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ORIGINAL PAPER

Determination of the cellulose and lignin content on wood fibre surfaces of eucalypts as a function of genotype and site

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Abstract We compared the chemical composition of wood fibres and fibre surfaces of several eucalypt species and hybrids originating from various growth sites in South Africa. The objective was to test for differences in chemical surface composition due to genetics or site with the ultimate aim to facilitate a tailor-made supply of wood for pulping that results in an optimal blend of fibres that can be pulped together with similar yields. This, however, requires a sound knowledge of the fibre properties. The surface functionality on the single fibre level is a key property, because it determines how good inter-fibre bonding will be when paper is formed, which depends amongst other fibre properties on the amount of free hydroxyl groups that are available and therefore on the cellulose content on the fibre surface. The cellulose and lignin content on the fibre surface were determined with chemical force microscopy, a variation of atomic force microscopy. Since the general bulk composition of the fibre and the surface composition might differ, both parameters were determined. We found significant differences in the cellulose and lignin content on fibre surfaces, with regard to genotype and site, respectively. In some, but not all, cases, the surface composition of wood fibres followed the bulk composition, and differences were generally more pronounced. Differences due to genotype were significant, especially with regard to the surface lignin content—but variation due to site was also distinctly recognisable. This variation in surface functionality could be the reason why some pulpwood blends result in a lower pulp yield and different quality.

Keywords Genetic effects · Site effects · Wood properties · Pulp fibres · Surface composition · Functional groups · Free hydroxyl groups · Chemical force microscopy

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Introduction

South Africa is the largest African producer of pulp and paper and produces approximately 1.9 million tons of wood pulp, 1.2 million tons of newsprint, printing and writing paper and more than 1 million tons of other paper products per year. South Africa has a relatively rich source of raw materials from its plantation forests in the KwaZulu-Natal and Mpumalanga regions. The warm climate of these areas leads to a faster tree growth than in most paper- and pulp-producing countries north of the equator (Mbendi Information Services 2009).

South Africa's major pulpwood source is *Eucalyptus*, and several eucalypt genotypes are commercially grown depending on the site characteristics. The growth regions for pulpwood production encompass different climate zones, ranging from warm, subtropical areas near the east coast to cooler sites on the escarpment with elevations above 1,000 m.

Species choice is usually based on climatic risk factors (e.g. snow, frost or drought risk), mean annual temperature (MAT), mean annual precipitation (MAP), soil characteristics as well as wood and pulping characteristics.

On subtropical sites near the coast (MAT > 19°C), *E. grandis* × *urophylla* and *E. grandis* × *camaldulensis* are the most widely planted genotypes. These hybrids have a superior disease resistance compared to pure species, such as *E. grandis*, which can also be grown in this zone. In addition, *E. grandis* × *urophylla* has excellent pulping properties and is thus the preferred genotype in the subtropical area.

In the warm, temperate climates (KwaZulu-Natal Midlands and Mpumalanga escarpment with MAT 16–19°C), *E. grandis*, *E. dunnii*, *E. saligna* and *E. smithii* are commonly planted. *E. grandis* has a fast growth rate but may be prone to drought risk on sites with low levels of available soil water, and it is sensitive to snow damage and to frost when young. *E. smithii* has highly desirable pulp properties but is relatively more susceptible to *Phytophthora* root rot on soils with poor drainage.

On cold temperate sites with a MAT of 15–16°C, *E. dunnii*, *E. grandis* × *nitens*, *E. saligna* and *E. smithii* can still be grown since they are moderately hardy to frost. However, on sites with a MAT below 15°C, species tolerant of snow—such as *E. nitens*—or frost and drought—such as *E. macarthurii*—are often preferred, despite having slightly less desirable pulping properties, especially in the latter case. Other species with desirable wood properties that show promise for cold temperate sites are *E. benthamii* and *E. badjensis* (Smith et al. 2005).

The pulp and paper quality of these species depends strongly on fibre properties, such as fibre length, diameter and chemical composition. The fibre dimensions determine how well the inter-fibre contact will be when paper is formed, and the chemical composition, i.e. the availability of free hydroxyl groups to form hydrogen bonds, determines how well these fibres will bind together, which in turn affects the paper quality. The importance of fibre properties—in addition to other tree improvement efforts—for any further use such as pulping has been highlighted by Lundqvist (2002).

