other factors such as carbon and water exchange that takes place between the plants and the atmosphere (Cutini *et al.*, 1998). In plantations with fast-growing trees (poplar and willow), biomass production is linearly related to intercepted radiation and photosynthetically active radiation (PAR), which are generally assessed via the LAI (Pearce *et al.*, 1965; Cannell *et al.*, 1988; Gardner *et al.*, 2001; Chauhan *et al.*, 2010).

Methods for *in situ* LAI measurement can be grouped in two main categories i.e., direct and indirect (Jonckheere *et al.*, 2004; Weiss *et al.*, 2004). Direct or destructive (litter collections or harvesting of leaves) method uses allometric regression models (Liberloo *et al.*, 2004; Kurata *et al.*, 2005; Broeckx *et al.*, 2012). Allometric equations are based on relationships between the foliage mass and the woody structures (stem diameter and/or tree height). Direct methods where representative leaf samples are measured is the only way to retrieve the actual leaf area. Besides the effort involved in collecting and measuring the samples, direct methods require an additional effort to estimate field plant density and optimal sample size (Confalonieri *et al.*, 2006). This direct method is a form of litter collection procedure, where the leaf litter is collected on traps during the period of leaf fall. In this method, the total dry weight of leaves is recorded. Hence this method can be renamed as leaf harvest direct method. The leaf harvest direct method is much less labor-intensive than the destructive methods (Jonckheere *et al.*, 2005). The success of this direct method, especially for broad-leaved species, can be explained by the relatively moderate amount of labor involved. On the other hand, the indirect methods estimate LAI using optical modes, such as remote sensing, radiation transmitted through the canopy (i.e. canopy gap fraction), plant canopy analyzer (SunScan and LAI 2000), hemispherical photography that are based on the tight relationship between canopy light transmittance and LAI (Herbert, 1965; Chen *et al.*, 1991; Welles and Chohen, 1996; Peper and McPherson, 1998; Schwalbe, 2005; Fu *et al.*, 2009). Plant Canopy

Analyzer (Delta-T Devices, Cambridge, UK) is one of the most widely used optical instruments for *in situ* LAI estimation. The SunScan Plant Canopy Analyzer is a portable instrument for measuring the light level of photosynthetically active radiation (PAR), interception of solar radiation and some types of canopy that estimate the LAI. The SunScan Plant Canopy Analyzer, which simultaneously measures the photosynthetically active radiation incident at the top of a crop canopy and at the bottom, has been used to measure LAI (Lambert *et al.*, 1999). The Plant Canopy Analyzer, SunScan has been widely used for the ecophysiology of agricultural crops. This method gives the best results and provide a good performance for homogenous woody canopies and cereal crops, but there are important errors that commonly occur when there are large gaps within the vegetation (Lang *et al.*, 1991; Potter *et al.*, 1996). LAI is a good and reliable estimator of woody biomass in short rotation coppice (SRC) culture of poplars (Ceulemans, 1990; Barigah *et al.*, 1994; Tharakan *et al.*, 2005; Verlinden *et al.*, 2015). LAI can be measured as one of the key parameter for retrieving plant biomass and net primary productivity for large-scale plantations and extrapolating spatial and temporal yield. In this study, we compared two approaches to quantify the LAI of a short-rotation coppice culture with poplar, i.e. an indirect approach (via light transmission) and a direct approach (via litter fall and allometric relationships with specific leaf area, SLA). The present study is aimed to evaluate the suitability and reliability of the SunScan Plant Canopy Analyzer to estimate seasonal patterns of LAI, relation between LAI and above ground woody dry biomass and to determine the SLA, in uncoppiced and coppiced short rotation poplar culture.

