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Variability of stomatal conductance, leaf anatomy, and seasonal leaf wettability of young and adult European beech leaves along a vertical canopy gradient

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Abstract This study assessed the variation of leaf anatomy, chlorophyll content index (CCI), maximal stomatal conductance (g_s^{max}) and leaf wettability within the canopy of an adult European beech tree (*Fagus sylvatica* L.) and for beech saplings placed along the vertical gradient in the canopy. At the top canopy level (CL_{28m}) of the adult beech, CCI and leaf anatomy reflected higher light stress, while g_s^{max} increased with height, reflecting the importance of gas exchange in the upper canopy layer. Leaf wettability, measured as drop contact angle, decreased from $85.5° \pm 1.6°$ (summer) to $57.5° \pm 2.8°$ (autumn) at CL_{28m} of the adult tree. At CL_{22m}, adult beech leaves seemed to be better optimized for photosynthesis than the CL_{28m} leaves because of a large leaf thickness with less protective and impregnated substances, and a higher CCI. The beech saplings, in contrast, did not adapt their stomatal characteristics and leaf anatomy according to the same strategy as the adult beech leaves. Consequently, care is needed when scaling up experimental results from seedlings to adult trees.

Abbreviations

Ab	Adaxial
Ad	Adaxial
CL_{xm}	Canopy level at x meter
CCI	Chlorophyll content index
DCA	Drop contact angle
Fv/Fm	Maximum quantum efficiency of photosystem II
g_s^{max}	Maximal stomatal conductance
K	Coefficient of palisadeness
Rs	Stomatal resistance
SPS	Stomatal pore surface

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Introduction

Plant leaves exchange gases and dissolved compounds with the atmosphere through a cuticular and a stomatal pathway. Leaf characteristics such as anatomical structure, pigment content, and stomatal apparatus are generally related to gaseous exchange processes, primarily photosynthesis, while cuticular properties mainly determine the exchange of dissolved compounds (Rennenberg and Gessler 1999). Stomata control the gas diffusion towards the leaf interior,

after which leaf anatomy, next to leaf morphology and biochemical factors, determines the internal conductance of carbon dioxide (CO_2) to the sites of carboxylation (Warren 2008). In process models, leaf parameters determining the stomatal pathway are mostly kept constant over a whole canopy, thereby considering the canopy to function as one big leaf (de Pury and Farquhar 1999). This big-leaf approach, however, often overestimates or underestimates canopy processes, and is more restricted to circumstances where structural complexity and canopy microclimate are irrelevant factors. For tree species such as European beech (*Fagus sylvatica* L.), where the self-shading effect of the leaves creates a high variation in irradiance, temperature, and humidity in the canopy (Niinemets and Valladares 2004; Herbinger et al. 2005), a multi-layer approach is more recommended.

For the cuticular pathway of canopy exchange, it is suggested to include canopy wetness in spatial modelling of pollutant biosphere–atmosphere exchange (Klemm et al. 2002). A thin water film on the cuticle decreases the value of cuticular resistance for the dry deposition of water-soluble gases (Gessler et al. 2000; Erisman and Draaijers 2003). Consequently, dry deposition rates to a wet canopy of atmospheric trace gases and pollutants with a high water solubility such as SO_2, NH_3, and HNO_3 (Klemm et al. 2002), but also of ozone (O_3), which is hardly soluble in water (Lamaud et al. 2002), are increased. Hence, the concentration gradient between the water layer and the leaf interior increases, promoting the uptake and/or leaching of those dissolved ions (Burkhardt et al. 2009). When cuticles are damaged, leaf wettability and permeability will increase, resulting in higher ion uptake rates by leaves (Adriaenssens et al. 2011) and enhanced foliar leaching of base cations (Tukey 1970). Water films also influence photosynthesis, as the diffusion of CO_2 through water is four orders of magnitudes slower than through air (Meidner 1986). In earlier research (Turunen and Huttunen 1990; Kupcinskiene and Huttunen 2005; Kardel et al. 2012), an increasing wettability of the leaves during the growing season was attributed to cumulative effects of natural and non-natural degradation and erosion of the waxy cuticle caused by weather conditions, insect damage, fungi, or atmospheric depositions.

Besides meteorological variables such as air humidity, pollutant concentrations and air turbulence patterns also vary from the top to the bottom of the canopy (Lovett and Lindberg 1992; Hansen 1996). This results in different levels of leaf acclimations between the canopy layers of a tree. Against stressors with a variable intensity along the canopy, e.g. O_3, leaves react with variable avoidance (e.g., by leaf anatomy and stomatal characteristics), defense (e.g., biochemical defense), compensation, and repair strategies (Kolb et al. 1997; Kolb and Matyssek 2001).

Microclimate has been pointed out as one of the factors influencing the defense capacity of beech leaves against oxidative stress caused by high irradiance, low temperatures, drought, or air pollution (Wieser et al. 2003). Thus, due to their location in the canopy and related specific microclimate, leaves differ in leaf anatomy (Terashima et al. 2006), leaf pigment and antioxidant concentrations (Herbinger et al. 2005), epicuticular waxes (Baker 1982), and derived parameters such as leaf wettability (Pandey and Nagar 2002) or stomatal (Eensalu et al. 2008) and internal (Terashima et al. 2006; Warren et al. 2007) leaf conductance. Leaf stress, caused by these different stressors, is often investigated by measuring the maximum quantum efficiency of photosystem II (Fv/Fm), which is inversely proportional to damage in the photosystem II reaction centres (Farquhar et al. 1989).

Some of these leaf characteristics and strategies are suggested to be related to tree size (and thus age), implying that scaling of leaf responses from seedlings to mature trees has several limitations due to the age effect (Kolb and Matyssek 2001; Wieser et al. 2003) and differences in microclimate (Kolb et al. 1997; Kolb and Matyssek 2001). Exploring the variations in these leaf parameters related to canopy processes could improve parameterization of multi-layer photosynthesis or canopy exchange models and give insight into the physiological complexity within one single tree crown.

In this study, the variation of morphological and physiological leaf properties related to gas exchange, photosynthesis, and the exchange of dissolved compounds were examined (1) along a vertical gradient within the canopy of an adult beech tree and (2) for beech saplings placed along this gradient. Thus, the beech saplings had developed their leaves under similar microclimatic conditions as the mature beech and it could be examined whether saplings responded to this environment by similar patterns as the adult tree. The aims of this research were (1) to assess the vertical variation in leaf wettability, stomatal characteristics, and leaf anatomy of this mature beech tree; (2) to assess the vertical variation of the same leaf characteristics for beech saplings placed along the vertical canopy gradient of the beech tree; and (3) to determine the seasonal changes in leaf wettability within the canopy, as former research pointed out the consequences of cumulative damage of the protecting wax layer. We hypothesized that leaves adapt to the specific microenvironment along a canopy gradient which is in first instance characterized by a light gradient. Leaf wettability is also expected to change during the season, dependent on location in the canopy. Beech saplings grown at the same atmospheric conditions within the canopy are believed not to adapt in a similar way as an adult beech.