That wood quality and more specifically fibre properties depend strongly on the environment has been demonstrated by many studies. Gindl et al. (2000), for example, showed that the lignin content in the secondary cell wall correlated positively with temperature in terminal latewood tracheids. Leal et al. (2007) reported tree growth variability within five different conifer species in the Austrian Alps depending on species and altitude. Kaakinen et al. (2007) studied the effects of growth differences due to geographical location and nitrogen fertilisation on the chemical composition of Norway spruce (*Picea abies* (L.) Karst.) at two sites in Finland. They found that nitrogen fertilisation caused only small changes in the chemical composition, while the differences due to different sites were significantly larger.

Various studies have discussed wood and fibre quality of *Eucalyptus*. Clarke et al. (1997) studied a variety of wood characteristics including the average density, fibre length and chemical composition of nine eucalypt species in three provenances from established trial sites in South Africa. These authors revealed significant differences in density, fibre length and chemical composition between the species and between sites. Naidoo et al. (2006) found a negative correlation between moisture availability and wood density and vessel amount in *Eucalyptus grandis* in the warm temperate region of South Africa. Miranda and Pereira (2002) studied the differences in wood density, fibre morphology, chemical composition and pulp yield in four provenances of *Eucalyptus globulus* at three different sites in Portugal. Their findings suggest no significant effect of provenance and site on the wood density. However, provenance and site caused significant variation in fibre length, cell wall thickness and lumen diameter. With regard to the chemical composition, only the extractive content showed any significant provenance and site effects.

Based on the effects of site quality and fibre properties, this study explores further ways of scrutinising the fibres quality. This is done by the use of chemical force microscopy to characterise the chemical surface composition of pulpwood fibres and compare this to the chemical bulk composition, which might differ considerably (Koljonen et al. 2004; Hannuksela et al. 2003; Fardim and Holbom 2005a; Fardim et al. 2005b). Nevertheless, typically the bulk chemical composition is determined to describe pulpwood fibres. To our knowledge, information on the surface composition of wood fibres has so far not been investigated or published.

The surface properties of fibres are, however, especially important for further processing, such as paper making, as they determine, for example, the degree of inter-fibre bonding. The formation of hydrogen bonds between pulp fibres depends on available hydrophilic hydroxyl (–OH) groups and small inter-fibre distances. The removal of the hydrophobic lignin from the fibre surface during pulping exposes cellulose and hemicelluloses containing –OH groups, while precipitation of hydrophobic lignin hinders the formation of hydrogen bonds (Laine et al. 1994). Furthermore, any lignin still present in pulp fibres results in an increased fibre stiffness, which inhibits good inter-fibre bonding. The fibres become more flexible when lignin is removed, and simultaneously, the amount of free hydroxyl groups on the fibre surface is increased, which promotes the formation of hydrogen bonds between the fibres. These bonds are responsible for the mechanical strength of the

final paper product (Maximova et al. 2001), and e.g. the tensile strength a paper sheet depends mostly on the number of hydrogen bonds per unit volume available between the fibre surfaces.

The objective of this study was to reveal possible differences in chemical composition of the fibre surfaces with regard to site and genotype. The investigated species consisted of different *Eucalyptus* species grown at various sites in South Africa. These sites varied in the availability of water, or mean annual precipitation (MAP) and mean annual temperature (MAT). Both factors can be expected to have an influence on the chemical composition of wood fibres.

A difference in surface functionality could be expected to affect the pulp quality and explain a variation therein.

An important part of that objective was to determine whether the traditionally used bulk composition is comparable with the surface composition that we assessed with chemical force microscopy (CFM), a technique that allowed us to scrutinise whether the fibre surface differs in its cellulose and lignin content from the fibre bulk.

Chemical force microscopy (CFM) is an adaptation of atomic force microscopy (AFM), in which the tip is coated with specific functional groups. The adhesive force determined between the modified tip and the sample surface can be used to gain additional information about the chemical composition of the surface (Noy et al. 1997). The tip is modified with self-assembling monolayers (SAMs) of alkane thiol compounds, which allow control of the functionality and their chemical and mechanical stabilities (Baralia et al. 2005). Chemical force microscopy has been established on chemically modified, flat silicon surfaces by Noy et al. (1995). In their study, both the silicon substrates and the S_3N_4 tips were coated with gold and then modified with SAMs of alkane thiol compounds terminated with $–CH_3$, –COOH and $–NH_2$ functional groups. They found that the adhesive force was larger if the tip and substrate had the same functionality. Chemical force microscopy has also been used to study cellulose fibres in aqueous media by Bastidas et al. (2005), who determined the effect of the pH value on the adhesive force determined with $–CH_3$,- –COOH- and –OH-coated tips.