MATERIALS AND METHODS

Study Area

The study was carried out in Domanínek, the Bohemian-Moravian Highlands ($49^{\circ}31'N$, $16^{\circ}14'E$ and altitude 530 m a.s.l.), Czech Republic. For verification of performance of selected poplar hybrid clone J-105 (*Populus nigra* \times *P. maximowiczii*) an area of 2.85 ha was established in April 2002. The plantation was cultivated on arable land previously seeded with cereals and potatoes. Soil sampling took place prior to planting. Soil conditions at the experimental location are representative to the wider region with the deep luvic cambisol. The area is cool and relatively wet temperate with long term (2008–2013, reference year) mean annual air temperature and rainfall of 7.2°C and 609 mm, respectively. The climatic conditions between the years 2008 to 2013 are shown in Tab. I. Hardwood cuttings were planted in a double-row design with a spacing of 0.7 m within the rows and with an inter-rows distance of 2.5 m. The plant density was 9216 trees ha^{-1} . Plantation was divided in two parts

I: Climatic conditions (mean annual temperature and rainfall) between 2008–2013, specific leaf area (SLA), plant area index (PAI), wood area index (WAI), maximum indirect LAI (LAI_{max}), average leaf dry biomass ($t \cdot ha^{-1} \cdot year^{-1}$) and above-ground woody biomass (AGWB, $t \cdot ha^{-1} \cdot year^{-1}$) from 2008–2009 (uncoppiced) and 2010–2013 (coppiced)

Year	Mean annual air temperature ($^{\circ}C$)	Total annual rainfall (mm)	SLA _{ave} \pm SD ($cm^2 \cdot g^{-1}$)	PAI ($m^2 \cdot m^{-2}$)	WAI ($m^2 \cdot m^{-2}$)	LAI _{max} ($m^2 \cdot m^{-2}$)	Leaf dry matter _{ave} \pm SD ($t \cdot ha^{-1} \cdot year^{-1}$)	AGWB ($t \cdot ha^{-1} \cdot year^{-1}$)
2008	8.8	578	-	7.7	0.9	6.8	-	13.4
2009	7.26	779	138.9 \pm 12.4	8.3	1.0	7.3	4.20 \pm 0.37	16.5
2010	6.02	694	-	4.4	0.8	3.6	-	3.62
2011	7.42	521	-	6.4	0.9	5.5	-	6.87
2012	7.43	511	125.8 \pm 29.0	7.7	0.9	6.8	4.30 \pm 0.12	10.31
2013	7.36	530	126.9 \pm 23.3	10.7	1.2	9.5	5.33 \pm 0.35	13.56

uncoppiced (first rotation, harvested after 8 years in 2009) and coppiced (second rotation, 2010–2013).

Direct (Litterfall Collection) LAI Measurements

Projected LAI was calculated from litterfall data collected with three litter traps ($3 m^2$) known surface areas. Litter traps were positioned in the middle of inter-rows and set close to the locations of the indirect measurements by the SunScan Plant Canopy Analyzer. The contents of the traps represent the average amount of leaves falling in the stand. Litterfall was collected in 2009 (uncoppiced, first rotation, 2002–2009), 2012 and 2013 (coppiced, second rotation, 2010–2013). On an average, litterfall collection was done for 3–5 times in a year without loss of original weight. Collected leaves from litter traps were put into three different paper bags and oven dried at $70^{\circ}C$ until a constant weight was reached. A representative sample of leaves (~30 leaves) were taken from each bag and weighed again. These leaves were spread on a rectangle white board with known dimensions (length 1 m \times width 0.5 m) and photographs were captured using digital camera Sony DSC-W210 (Sony Electronics Inc., New York, USA) placed on a tripod stand with a known distance from the white board. This was carried out in 2009 and the same procedure was repeated by a Canon power shot A3100 (4X optical zoom, 12.1 megapixel, Malaysia) in 2012 and 2013, individually. Photographs were saved in high-quality JPEG file format and stored on memory card of the type secure digital card (SD card). The images were analyzed using the ImageJ 1.4.1 software (Rasband, 2009). All the color pictures were converted into black and white, as the black area was required to be analyzed in line with the software protocol (Glozer, 2008). This method was described to measure the leaf area by using an image histogram and photographic software tools. Thus, the area of all the leaves presented on the white board was determined (Bradshaw *et al.*, 2007) to obtain the SLA. Leaf area index is the product of yield ($g \cdot m^{-2}$) \times % leaves \times specific leaf area ($m^2 \cdot g^{-1}$) and is a dimensionless number. The average SLA and dry matter of leaves are shown in Tab. I. for uncoppiced (2009) and coppiced (2012 and 2013).