Materials and methods

Study area

The study tree, a 29-m high dominant European beech tree was located in a mixed deciduous stand in the Aelmoese-neie experimental forest (50°58′N, 3°49′E, 16 m a.s.l.) near Ghent (north of Belgium, approximately 60 km from the North Sea in the prevailing wind direction). The forest has a total area of 28 ha and dominant trees were about 90 years old at the study time. The mixed stand is dominated by beech and pedunculate oak (*Quercus robur* L.) and had a summer maximum LAI value of 5.5 in 1996 (Mussche et al. 2001). Mean annual precipitation (1980–2008) at a nearby weather station was 784 mm, and distributed equally over the year, and mean annual temperature was 10.4 °C (Royal Meteorological Institute of Belgium). Average annual deposition fluxes to this forest amounted to 36 kg N ha^{-1} year^{-1} and 24 kg S ha^{-1} year^{-1} for the period 1992–2002 (Neirynck et al. 2004). Leaf sampling took place during the summer of 2008 from a scaffold measuring tower holding platforms at 7, 14, 21 and 28 m along the canopy. At canopy heights of 8, 15, 22 and 28 m, subsequently called Canopy Level (CL$_x$, with x the height), the leaves were collected. On each of these platforms, including the ground level, (GL$_{1m}$), two potted beech saplings were placed in March 2008, before leaf development, after which they were periodically watered in response to periods of drought. The 3-year-old saplings were grown in a nursery and were of the same known provenance. The saplings were potted in 15-l black polypropylene pots at the end of February 2008 in a mixture of peat and slow-releasing fertilizer (8.6 g N, 0.02 g P, and 0.08 g K kg^{-1} dry soil year^{-1}) in order to eliminate potential effects of varying soil nutrient availability on leaf characteristics. Field measurements and sample collection were carried out from 2 June until 3 November 2008. During this period, mean air relative humidity (Vaisala HMP143, Vaanta, Finland) at CL$_{28m}$ and GL$_{1m}$ were 75.5 and 85.9 %, respectively. Mean air temperature (Vaisala HMP143, Vaanta, Finland) increased from 13.6 °C (GL$_{1m}$) until 14.9 °C (CL$_{28m}$), while the largest difference between the minimum and maximum air temperature was found at CL$_{28m}$ (Table 1). Cumulative leaf wetness, i.e. the
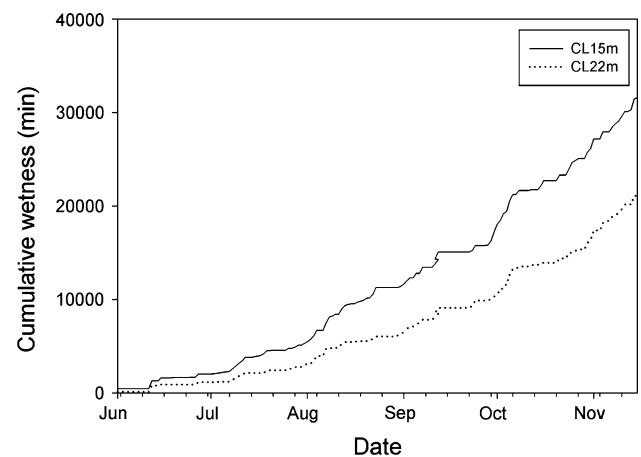
Table 1 Average, minimum, and maximum air temperature at ground level (GL) and different canopy levels (CL) from data registered every 20 s over the period 2 June–3 November 2008

Temperature (°C)	GL$_{1m}$	CL$_{8m}$	CL$_{15m}$	CL$_{22m}$	CL$_{28m}$
Average	13.6	14.4	14.5	14.7	14.9
Minimum	10.4	10.7	10.8	10.6	10.6
Maximum	17.0	18.3	18.6	19.1	20.5

Fig. 1 Cumulative leaf wetness duration (min) at canopy level (*CL*) 15 and 22 m over the measuring period. Values are based on average time of leaf wetness per day (min day^{-1})

cumulative time with wet leaves, was estimated by an electric grid surface wetness sensor (EE507-264, ELE International, Bedfordshire, UK) at CL$_{15m}$ and CL$_{22m}$ during the measuring period and also demonstrates the variation in microclimate along the vertical profile (Fig. 1).

Leaf parameters

Stomatal characteristics and maximal stomatal conductance (g_s^{max})

Stomatal imprints were taken on a sunny day (25 August 2008) by applying colourless nail varnish over an area of 2×3 cm^2 over de midrib on the abaxial leaf side (Kardel et al. 2010). For both tree ages, two branches on each CL were used to apply the nail varnish on each second, fifth, ninth and tenth leaf, counted from the terminal bud. Stomatal density and pore dimensions were measured by analysing the imprints with a light microscope connected to a camera (Olympus BX51, Tokyo, Japan) at a magnification of 40×10 and 100×10, respectively. Stomatal density was determined according to Balasooriya et al. (2009) and stomatal pore surface (SPS, μm^2) was calculated for widely opened stomata based on the assumption of elliptical stomatal pores:

$$SPS = 0.25 \cdot (L \cdot W \cdot \pi) \tag{1}$$

with L the stomatal pore length (μm) and W the stomatal pore width (μm). The model by Parlange and Waggoner (1970) was applied to calculate the theoretical maximum stomatal conductance (g_s^{max}) of water vapour, which is the inverse of the minimal stomatal resistance R_s (Balasooriya et al. 2009; Kardel et al. 2010). These obtained g_s^{max} values give theoretical maxima, as stomata do not operate at their maximum opening, neither all at the same time.

Leaf anatomy

Leaf anatomy was characterized by the thickness of the different leaf tissue layers, the fraction of palisade parenchyma in the total mesophyll layer, and the presence of phenolic compounds. On 11 August 2008, ten samples from both tree ages per CL were collected, distributed over two saplings or two adult beech branches. Segments of 9 mm diameter were punched out between the main vein and the leaf border in the middle of the leaf and fixed in FAA (formalin-acetic-alcohol, ethanol/acetic acid/formaldehyde (37–40 %)/distilled water 10:1:2:7). Next, the leaf tissues were dehydrated by placing them in a sequence of 35–50–65–80–90–100 % ethanol solution, followed by a 100 % isopropanol solution and an isopropanol/chloroform solution with consecutive ratios of 3:1, 1:1 and 1:3. Each step lasted one day. Then, leaf segments were put in the oven at 40 °C with paraffin and chloroform until the paraffin was melted and the chloroform evaporated, after which they were embedded in paraffin. Finally, cross sections were made with a rotation microtome (Jung, Heidelberg, Germany), and were coloured with safranin and astra blue. The thickness of the upper epidermis, palisade parenchyma, spongy parenchyma, and the lower epidermis were determined with a light microscope at a magnification of 20×10. The coefficient of palisadeness (K) was calculated as the percentage ratio of the thickness of the palisade parenchym (Rp) to the mesophyll (Rm). This coefficient is an index of the rate of gas exchange in plant leaves, whereby a lower K indicates a higher rate of gas exchange (Dineva 2006). Presence of phenolic compounds were not quantified but were examined by affinity for safranin (Reig-Arminana et al. 2004). Leaf anatomy was not determined at CL_{15m} because of time limitation and the relatively small expected difference between CL_{8m} and CL_{15m}.

Chlorophyll content index and chlorophyll fluorescence

Relative chlorophyll content was quantified as the chlorophyll content index (CCI), measured with the CCM-220 (Chlorophyll Content Meter, Opti-Sciences Inc., Hudson, USA). For both the adult tree and the saplings, ten fully developed, undamaged leaves were used at each CL to measure CCI on 19 August 2008. For each leaf, three measurements on different locations were taken, avoiding the mid-vein.

A portable fluorometer Plant Efficiency Analyser (Hansatech Instruments, Norfolk, UK) was used to determine the parameters of rapid kinetics of chlorophyll a fluorescence at the adaxial leaf side. For each CL, ten leaves of the adult tree and of the saplings were measured on 10 October 2008. A Kautsky induction curve measurement was performed by applying an irradiance of

3,290 µmol m^{-2} s^{-1} to each leaf after 25 min of dark adaptation with a leaf clip. The maximum quantum efficiency of photosystem II (Fv/Fm) was determined as the ratio of the variable fluorescence (Fv) to the maximum fluorescence at light saturation (Fm).