In this study, we determined the adhesive forces between AFM tips coated with $–CH_3$ and –COOH, respectively. In a previous study (Klash et al. 2009), we could show successfully that $–CH_3$ groups are more sensitive to lignin, while –COOH groups are more sensitive to cellulose and hemicelluloses. The thus functionalised tips can therefore be used to quantify the cellulose and lignin content on the surface of pulpwood fibres. Figure 1 shows a sketch of the experimental setup and an example of an AFM image that is used to quantify functional groups on the surface.

Materials and methods

Sample preparation

Samples of different eucalypt genotypes, namely *E. dunnii*, *E. grandis* (grown from genetically variable seedlings), *E. grandis* (clonal material) as well as *E. grandis* × *nitens* and *E. grandis* × *camaldulensis* hybrid clones, were collected from different growth sites with either cool temperate, warm temperate or subtropical (hot) MAT and on sites with a MAP that was either dry, moist or wet according to the classification by Smith et al. (2005). Not all species were available from all growth sites.

The MAT, MAP, average age, diameter at breast height (DBH), site index at 5 years (SI_5), basal area (BA) and mean annual increment (MAI) of the investigated species are given in Table 1.

The trees were between six (fast growing species) and eight (slower growing species) years old when harvested. Only straight trees without any reaction wood that would result in a different cellulose and lignin content were cut on each sample site, and discs were cut at DBH directly after harvesting. These discs were chipped, and fibres were prepared from the chips by a mild maceration in Jeffrey's solution for 4 h (Han et al. 1999). The maceration removed only the middle lamellae and left the mostly amorphous primary cell wall exposed. This has been shown by AFM images of the fibre surfaces in a previous publication by Meincken (2007).

The separated fibres were subsequently kept in distilled water. For AFM analysis, they were spread onto a glass slide and left to dry for 12 h. The adhesion due to capillary forces between the cells and the glass substrate was sufficient to keep the fibres in position for AFM analysis. The low concentration of fibres in the water ensured that the fibres did not overlap and only formed a single layer on the substrate. Images were acquired with the fast scan axis parallel to the longitudinal fibre axis, in order to minimise shear forces.

In order to verify the reproducibility of results, two sample sets were prepared for AFM measurements from randomly chosen wood chips, where enough material was available. This was, however, not possible for all samples.

Tip modification for CFM

Silicon force modulation cantilevers from nanosensors were modified according to Bastidas et al. (2005). Tips with the following functional groups were prepared:

- methyl ($–CH_3$) groups, from 1-octadecanethiol $CH_3(CH_2)_{17}SH$ (Acros).

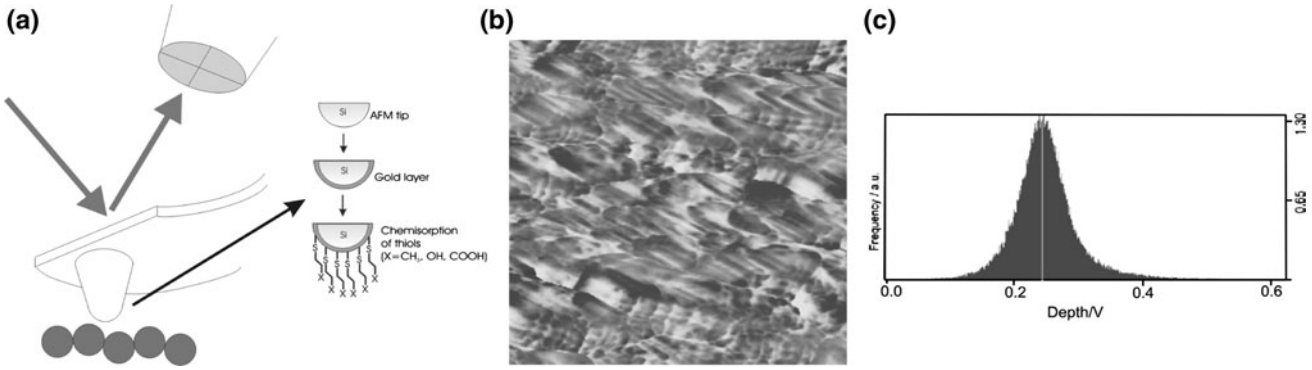

Fig. 1 **a** Schematic drawing of an AFM with a chemically modified tip, **b** adhesive force image obtained with a COOH-coated tip that shows the distribution of cellulose as brighter areas and **c** representation of the image as histogram to determine the average grey value