Indirect (SunScan Plant Canopy Analyzer) LAI Measurements

LAI was measured in uncoppiced and coppiced plantations during the whole growing season (mid-April to mid-October) by using the SunScan Plant Canopy Analyzer (Delta-T Devices, Cambridge, UK). This indirect measurement technique is based on simultaneous measurement of the incident photosynthetically active radiation (PAR) above and the transmitted below the canopy. The incident PAR was measured by the Beam Fraction Sensor (BFS), which incorporates multiple photodiodes, of which one is always shaded. The BFS was connected by a 50 m long cable (2009 and 2012) and by a radio link (2013) to the ceptometer via a 1 m long measuring probe with 64 equally spaced photodiodes. More details of SunScan Plant Canopy Analyzer are mentioned in SunScan Canopy Analysis System user manual (Delta-T Devices Ltd.). Moreover, the model assumes that the canopy is not a black body and that it uniformly reemits radiation from the leaf elements in all directions. During the measuring routine, the BFS was placed horizontally levelled on a 1.5 m high tripod in the open grassland south of the plantation. The selected locations of SunScan Plant Canopy Analyzer measurements were three spots close to litter collections in the plantations. Sampling was carried out on a weekly basis at each place. For data collection, approximately 30 readings were taken within the double row and inter-rows at each place. The measurements were done either early in the morning or late in the afternoon. The values thus obtained by the SunScan Plant Canopy Analyzer are considered to be the plant area index (PAI) during the whole growing season (mid-April to mid-October). Therefore, at the end of the growing season (defoliated all leaves from plant canopy); the same measurement was carried out by the SunScan Plant Canopy Analyzer to determine the wood area index (WAI). The WAI was subsequently deducted from the measured value (PAI), and finally the LAI was obtained. PAI and WAI are shown in Tab. I. For gap-filling, we interpolated the weekly LAI data with help of daily mean air temperature and obtained daily values of LAI.

II: Comparison of the coefficient of determination (R^2) values between two different cases, i.e. LAI_{max} vs. biomass and LAI_{Avg} vs. biomass

R^2	Biomass x LAI_{max}	Biomass x LAI_{Avg}
	0.6692	0.9268

Woody Biomass Estimates

The allometric equation was developed by Fischer (2012) to estimate the biomass in uncoppiced and coppiced rotation thus obtaining an improved average value for both LAI and the woody biomass measurement. Coefficient of determination (R^2) values for two different regressions namely. LAI_{max} vs biomass and LAI_{Avg} vs biomass were estimated (Tab. II).

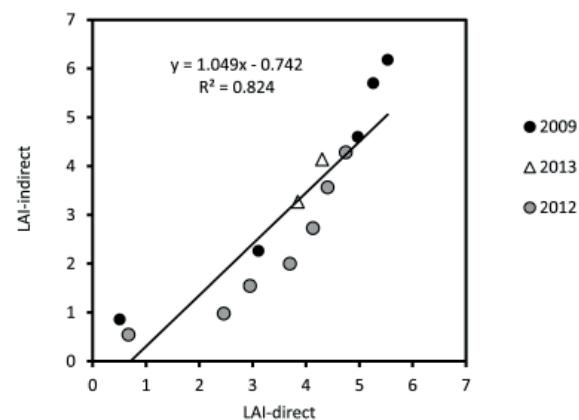
Statistical Analysis

Linear regression analysis of indirect LAI against direct LAI was done to evaluate the above procedures and to standardize method for SunScan Plant Canopy Analyzer. Coefficient of determination (R^2) was calculated to fit a slope of the linear regression. We also analysed the linear regression (R^2) of annual average LAI vs annual cumulative woody dry biomass and determined p value ($p > 0.001$) followed by post hoc Fischer test. Specific leaf area in uncoppiced and coppiced was based on the coefficient of variation statistics as a normalized measure of the relative dispersion of different years. Standard deviations of the mean of SLA were determined to non-significant difference p value ($p < 0.05$) followed by post hoc Fischer test. All statistical analyses were conducted using STATISTICA 10 (StatSoft, Inc., USA).