Drop contact angle

Leaf wettability was determined on both the adaxial (ad) and the abaxial (ab) side of a leaf by measuring the contact angle between a drop (drop contact angle, DCA) with a standardized volume and the leaf surface. This parameter indicates the hydrophobicity of the leaf surface determined by the physical and chemical composition of the cuticle (Holloway 1969). On each CL, ten fully developed, undamaged leaves from two beech saplings or two branches of the adult beech were collected and transported to the laboratory with the petiole wrapped in wet paper. Images were taken in laboratory conditions with a Canon 5D digital camera and macro objective (Sigma EX 105 mm, Setagaya-ku, Japan) within 30 s after placing a drop of 7.5 µl deionized water with a micropipette on the middle part of the horizontal leaf surface. DCA_{ad} and DCA_{ab} were measured with ImageJ software (http://rsbweb.nih.gov/ij/) as the average of the contact angles on the left and right side of the drop. Leaf wettability was measured in June, August, and October over the growing season to investigate its seasonal variation.

Statistical analysis

Statistical analysis was performed with S-PLUS 8.0 and SPSS 17 software. The modified Levene test (S-PLUS) was used to test homoscedasticity and determined the further statistical process. Analyses of variance (ANOVA) were applied to investigate the effects of CL (for stomatal parameters, leaf anatomical parameters, and Fv/Fm), and also sampling time in case of DCA. In case of significant effects, post hoc comparisons were performed in order to detect the variation pattern of each leaf parameter. Tukey's honestly significant difference test (CCI), Dunnett's C test (g_s^{max}), or both tests (stomatal parameters, DCA) were used. The differences between means at different sampling times were investigated by Tukey's honestly significant difference test (DCA). For all analyses a significance level of $P < 0.05$ was used.

Results

Stomatal characteristics and maximal stomatal conductance (g_s^{max})

For the adult beech, the stomatal characteristics changed significantly between the different canopy levels (Table 2).

Table 2 Mean stomatal characteristics (g_s^{max}: theoretical maximal stomatal conductance), cross-sectional thickness of anatomical leaf layers, and coefficient of palisadeness (K) of the leaves of an adult beech tree at different canopy levels (CL) and of beech saplings at ground level (GL) and different CLs

Parameter	GL$_{1m}$		CL$_{8m}$		CL$_{15m}$		CL$_{22m}$		CL$_{28m}$	
	Sapling	Adult	Sapling	Adult	Sapling	Adult	Sapling	Adult	Sapling	Adult
Stomatal characteristic ($n = 8$)										
Length (µm)	10.4[b]	–	9.5[a]	10.6[a]	10.1[ab]	10.9[a]	10.4[b]	10.9[a]	9.5[a]	12.2[b]
Width (µm)	5.3[b]	–	5.3[b]	6.0[a]	5.7[c]	6.0[a]	5.4[bc]	5.8[a]	4.8[a]	6.6[b]
Stomatal pore surface (µm^2)	43.5[b]	–	39.6[ab]	50.0[a]	45.2[b]	51.5[a]	44.1[b]	50.0[a]	36.0[a]	64.0[b]
Density (mm^{-2})	420[c]	–	416[bc]	154[a]	399[bc]	223[b]	386[ab]	268[c]	364[a]	229[b]
g_s^{max} (mol H$_2$O m^{-2} s^{-1})	1.84[b]	–	1.66[b]	0.77[a]	1.81[b]	1.16[b]	1.71[b]	1.35[c]	1.32[a]	1.47[d]
Thickness (µm) ($n = 10$)										
Upper epidermis	11.94[b]	–	11.28[a]	11.23[b]	n.d.	n.d.	11.23[a]	14.32[c]	10.79[a]	9.10[a]
Palisade parenchyma	22.24[a]	–	24.66[b]	26.49[a]	n.d.	n.d.	29.27[c]	28.51[b]	39.95[d]	26.97[a]
Spongy parenchyma	37.16[ab]	–	36.41[a]	32.84[a]	n.d.	n.d.	41.15[b]	51.43[c]	46.55[c]	45.20[b]
Lower epidermis	10.29[a]	–	9.56[a]	10.51[b]	n.d.	n.d.	10.07[a]	11.45[c]	10.64[a]	8.24[a]
Total	81.63[a]	–	81.91[a]	81.71[a]	n.d.	n.d.	91.72[b]	105.72[c]	107.93[c]	89.51[b]
Coefficient of palisadeness (%)	37.6[a]	–	40.7[b]	45.0[b]	n.d.	n.d.	41.6[b]	35.8[a]	46.5[c]	37.3[a]

Different lowercase letters within a row indicate significant ($P < 0.05$) differences in leaf characteristics between different CLs for a given age

n number of leaves for both ages at each CL, – no adult leaves at ground level, *n.d.* not determined

At CL$_{28m}$, the stomatal pore surface of the adult tree was significantly higher compared to all other CLs, while stomatal density increased significantly from CL$_{8m}$ to CL$_{22m}$. These significant changes in stomatal characteristics of the adult beech resulted in a significant increase of g_s^{max} with CL from 0.77 mol H$_2$O m^{-2} s^{-1} at CL$_{8m}$ to 1.47 mol H$_2$O m^{-2} s^{-1} at CL$_{28m}$. For the beech saplings, less significant variation was found along the canopy profile. Except that both the stomatal pore surface and stomatal density were significantly lower at the top compared to most of the lower CLs. This led to a significantly lower g_s^{max} at the top level compared to all lower levels.

Leaf anatomy

The adult tree developed the thickest leaf at CL$_{22m}$ (Table 2; Fig. 2), where all histological layers were significantly thicker compared to the other CLs. The coefficient of palisadeness (K) was lowest at CL$_{22m}$ (36 %), but not significantly lower than the value at CL$_{28m}$. In contrast, total leaf thickness of the beech saplings significantly increased with CL to a total thickness of 108 µm at CL$_{28m}$. However, these thickest sapling leaves showed the highest mean K value (47 %) at the top level, thus forming a higher fraction of palisade parenchyma. Consequently, the relation of K to the total leaf thickness differed between the adult tree and the saplings (Fig. 3). For the sapling leaves the thickness of both the upper and lower epidermis was similar at the different CLs, in contrast to the adult tree. At 22 m, the adult tree leaves developed the thickest upper

and lower epidermis, while the top leaves at 28 m showed significantly thinner epidermises (Table 2). The adult tree leaves at the lowest CL showed in-between values for epidermal thickness. Cells of the top leaves of the adult tree seemed to have a denser content with a high affinity for the reddish safranin. The mesophyll cells of the top adult tree leaves also had a tendency towards more irregular angular-shaped cells than those of the lower CLs. In addition, a thickening of the cuticle (also with a high affinity for the reddish safranin) could be observed for leaves at CL$_{22m}$ and CL$_{28m}$, both for the saplings and the adult tree.

Chlorophyll content index and chlorophyll fluorescence

Along the vertical gradient, the adult tree had an increasing CCI until CL$_{22m}$ (25.0 ± 0.4; mean $\pm$ standard error) (Fig. 4). For the saplings this maximum was situated at CL$_{15m}$ (24.1 ± 0.4). The lowest CCI was measured at the top level for both ages, 13.3 ± 0.4 and 9.8 ± 0.3 for the adult and the saplings, respectively.

The Fv/Fm parameter showed also a similar trend for both tree ages, whereby a significantly lower Fv/Fm value was observed at 28 m compared to the lower CLs (Fig. 5).