Table 1 Medium annual temperature (MAT), medium annual precipitation (MAP), age, diameter at breast height (DBH), site index at 5 years (SI_5), basal area (BA) and mean annual increment (MAI) of the sampled *Eucalyptus* genotypes

Species	MAT	MAP	Age [years]	DBH [cm]	SI_5 [m]	BA [m^2/ha]	MAI [m^3/ha/year]
E. grandis	Warm	Moist	6	14.5	18.1	17.8	23.5
E. grandis clone	Cool	Moist	8	19.9	19.9	12.4	14.9
E. dunnii	Warm	Moist	8	14.0	13.8	17.8	15.2
E. dunnii	Warm	Wet	7	18.0	17.4	16.8	21
E. grandis × *camaldulensis*	Cool	Moist	8	13.2	15.3	18.7	17.5
E. grandis × *nitens*	Cool	Moist	7	20.6	21.0	21.4	41.4
E. grandis × *nitens*	Warm	Moist	7	15.6	16.2	10.7	24.1
E. grandis × *nitens*	Hot	Dry	7	17.7	17.0	11.8	12.9

- carboxylic (–COOH) groups, from 11-mercaptoundecanoic acid $HOOC(CH_2)_{10}SH$ (Aldrich).

The silicon tips were first coated with gold with a sputter coater and cleaned under a 254-nm UV lamp for 1 h to ensure that all organic material was removed. The gold-coated tips were subsequently immersed in reagent alcohol for 30 min and then in a 3 mM $CH_3(CH_2)_{17}SH$ or $HOOC(CH_2)_{10}SH$ alcoholic solution for 2 h at room temperature. The coated tips were then rinsed with *n*-heptane (Aldrich) and alcohol and gently dried in an Argon stream.

AFM imaging

AFM images were acquired with a Multimode AFM from Veeco combined with a digital pulsed force mode (DPFM) controller from Witec. This leads to the simultaneous acquisition of a topography and an adhesive force image, which displays the adhesive force between the substrate and the tip on each image pixel. Images were acquired with a scan range of 2 × 2 μm and a scan speed of 0.7 lines/s.

In order to compensate for the environmental effect of humidity and temperature, which could lead to a change in surface polarity, the tip was calibrated on a mica surface before each measurement.

The average polarity of a single fibre was determined from one adhesion image, which consists of 65,536 (256 × 256) single measurements in the observed area. This was done for ten different fibres, resulting in one average adhesive force value to describe the sample.

Bulk chemical composition

Typically, hardwoods contain about 40-50% cellulose, 20-30% hemicelluloses, 18–25% lignin and less than 10% extractives (Walker 2006).

To determine the chemical bulk composition, the wood chips were ground into flour in a Retsch Ultra centrifugal mill and screened for size. The fraction retained in the 80 mesh sieve was extracted in triplicate for 4 h in a Soxhlet apparatus with 1:2 v/v 95% ethanol/cyclohexane (E/C) mixture and then with distilled hot water (ASTM 2001a; Fengel and Przyklenk 1983; TAPPI 1988a).

The solvent was evaporated at 40°C under vacuum, after which the residue was oven-dried at 102 ± 3°C for 20 min to determine the dry mass extractives content.

Three replicates of 0.5 mg extractive-free wood were then hydrolysed with 7.5 ml, 72% (w/w) sulphuric acid at 20°C for 2 h. The acid was diluted to a concentration of 3%

by adding 280 ml of distilled water. The residue was cooled and washed in a porosity 2 filtering crucible with 250 ml of boiling water. The solid residue was dried at 102 ± 3°C to a constant weight to determine the percentage of acid-insoluble, i.e. Klason lignin (ASTM 2001b; TAPPI 1988b).

The cellulose content was determined according to the Seifert method (Browning 1967) using acetylacetone from Sigma–Aldrich and dioxane, hydrochloric acid, methanol and diethyl ether from Merck Chemicals.

Statistical analysis

The resulting data were not evaluated with ANOVA, because the sample set was not complete, i.e. not all genotypes were available from all growth sites. For the average adhesive force values determined from ten AFM images per sample, an average value and a 95% confidence interval were calculated with a *t*-test.