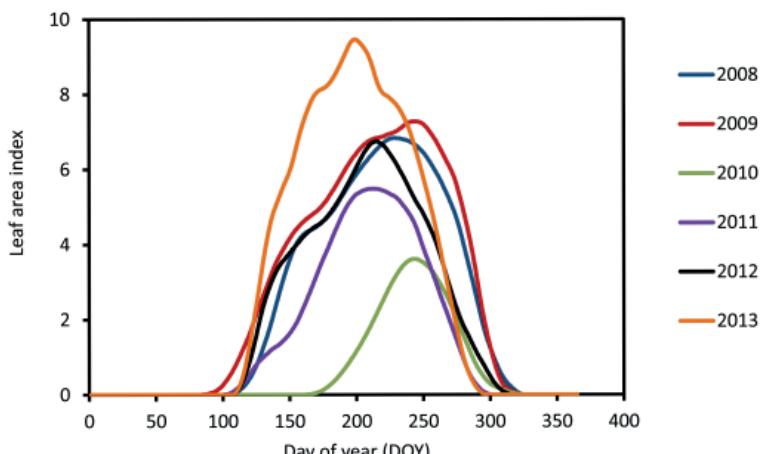
RESULTS

SunScan plant canopy analyzer measures whole PAI. Therefore, at the end of growing season when trees were defoliated (total leaf fall), the same

measurement was carried out by SunScan Plant Canopy Analyzer to determine the WAI. These wood area indexes were subtracted from the measured PAI (Tab. I) and LAI were obtained. The values of LAI estimated indirectly with SunScan Plant Canopy Analyzer were plotted for scatter analysis against the direct LAI estimation through litter collection. LAI directly determined from litter collection was considered as the true value, and the LAI from the indirect method was then compared and successfully validated against direct method (litterfall). Regression analysis between direct and indirect measurements of LAI showed very good agreement (Fig. 1, 82%) and highly significant linear correlation ($R^2 = 0.82$, $p = 0.00001$) was followed by post hoc Fischer test ($p < 0.001$). After the successful indirect LAI method validation, measurements were carried out on uncoppiced and coppiced short rotation poplar culture to determine LAI during



1: Relationship between indirect (SunScan Plant Canopy Analyzer) leaf area index (LAI) measurements and direct LAI values obtained through litter collections in uncoppiced (first rotation after 8th year plant growth, 2009) and coppiced (second rotation, 3rd and 4th year plant growth, 2012 and 2013, respectively) in short-rotation coppice (SRC) poplar cultures. The relationship is ~1 ($R^2 = 0.82$) and $p = 0.00001$ ($p < 0.001$)



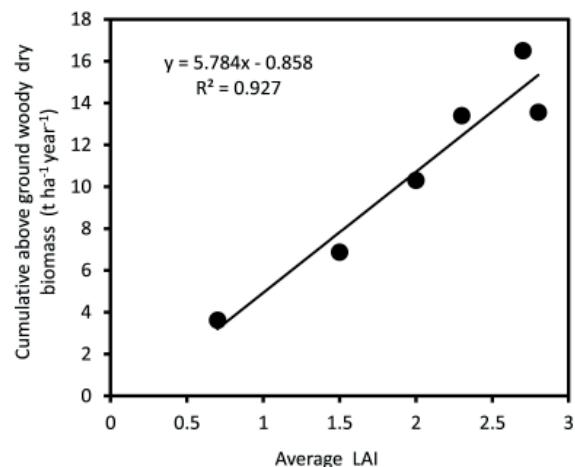
2: Time course of leaf area index (LAI) measured weekly by the SunScan Plant Canopy Analyzer during the growing seasons in uncoppiced (first rotation, 7th and 8th year plant growth, 2008 and 2009, respectively) and coppiced (second rotation, 1st, 2nd, 3rd and 4th year plant growth, 2010, 2011, 2012 and 2013, respectively)