Drop contact angle

Along the vertical gradient there were clear differences in DCA for both ages and both leaf sides (Table 3). During summer (29 August), the leaves of the saplings showed the highest adaxial and abaxial DCAs at CL$_{22m}$ and CL$_{28m}$.
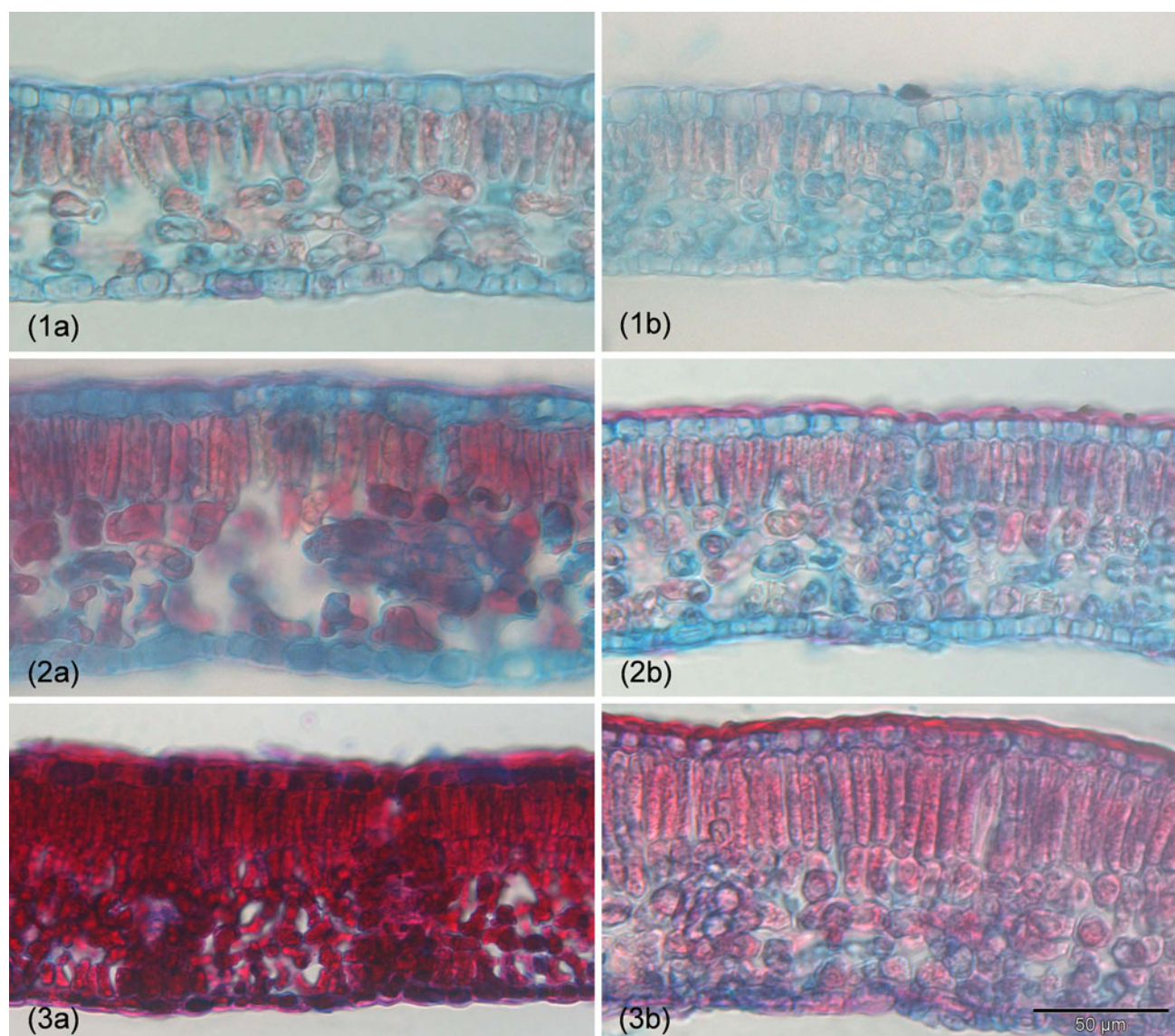
Fig. 2 Cross section of the leaves (magnification ×200) at canopy levels CL_{8m} (**1**), CL_{22m} (**2**) and CL_{28m} (**3**) of a mature beech tree (**a**) and beech saplings (**b**)

This also held true for the adaxial leaf sides of the adult tree. During autumn (21 October), the DCA_{ad} were overall significantly lower compared to spring measurements (15 June), except at CL_{22m} for the adult tree and at CL_{28m} for the saplings. The DCA_{ad} measured at CL_{28m} showed the largest decline, from $82.6° \pm 0.9°$ (summer) to $61.6° \pm 3.5°$ (autumn) for the saplings and from $85.5° \pm 1.6°$ (summer) to $57.5° \pm 2.8°$ (autumn) for the adult tree. As a consequence of this decline at the top CL, the autumn DCA_{ad} was highest at the canopy level at 22 m for both ages, where the DCA_{ad} also was recorded high during summer measurements.

For DCA_{ab}, less significant variation existed through time, so DCA_{ab} was generally higher than DCA_{ad} at the end of the growing season. From summer to autumn DCA_{ab} at CL_{28m} however declined significantly from $90.7° \pm 2.5°$ to

$75.8° \pm 3.4°$ for the saplings and from $84.7° \pm 2.9°$ to $72.0° \pm 4.9°$ for the adult tree, showing a similar behaviour as for the adaxial leaf side. During summer measurements, DCA_{ab} did not differ significantly along the vertical gradient of the adult tree. However, due to the decline at the top level the autumn DCA_{ab} was significantly lower here compared to the lower CLs for both ages.

Discussion

Leaf parameter variability within the adult beech

The first aim of this research was to assess the vertical variation of several leaf characteristics along the canopy of
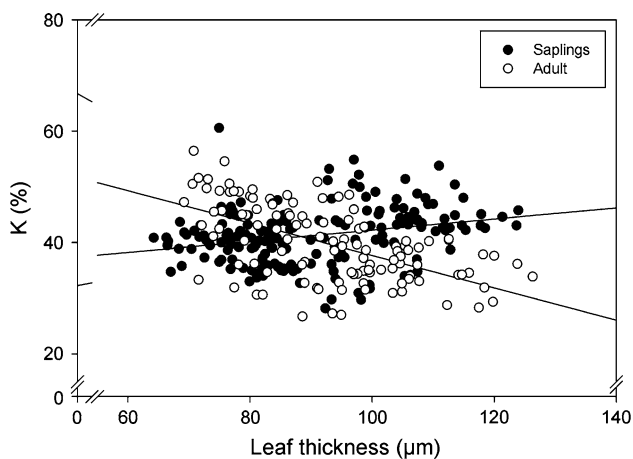
Fig. 3 Coefficient of palisadeness (K) in relation to leaf thickness for both an adult beech tree ($r^2 = 0.34$, $P < 0.0001$) and beech saplings ($r^2 = 0.10$, $P < 0.0001$) for all canopy levels considered
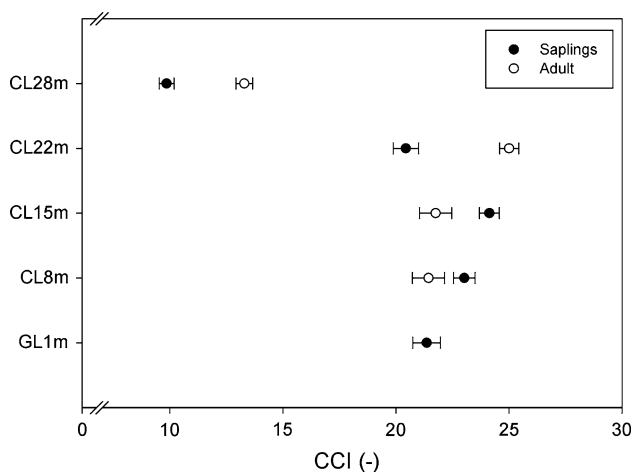

Fig. 4 Mean chlorophyll content index (CCI; 19 August 2008) of the leaves of an adult beech tree at different canopy levels (*CL*) and of beech saplings at ground level (*GL*) and different *CL*s. *Error bars* indicate standard error ($n = 30$)

a mature beech tree. Hereby we consider, apart from the top leaves at CL_{28m}, also the leaves at CL_{22m} as 'sun' leaves, as direct sunlight is still arriving at some locations due to the scaffolding tower standing close to the tree.