Results and discussion

The bulk chemical composition of eucalypt genotypes from sites with cool or medium MAT and medium MAP values is given in Table 2.

The amount of cellulose did not differ significantly between the investigated genotypes and ranged from 44 to 48%. The *E. grandis* clone had with 48% the highest cellulose content, followed by *E. grandis* and the two hybrids, *E. grandis* × *nitens* and *E. grandis* × *camaldulensis* with about 46%. The lowest cellulose content was found in *E. dunnii* with 44%. The lignin content followed the same trend–the *E. grandis* clone showed with 21% the largest amount of lignin and *E. dunnii* with 13% the lowest. The maximum variation in the bulk lignin content between the species was 8%.

The chemical surface composition of the fibres determined with CFM revealed considerably larger differences between the species than the bulk composition. Figure 2 displays the adhesive forces and 95% confidence intervals determined with –COOH (cellulose sensitive) or $–CH_3$ (lignin sensitive) functionalised tips on the fibre surfaces of wood originating from similar growth sites but from different species. The determined adhesive force values are directly proportional to the amount of cellulose or lignin present on the surface, although they do not yield a percentage value for the cellulose or lignin amount.

The adhesive forces of different *Eucalyptus* genotypes from comparable sites with a cool/moist MAT/MAP are shown in Fig. 2a. It shows that similar adhesive force values were obtained with the CH_3-coated tip on fibres from *E. grandis* clone and *E. grandis* × *camaldulensis*, and they were significantly higher than the values observed on fibres from *E. grandis* × *nitens.* This indicates that fibres of *E. grandis* × *nitens* have a lower lignin content on the surface than that of *E. grandis* clone and *E. grandis* × *camaldulensis*, and this result was confirmed in a second set of measurements on *E. grandis* × *nitens* fibres.

The adhesive forces determined with a COOH-coated tip showed similar values for all four species within the 95% confidence interval. Although the adhesive forces determined for *E. grandis* × *nitens* in the two measurement sets differ, the COOH-coated tip showed a higher sensitivity towards the fibre surface than the CH_3-coated tip for both measurements. This suggests a higher cellulose content on the fibre surfaces compared to the lignin content. Fibres from *E. grandis* clone and *E. grandis* × *camaldulensis* on the other hand showed a comparable cellulose and lignin content on the surface.

Comparison between the chemical surface composition of the fibres determined with CFM and the bulk composition showed that the amount of cellulose did not differ significantly between the examined genotypes for both methods. The amount of lignin on the surface, however, showed considerable differences between the species with a higher (and similar) lignin content determined on fibres from *E. grandis* clone and *E. grandis* × *camaldulensis* than for *E. grandis* × *nitens*.

Figure 2b shows the surface functionality of different *Eucalyptus* genotypes from comparable sites with a warm/moist MAT/MAP. It can be seen that the adhesive force values were well reproducible where two measurement sets were performed.

As for the cool/moist climate, *E. grandis* and *E. grandis* × *nitens* displayed a slightly higher sensitivity towards

Table 2 Chemical bulk composition of various *Eucalyptus* genotypes from cool (c) or warm (w) MAT sites with standard deviation in brackets

Species	Lignin (%)	Cellulose (%)	E/C extractives (%)	H_2O extractives (%)
E. grandis (w)	14.8 (4.5)	45.6 (2.2)	2.2 (0.7)	1.9 (0.9)
E. grandis clone (c)	20.9 (1.3)	47.6 (2.5)	3.3 (1.7)	2.0 (2.0)
E. dunnii (w)	12.7 (3.1)	44.1 (2.2)	5.4 (0.1)	2.7 (1.4)
E. grandis × *nitens* (w)	16.5 (1.2)	45.2 (2.8)	4.3 (2.3)	1.8 (1.5)
E. grandis × *camaldulensis* (c)	15.5 (0.7)	45.9 (3.0)	4.7 (1.7)	3.6 (1.7)