growing seasons (2008–2013) as depicted in Fig. 2. During the six years of measurements including uncoppiced (2008 and 2009) and coppiced (2010–2013), coppicing enhanced LAI, where LAI_{max} was estimated to be 9.5 in coppiced and 7.3 in uncoppiced. The annual LAI_{max} in uncoppiced and coppiced is shown in Tab. I. The leaf area index is a determinant of woody dry biomass. Coefficient of determination (R^2) values for two different regressions namely LAI_{max} vs biomass and LAI_{Avg} vs biomass is shown in Tab. II. Yearly average LAI determined the standing woody dry biomass ($t \text{ ha}^{-1}\text{year}^{-1}$) in uncoppiced and coppiced short rotation poplar culture. The linear regression of the annual average LAI vs cumulative above-ground woody dry biomass ($R^2 = 0.93$) is shown in Fig. 3. This shows that above ground woody dry biomass

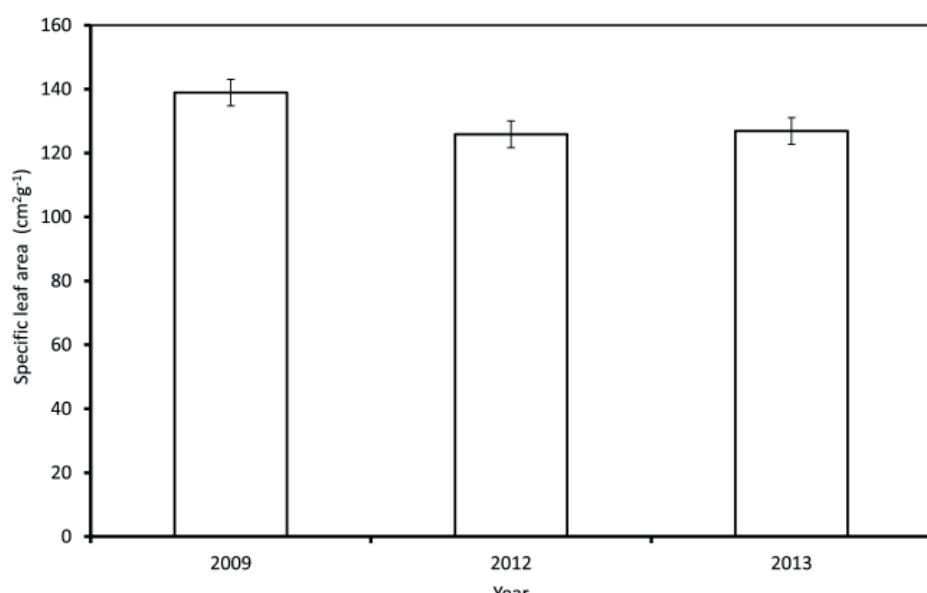
is dependent on annual average LAI enhancement. The annual above-ground woody dry biomass in uncoppiced (after 7th and 8th years growth) and coppiced (after 1st, 2nd, 3rd and 4th years of growth) is shown in Tab. I. Specific leaf area was used to estimate the LAI by direct method that was evaluated in uncoppiced (after 8 years of growth) and coppiced (after 3rd and 4th years of growth, respectively). Average SLA (Fig. 4) varied in uncoppiced and coppiced because of temporal and spatial variability but, i.e. not statistically significantly different on level $p < 0.05$. In plantation, canopy homogeneity was found because of canopy homogeneity in the plantation, it has been proved after the three years (mature plant) of plant growth there is no significant difference in SLA.