Stomatal characteristics and maximal stomatal conductance (g_s^{max})

For stomatal characteristics it is well known that they react plastically to their environment, thereby adapting to environmental factors such as light, temperature, water availability, and atmospheric pollutant concentrations (Eensalu et al. 2008; Kardel et al. 2010; Wuytack et al. 2010). While previous reports mainly focused on the comparison between sun and shade leaves, the present study illustrates the gradual increase of the theoretical conductance (g_s^{max})
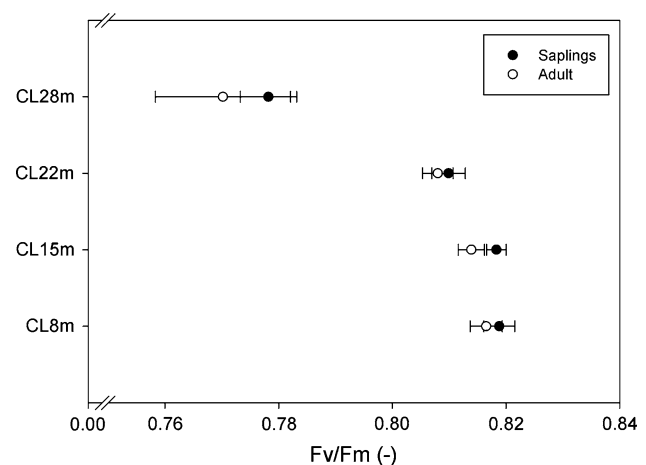

Fig. 5 Mean Fv/Fm value (10 October 2008) of the leaves of an adult beech tree and beech saplings at different canopy levels (*CL*). *Error bars* indicate standard error ($n = 10$)

along four CLs in a mature beech canopy. The observed higher g_s^{max} of sun leaves compared to shade leaves is consistent with other reported findings on stomatal conductance based on stomatal dimensions (e.g., *Betula pendula* (Eensalu et al. 2008) or gas exchange measurements (e.g., *Fagus sylvatica* (Herbinger et al. 2005). The reason for an increasing g_s^{max} from CL_{8m} to CL_{22m} is due to an increasing stomatal density, as also observed by Eensalu et al. (2008), while the further g_s^{max} increase at the upper CL is attributed to the significant enlargement of the stomatal pore surface. Nevertheless the high irradiance and high water vapour pressure deficit at this top level, leaves do not invest in smaller and thus better controllable stomatal pores (besides a higher stomatal density). This suggests the major importance of gas exchange processes at the upper CL. Apart from that, an increasing g_s^{max} trend enhances transpiration. As leaf physiology is closely linked to the hydraulic architecture of a tree (Meinzer et al. 2001), this increased potential for transpiration with height might have a mitigating effect against the increasing aboveground plant hydraulic resistance with tree height.

CCI and chlorophyll fluorescence

At CL_{28m} the maximum quantum efficiency of photosystem II (Fv/Fm) was significantly lower compared to the lower CLs near the end of the growing season (Fig. 5), indicating an irreversible photoinhibition of photosystem II for the leaves at this top level. This is explained by the fact that the primary site of attack in stress due to strong light is the reaction centre of photosystem II whereby its efficiency is reduced (Larcher 2003). Besides photoinhibition stress, also heat and water stress co-occur at upper canopy layers, which can restrict the photosynthetic capacity of those leaves (Niinemets 2007). Moreover, also atmospheric

Table 3 Mean drop contact angle (DCA, °) at the adaxial (ad) and abaxial (ab) side of the leaves of an adult beech tree at different canopy levels (CL) and of beech saplings at ground level (GL) and at different CLs

	15 June		29 August		21 October	
	Sapling	Adult	Sapling	Adult	Sapling	Adult
DCA_{ad}						
GL_{1m}	86.7[bB]	–	60.2[aA]	–	66.6[bcA]	–
CL_{8m}	87.1[bC]	86.6[cB]	60.3[aB]	59.6[aA]	51.2[aA]	54.5[aA]
CL_{15m}	69.7[aB]	74.4[abB]	67.9[aAB]	65.9[abAB]	57.0[abA]	58.5[aA]
CL_{22m}	82.7[bB]	73.5[aA]	85.4[bB]	69.8[bA]	72.4[cA]	75.1[bA]
CL_{28m}	73.4[abB]	78.0[bcB]	82.6[bB]	85.5[cC]	61.6[bA]	57.5[aA]
DCA_{ab}						
GL_{1m}	91.7[cB]	–	77.2[aA]	–	86.8[bAB]	–
CL_{8m}	76.3[aA]	69.5[aA]	88.6[abB]	86.5[aB]	81.0[bAB]	84.1[bAB]
CL_{15m}	74.6[aA]	86.4[bA]	83.3[aB]	82.8[aA]	84.3[bB]	84.1[bA]
CL_{22m}	89.3[bcA]	90.0[bA]	97.7[cB]	85.1[aA]	83.5[bA]	87.0[bA]
CL_{28m}	80.0[abA]	81.7[bAB]	90.7[bB]	84.7[aB]	75.8[aA]	72.0[aA]

Different lowercase letters within a column indicate significant ($P < 0.05$) differences in DCA_{ad} or DCA_{ab} between different CLs per measurement time; different capital letters within a row indicate significant ($P < 0.05$) differences in DCA_{ad} or DCA_{ab} for sapling or adult between the different measuring times

– No adult leaves at ground level

turbulence and boundary layer conductance are higher at the top of the canopy, which causes a higher water vapour pressure deficit (Niinemets and Valladares 2004). For the relative chlorophyll content, we found that leaves at CL_{22m} had higher values than the leaves at the lower more shaded CLs. This is in accordance with Hallik et al. (2009) who found a slightly positive or insignificant correlation between current light availability and chlorophyll content per leaf area for three temperate deciduous trees, and with Lichtenthaler et al. (2007) who found a higher chlorophyll content per leaf area for sun leaves compared to shade leaves of deciduous trees. However, in the latter study sun leaves of European beech had a slightly lower chlorophyll content per leaf area than shade leaves. In fact, this observation of Lichtenthaler et al. (2007) corresponds to our observation of the top leaves at CL_{28m}, where CCI is clearly lowest of all CLs. This could be explained by photoinhibition stress, whereby chloroplast pigments can be destroyed due to oxygen species that accumulate (Larcher 2003).

Leaf anatomy

Also leaf anatomy strongly adapts under these high sun-exposed conditions (Figs. 2, 3a). First, increasing cell wall thickness and tighter packing of mesophyll cells are a strategy to limit excessive transpiration by higher water use efficiency (Bussotti 2008). Warren (2008) found that sun leaves of *F. sylvatica* have lower mesophyll porosity but on the other hand, a higher internal CO_2 diffusion conductance

compared to shade leaves. They concluded that the liquid-phase conductance through the mesophyll cell walls limits internal leaf conductance more than the gas-phase conductance in the intercellular spaces. Secondly, lower leaf porosity can also be seen as a limitation for easily performable gas-phase diffusion (Dineva 2004) and thus an avoidance or acclimation leaf strategy to pollutant gases such as O_3 in environments subjected to oxidative stress (Bussotti 2008). Also characteristic for leaves under influence of drought, O_3 (Bussotti et al. 1998; Reig-Arminana et al. 2004), or air pollution in general (Gostin 2009) is the presence of tannins and antioxidants acting as biochemical foliar defense capacities near the top of the canopy. The reddish safranin staining, clearly visible in the leaves at CL_{28m} (Fig. 2), indicates the presence of (ligni-fied) substances which are generally related to secondary cell walls. Because almost no secondary cell walls occur in leaves, this staining is designated to phenolics and other antioxidants which occur in vacuoles and cell walls (R. Caubergs, University of Antwerp, personal communication). For beech, tannins are the phenolics that have been identified as the main substances which impregnate leaves under stress conditions (Bussotti et al. 1998; Grossoni et al. 1998) and have an affinity with safranin (Reig-Arminana et al. 2004). This observation is in accordance with other studies showing accumulated antioxidants or other protective components at higher canopy heights (Hansen et al. 2002; Wieser et al. 2003) due to a higher requirement for photoprotection of leaves exposed to higher (UV) irradi-ances (Herbinger et al. 2005). The thickest leaves of the

adult tree were found at CL_{22m} (Fig. 2a; Table 2). In general, thick leaves have a high photosynthetic potential and lower light-saturated rate of leaf photosynthesis per unit area (Gerosa et al. 2003). While the palisade parenchyma acts as a light guide, the diffusible spongy tissue scatters and re-orients the light, hereby lengthening the light path and increasing the probability of light interception by a chloroplast and absorption for photosynthesis (Vogelmann et al. 1996). We assume that the co-occurring stresses at the top level are less severe, and thus have a smaller impact on the leaves at CL_{22m}, while on the other hand light intensity at this CL is still high so that leaves can invest more in photosynthetic components rather than protective walls and structures, which is the case for the leaves at CL_{28m} (Figs. 2, 3a).

Leaf wettability and leaf wetness

At each measuring moment, leaf wettability at the adaxial leaf side is clearly variable along the canopy gradient of the adult tree (Table 3), e.g. during June and August, the higher DCA_{ad} at CL_{28m} of the adult tree suggests a higher water repellency of the fully sun-exposed leaves. This is probably due to the formation of a thicker, more water-repellant cuticle (Fig. 2). It is indeed well known that leaves more exposed to direct sunlight and wind speed will protect themselves against the negative influences of, e.g. (UV) radiation, and excessive transpiration by developing a thicker, and often more water-repellant cuticle (Baker 1982), which results in a higher DCA (Pandey and Nagar 2002). Further, our findings of a higher duration of leaf wetness (min day^{-1}) in the middle canopy (Fig. 1) compared to the upper canopy illustrate microclimate variation in the canopy, which is concordant with Dietz et al. (2007) who found a similar result for a tropical montane forest after rainfall events. These observations thus confirm the hypothesis of Burkhardt et al. (2009) who suggested that a vertical gradient in leaf surface wetness can be expected since leaf surfaces at different heights within the canopy are differently affected by ambient atmospheric humidity, precipitation, soil evaporation, and plant transpiration. Leaves at CL_{15m}, which remain wet longer than those at CL_{22m} (Fig. 1) generally have a significantly lower DCA_{ad} (Table 3). Both parameters, leaf wettability and duration of leaf wetness, are thus influenced by microclimate and could affect the exchange of dissolved compounds. In a joint research, DCA_{ad} was found to be negatively correlated with foliar uptake of ^{15}N-labelled ammonium and nitrate originating from wet deposition for different tree sapling species and phenological stages (Adriaenssens et al. 2011), suggesting a higher importance of this foliar N uptake in lower crown layers.

Leaf parameter response of the saplings along the canopy gradient

For most species, adult trees exhibit a lower stomatal conductance than seedlings of the same species when height differences are at least 4 m, which is explained by the difference in water availability between shallow-rooted saplings and more deep-rooted mature trees (Kolb et al. 1997; Kolb and Matyssek 2001). Although the beech saplings were provided with water according to drought periods, watering was not sufficient at the top CL which led to drought stress. In addition to a higher atmospheric drought caused by higher wind speed and irradiation, the saplings also experienced a soil drought. Effects of this drought were seen during the experiments by relatively drier leaves and drier pot soil, which can be considered as a limitation for the beech saplings to better adapt to this environment. In contrast to the adult beech tree where maximal g_s^{max} was found at CL_{28m}, g_s^{max} of the saplings showed a significantly lower value at CL_{28m} compared to the saplings on the lower CLs. The minimal g_s^{max} of the samplings at CL_{28m} is both due to a low stomatal density and a lower stomatal pore surface (Table 2). Small stomata have the advantage of closing faster than larger stomata, which results in greater stomatal control, e.g. in case of gas diffusion control (Balasooriya et al. 2009). Between GL_{1m} and CL_{22m} there were no significant differences in g_s^{max} for the saplings, which indicates that their stomata did not adapt in a similar way to the differences in microclimate as the mature beech did. Besides the influence of water supply at the top level, intrinsic (hormonal) signals, driven by growth conditions from the previous growing season, also play an important role in the development of stomata (Lake et al. 2001). We assume that this is one of the reasons why the stomata of the saplings did not adapt to the canopy gradient during the first growing season in this environment.

The CCI results of the saplings show the lowest relative chlorophyll content at CL_{28m}. Less photosynthetic pigments in sun-exposed leaves compared to shade-exposed leaves have also been demonstrated for beech saplings grown at higher crown positions in a similar setup (Wieser et al. 2003). The dissimilarity with the adult beech was that the maximum CCI for the saplings was observed at CL_{15m} instead of at higher CLs. This indicates that the saplings, placed on the platforms before the start of the growing season, had an optimal chlorophyll content at lower and cooler CLs compared to the adult beech. This observation might suggest that, as beech is a shade-tolerant and late-succession species, the development of beech saplings is better adapted to shaded environments.

Similar to the CCI, the leaf anatomy of the beech saplings followed a different trend compared to the adult tree. The saplings developed a higher fraction of palisade

parenchyma (K) at CL_{28m} (Table 2), where also the lowest Fv/Fm value was found. The cross sections of the saplings at CL_{28m} (Figs. 2, 3b) show that, besides a thicker cuticle also phenolic substances are produced although less dominant than for the adult tree. In other studies with beech saplings, environmental stress (wind action, nutritional deficiencies, high levels of solar radiation, and high O_3 levels) was also determined as a cause for increasing the thickness of both the cutinized layer of the cuticle and the (palisade) mesophyll as well as for the increased production of phenolics (Bussotti et al. 1998; Grossoni et al. 1998). The thicker leaves with a high K value due to an increase in palisade thickness at this top level can be seen as a xeromorphic adaptation to protect the leaf against high radiation and to reduce water loss by transpiration. However, the higher drought stress originating from the soil of the potted saplings is considered as an extra stress factor which could have triggered this xeromorphic adaptation. Since in this study, although not statistically tested, clear differences in leaf anatomy could be observed between the adult tree and the beech saplings, care should be taken in extrapolating the results of studies on saplings to adult trees.

With respect to leaf wettability, the saplings followed the same trend as the adult beech with the highest DCA at 22 and 28 m in August on both leaf sides and the highest DCA_{ad} at 22 m in autumn (Table 3).