DISCUSSION

One of the most reliable, direct and non-destructive methods for LAI estimation is to collect leaves in litter traps (McShane *et al.*, 1983; Bouriaud *et al.*, 2003). These methods are of high accuracy; they are frequently used in validation of other indirect approaches (Lovell *et al.*, 2003; Jonckheere *et al.*, 2004; Liberloo *et al.*, 2004). The direct methods are still considered the best choice for accurate measurements of LAI, but as these are time consuming (Thimonier *et al.*, 2010), the researchers prefer to use an alternate indirect procedure for rapid LAI measurements, for example- optical hemispherical photography, remote sensing, and plant canopy analyzers (SunScan LI-2000 and LI-2200). In the current study, the results prove that the SunScan Plant Canopy Analyzer method has good potentiality for indirect measurements in SRC poplar plantations. The method has been



3: Relationship between above-ground woody biomass (AGWB) and annual average leaf area index (LAI_{Avg})



4: Specific leaf area (SLA) of 30 leaves three times during leaf fall in uncoppiced (after 8 year plant growth, 2009) and coppiced (after 3rd and 4th year plant growth, 2012 and 2013, respectively). Bars are standard deviations of the mean and $p = 0.11383$ ($p < 0.05$). Fresh leaf area was measured by image analysis and dry mass was measured after 48 h of oven drying at 70 °C

successfully validated ($R^2 = 0.82$) through litter collections or direct method (Fig. 1) in our study. The value thus obtained is in consensus with value ($R^2 > 0.74$) obtained on maize for all the stages and seasons (Oguntunde *et al.*, 2012). Similar results ($R^2 = 0.96$) were successfully validated for cereal crop (sugarcane) in South Africa (Chiroro *et al.*, 2006). A review of the indirect and direct methods can be found in Welles and Cohen (1996) and Bréda (2003). The good performance of the SunScan Plant Canopy Analyzer device lends to support the manufacture claim and gives a good estimate of the LAI especially in cereal crops (Potter *et al.*, 1996).

In our study, the values of LAI_{max} observed during the consequent growing seasons in uncoppiced and coppiced were comparable to values reported for various willows (Tharakan *et al.*, 2008; Petzold *et al.*, 2010) and poplar SRC cultures (Ceulemans *et al.*, 1996; Liberloo *et al.*, 2006; Fischer *et al.*, 2013). The seasonal patterns of LAI were evaluated in uncoppiced and coppiced, coppicing enhanced

the LAI. Rae *et al.* (2004), Tharakan *et al.* (2008) and Broeckx *et al.* (2015) found the similar results in different SRC poplar cultures. Our present study shows that above ground woody dry biomass is determined by the annual average LAI enhancement, LAI is reliable indicator of biomass production (Ceulemans, 1990; Barigah *et al.*, 1994; Tharakan *et al.*, 2005; Verlinden *et al.*, 2015). The annual average LAI showed a strong positive correlation with cumulative above-ground dry woody biomass productivity as shown in Tab. I and Fig. 3. For estimating the direct LAI, average specific leaf area was determined in coppiced and uncoppiced. The average SLA for coppiced, ranged between 125.8 to 126.9 $\text{cm}^2 \cdot \text{g}^{-1}$ and in uncoppiced the maximum value obtained was 138.9 $\text{cm}^2 \cdot \text{g}^{-1}$ (Fig. 4). Our results are in agreement with previous study performed by Verlinden *et al.* (2013) in poplar genotypes where they found the average SLA in two growing seasons to be 114 and 138 $\text{cm}^2 \cdot \text{g}^{-1}$, individually.

CONCLUSION

The SunScan Plant Canopy Analyzer indirect LAI measurement method presented can be applied for rapid and reliable indirect LAI measurements in homogenous canopy row crops like jatropha, willows and cereal crops such as sugarcane and maize etc. having similar plantation spacing, canopy height and architecture. SunScan Plant Canopy Analyzer is resistant to weather changes and can be used to measure versatile weather conditions. However, important errors that commonly occur due to large gaps within the vegetation, further testing is needed for cross validation under different short rotation coppice management plantations among other indirect measurement methods such as Fisheye, LAI 2200 and LAI 2000 etc. In our study the above ground woody dry biomass increased as a function of increasing annual average LAI, thus considering LAI to be a reliable determinant of above-ground woody dry biomass in SRC culture of poplars. Specific leaf area values used to estimate the direct LAI measurements were not significantly different due to the fast growth and homogeneity in vegetation canopy.

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