Seasonal effect on leaf wettability for both ages

In summer, when the leaves are fully developed, we found that the highest DCA_{ad} are at the two upper canopy levels. This can be explained by the higher light intensity. However, several events can alter the leaf wettability through time. First, differences in air temperature, relative air humidity, soil moisture and irradiance (Baker 1982), and the frequency of precipitation events (Brewer 1996) can modify the epicuticular waxes. A higher wetness duration causes a longer interaction time between the leaf cuticle and the (hygroscopic) ions deposited on it, which could lead to additional physicochemical degradations of the waxes and a higher cuticle permeability (Burkhardt and Eiden 1994; Cape 2008). The higher accumulated leaf wetness duration at CL_{15m} might, therefore, at least partially explain the decrease in DCA_{ad} from summer to autumn. However, DCA_{ad} not only decreased significantly from spring to autumn at CL_{15m}, but also at CL_{8m} and CL_{28m} for the adult tree. At CL_{8m} the explanation of longer wetness duration might also hold true, while at CL_{28m}, leaves will dry faster because of a higher air temperature (Table 1) and wind velocity. Secondly, atmospheric turbulence and boundary layer conductance are higher at the top of the canopy, which can enhance the dry deposition of particles and gases (Lovett and Lindberg 1992; Hansen

1996). This gradient of increasing accumulated components with height also corresponds to the extent of leaf damage by several air pollutants (Fritsche et al. 1989). A decrease in DCA_{ad} at the top level can thus be explained by either a higher physical erosion of epicuticular waxes due to rain and wind forces, or a chemical degradation due to deposition of hygroscopic salt particles (Burkhardt 2010) or air pollutants (Schreuder et al. 2001; Padgett et al. 2009). At CL_{22m} the DCA_{ad} was highest for both tree ages in autumn and did not change significantly through time for the adult tree. This can be explained by the development of a thicker waxy cuticle, better resistant to degradation, near the highly irradiated top levels CL_{22m} and CL_{28m} on the one hand (Fig. 2), and less severe wax degradation at CL_{22m} compared to CL_{28m} on the other hand. We assume that a higher decrease in DCA_{ad} compared to DCA_{ab} during the whole growing season is due to a higher chemical and physical degradation of the waxes on the upper leaf side. Furthermore, it is likely that leaves develop a lower wettability on their abaxial side to enhance the diffusion of CO_2 through the stomata, while this is less important at the adaxial side lacking stomata. It can be expected that species with stomata on both sides of the leaf exhibit a different strategy. Our results suggest a higher adaxial cuticle permeability towards the end of the growing season, which could further influence nitrogen uptake and the leaching of K^+ (Sase et al. 2008) or other base cations from forest canopy leaves (Hansen 1996). The relationship between foliar uptake of wet deposited N and leaf surface properties at different canopy heights, as well as its seasonal dynamics, still remains to be investigated. Consequently, both the adult tree and the saplings show similar trends on development and degradation of the waxy cuticle along the canopy, which implicates that microclimatic factors such as irradiation (cuticle development), physical abrasion, and damage by deposition processes (cuticle degradation) at the specific canopy level are determining this parameter, and not plant size or age related factors.

Conclusions

This study demonstrated that leaf characteristics related to photosynthesis and the exchange of gases and dissolved compounds vary significantly between different canopy layers of an adult beech tree. The theoretical stomatal conductance gradually increased with height in the beech canopy, emphasizing the importance of gas exchange in higher canopy layers. However, other leaf parameters, e.g., leaf anatomy, Fv/Fm, CCI, and leaf components protecting against oxidative stress, reflected high microclimatic stress at the top level of the 28 m high beech. The leaves at CL_{22m}, which can be considered as 'sun' leaves as well,

diverged from those at CL_{28m} by developing thicker leaves, less protective leaf compounds, and a higher CCI. These different leaf characteristics point out that the most efficient canopy layer for photosynthesis is below the top, as in this layer the extreme conditions experienced at the top are less severe, and light intensities are still high. Further, it can be concluded that leaf wettability at the adaxial leaf side varies along a canopy gradient, and also throughout the growing season. Apart from the wettability parameter, the beech saplings seem to maintain optimal leaf properties in lower and thus cooler environments compared to the adult tree. The different responses in leaf anatomy and in stomatal characteristics of the beech saplings to their microenvironment, compared to adult beech leaves, underline that scaling of leaf responses from beech saplings to mature trees should be done with caution. We conclude that the observed variability in leaf parameters (1) along a canopy gradient, and (2) through the growing season might have an impact on processes like photosynthesis and canopy exchange and should thus be incorporated in mechanistic tree or forest models.

References

Adriaenssens S, Staelens J, Wuyts K, De Schrijver A, Van Wittenberghe S, Wuytack T, Kardel F, Verheyen K, Samson R, Boeckx P (2011) Foliar nitrogen uptake from wet deposition and the relation with leaf wettability and water storage capacity. Water Air Soil Pollut 219:43–57

Baker EA (1982) Chemistry and morphology of plant epicuticular waxes. In: Cutler DJ, Alvin KL, Price CE (eds) The plant cuticle. Academic Press, London, pp 139–165

Balasooriya BLWK, Samson R, Mbikwa F, Vitharana UWA, Boeckx P, Van Meirvenne M (2009) Biomonitoring of urban habitat quality by anatomical and chemical leaf characteristics. Environ Exp Bot 65:386–394

Brewer CA (1996) What's so bad about being wet all over—investigating leaf surface wetness. Am Biol Teach 58:414–417

Burkhardt J, Eiden R (1994) Thin water films on coniferous needles. Atmos Environ 28:2001–2011

Burkhardt J, Flechard CR, Gresens F, Mattsson M, Jongejan PAC, Erisman JW, Weidinger T, Meszaros R, Nemitz E, Sutton MA (2009) Modelling the dynamic chemical interactions of atmospheric ammonia with leaf surface wetness in a managed grassland canopy. Biogeosciences 6:67–84

Burkhardt J (2010) Hygroscopic particles on leaves: nutrients or desiccants? Ecol Monogr 80:369–399

Bussotti F (2008) Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: a critical overview. Glob Change Biol 14:2727–2739

Bussotti F, Gravano E, Grossoni P, Tani C (1998) Occurrence of tannins in leaves of beech trees (Fagus sylvatica) along an ecological gradient, detected by histochemical and ultrastructural analyses. New Phytol 138:469–479

Cape JN (2008) Interactions of forests with secondary air pollutants: some challenges for future research. Environ Pollut 155:391–397

de Pury DGG, Farquhar GD (1999) A commentary on the use of a sun/shade model to scale from the leaf to a canopy. Agric Forest Meteorol 95:257–260

Dietz J, Leuschner C, Holscher D, Kreilein H (2007) Vertical patterns and duration of surface wetness in an old-growth tropical montane forest, Indonesia. Flora 202:111–117

Dineva SB (2004) Comparative studies on the leaf morphology and structure of white ash Fraxinus americana L. and London plane tree Platanus acerifolia Willd growing in pollutes area. Dendrobiology 52:3–8

Dineva SB (2006) Development of the leaf blades of Acer platanoides in industrially contaminated environment. Dendrobiology 52:3–8

Eensalu E, Kupper P, Sellin A, Rahi M, Sober A, Kull O (2008) Do stomata operate at the same relative opening range along a canopy profile of Betula pendula? Funct Plant Biol 35:103–110

Erisman JW, Draaijers G (2003) Deposition to forests in Europe: most important factors influencing dry deposition and models used for generalisation. Environ Pollut 124:379–388

Farquhar GD, Wong SC, Evans JR, Hubick KT (1989) Photosynthesis and gas exchange. In: Jones HG, Flowers TJ, Jones MB (eds) Plants under stress. Cambridge University Press, Cambridge, pp 47–69

Fritsche U, Gernert M, Schindler C (1989) Vertical profiles of air-pollutants in a spruce forest—analysis of adherent water, throughfall and deposits on surrogate surfaces. Atmos Environ 23:1807–1814

Gerosa G, Marzuoli R, Bussotti F, Pancrazi M, Ballarin-Denti A (2003) Ozone sensitivity of Fagus sylvatica and Fraxinus excelsior young trees in relation to leaf structure and foliar ozone uptake. Environ Pollut 125:91–98

Gessler A, Rienks M, Rennenberg H (2000) NH_3 and NO_2 fluxes between beech trees and the atmosphere—correlation with climatic and physiological parameters. New Phytol 147:539–560

Gostin IN (2009) Air pollution effects on the leaf structure of some Fabaceae species. Not Bot Hortic Agrobot 37:57–63

Grossoni P, Bussotti F, Tani C, Gravano E, Santarelli S, Bottacci A (1998) Morpho-anatomical alterations in leaves of Fagus sylvatica L. and Quercus ilex L. in different environmental stress condition. Chemosphere 36:919–924

Hallik L, Kull O, Niinemets U, Aan A (2009) Contrasting correlation networks between leaf structure, nitrogen and chlorophyll in herbaceous and woody canopies. Basic Appl Ecol 10:309–318

Hansen K (1996) In-canopy throughfall measurements of ion fluxes in Norway spruce. Atmos Environ 30:4065–4076

Hansen U, Fiedler B, Rank B (2002) Variation of pigment composition and antioxidative systems along the canopy light gradient in a mixed beech/oak forest: a comparative study on deciduous tree species differing in shade tolerance. Trees Struct Funct 16:354–364

Herbinger K, Then C, Low M, Haberer K, Alexous M, Koch N, Remele K, Heerdt C, Grill D, Rennenberg H, Haberle KH, Matyssek R, Tausz M, Wieser G (2005) Tree age dependence and within-canopy variation of leaf gas exchange and antioxidative defence in Fagus sylvatica under experimental free-air ozone exposure. Environ Pollut 137:476–482

Holloway PJ (1969) Chemistry of leaf waxes in relation to wetting. J Sci Food Agr 20:124–128

Kardel F, Wuyts K, Babanezhad M, Vitharana UWA, Wuytack T, Potters G, Samson R (2010) Assessing urban habitat quality based on specific leaf area and stomatal characteristics of *Plantago lanceolata* L. Environ Pollut 158:788–794

Kardel F, Wuyts K, Babanezhad M, Wuytack T, Adriaenssens S, Samson R (2012) Tree leaf wettability as passive bio-indicator of urban habitat quality. Environ Exp Bot 75:277–285

Klemm O, Milford C, Sutton MA, Spindler G, van Putten E (2002) A climatology of leaf surface wetness. Theor Appl Climatol 71:107–117

Kolb TE, Fredericksen TS, Steiner KC, Skelly JM (1997) Issues in scaling tree size and age responses to ozone: a review. Environ Pollut 98:195–208

Kolb TE, Matyssek R (2001) Limitations and perspectives about scaling ozone impacts in trees. Environ Pollut 115:373–392

Kupcinskiene E, Huttunen S (2005) Long-term evaluation of the needle surface wax condition of *Pinus sylvestris* around different industries in Lithuania. Environ Pollut 137:610–618

Lake JA, Quick WP, Beerling DJ, Woodward FI (2001) Plant development—signals from mature to new leaves. Nature 411:154

Lamaud E, Carrara A, Brunet Y, Lopez A, Druilhet A (2002) Ozone fluxes above and within a pine forest canopy in dry and wet conditions. Atmos Environ 36:77–88

Larcher W (2003) Plants under stress. In: Larcher W (ed) Physiological plant ecology, 4th edn. Springer, Berlin, pp 345–450

Lichtenthaler HK, Ač A, Marek MV, Kalina J, Urban O (2007) Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiol Bioch 45:577–588

Lovett GM, Lindberg SE (1992) Concentration and deposition of particles and vapors in a vertical profile through a forest canopy. Atmos Environ 26:1469–1476

Meidner H (1986) Cuticular conductance and the humidity response of stomata. J Exp Bot 37:517–525

Meinzer FC, Clearwater MJ, Goldstein G (2001) Water transport in trees: current perspectives, new insights and some controversies. Environ Exp Bot 45:239–262

Mussche S, Samson R, Nachtergale L, De Schrijver A, Lemeur R, Lust N (2001) A comparison of optical and direct methods for monitoring the seasonal dynamics of leaf area index in deciduous forests. Silva Fenn 35:373–384

Neirynck J, Genouw G, Coenen S, Roskams P (2004) Depositie en luchtkwaliteit in Vlaamse bosgebieden. Institute for Forestry and Game Management, No. IBW 2004-1, Geraardsbergen, Belgium

Niinemets U (2007) Photosynthesis and resource distribution through plant canopies. Plant Cell Environ 30:1052–1071

Niinemets U, Valladares F (2004) Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. Plant Biol 6:254–268

Padgett PE, Cook H, Bytnerowicz A, Heath RL (2009) Foliar loading and metabolic assimilation of dry deposited nitric acid air pollutants by trees. J Environ Monitor 11:75–84

Pandey S, Nagar PK (2002) Leaf surface wetness and morphological characteristics of *Valeriana jatamansi* grown under open and shade habitats. Biol Plantarum 45:291–294

Parlange J-Y, Waggoner PE (1970) Stomatal dimensions and resistance to diffusion. Plant Physiol 46:337–342

Reig-Arminana J, Calatayud V, Cervero J, Garcia-Breijo FJ, Ibars A, Sanz MJ (2004) Effects of ozone on the foliar histology of the mastic plant (*Pistacia lentiscus* L.). Environ Pollut 132:321–331

Rennenberg H, Gessler A (1999) Consequences of N deposition to forest ecosystems—recent results and future research needs. Water Air Soil Pollut 116:47–64

Sase H, Takahashi A, Sato M, Kobayashi H, Nakata M, Totsuka T (2008) Seasonal variation in the atmospheric deposition of inorganic constituents and canopy interactions in a Japanese cedar forest. Environ Pollut 152:1–10

Schreuder MDJ, van Hove LWA, Brewer CA (2001) Ozone exposure affects leaf wettability and tree water balance. New Phytol 152:443–454

Terashima I, Hanba YT, Tazoe Y, Vyas P, Yano S (2006) Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. J Exp Bot 57:343–354

Tukey HB (1970) Leaching of substances from plants. Ann Rev Plant Physiol 21:305–324

Turunen M, Huttunen S (1990) A review of the response of epicuticular wax of conifer needles to air-pollution. J Environ Qual 19:35–45

Vogelmann TC, Nishio JN, Smith WK (1996) Leaves and light capture: light propagation and gradients of carbon fixation within leaves. Trends Plant Sci 1:65–70

Warren CR (2008) Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO_2 transfer. J Exp Bot 59:1475–1487

Warren CR, Low M, Matyssek R, Tausz M (2007) Internal conductance to CO_2 transfer of adult *Fagus sylvatica*: variation between sun and shade leaves and due to free-air ozone fumigation. Environ Exp Bot 59:130–138

Wieser G, Hecke K, Tausz M, Haberle KH, Gramsc TEE, Matyssek R (2003) The influence of microclimate and tree age on the defense capacity of European beech (*Fagus sylvatica* L.) against oxidative stress. Ann Forest Sci 60:131–135

Wuytack T, Verheyen K, Wuyts K, Kardel F, Adriaenssens S, Samson R (2010) The potential of biomonitoring of air quality using leaf characteristics of white willow (*Salix alba* L.). Environ Monit Assess 171:197–204