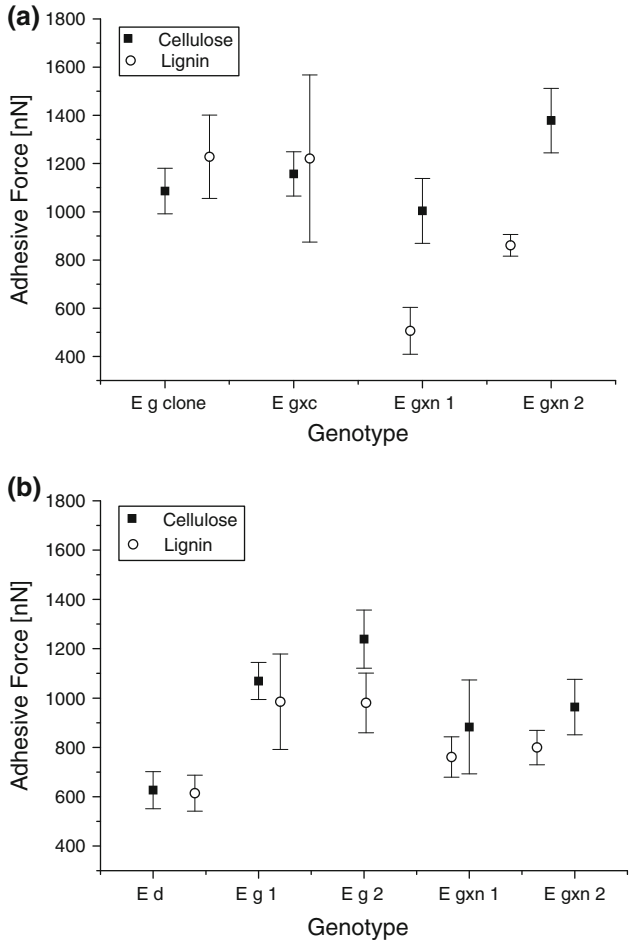

Fig. 2 **a** Different *Eucalyptus* genotypes from comparable sites with a cool/moist MAT/MAP, **b** different *Eucalyptus* species from comparable sites with warm/moist MAT/MAP

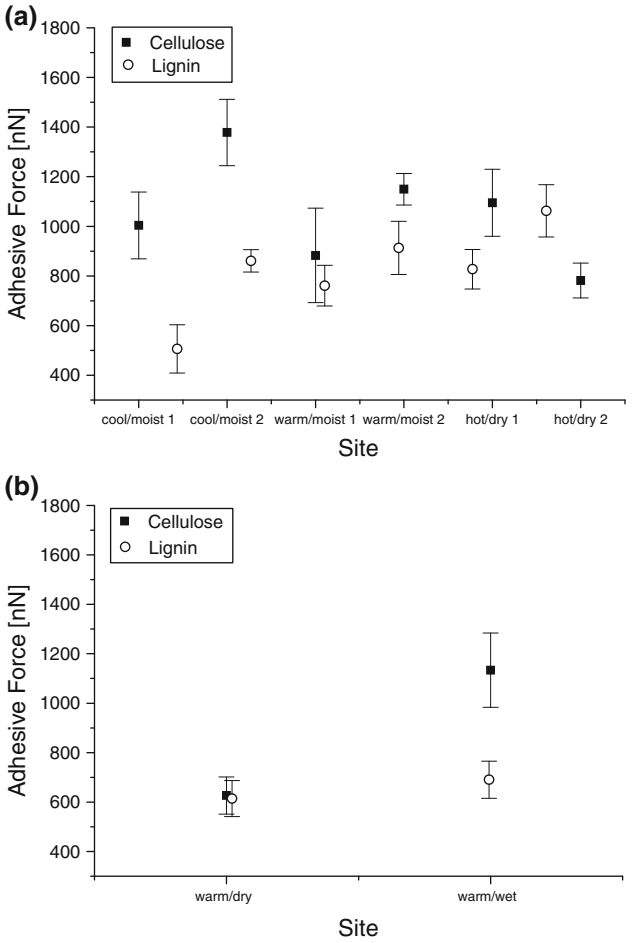

Fig. 3 Same *Eucalyptus* genotypes from sites with differing climate. **a** *E. grandis* × *nitens* hybrids, **b** *E. dunnii*

the COOH-coated tip than towards the CH_3-coated tip, although this was only significant in the second measurement set. This can be attributed to a higher cellulose content on the fibre surface. Both the cellulose and lignin content were significantly lower in *E. dunnii* than in *E. grandis* and *E. grandis* × *nitens*. This distribution was confirmed, although not quite as pronounced, by the bulk chemical composition. The lignin content on the surfaces of *E. grandis* fibres was significantly higher than on fibres from *E. dunnii* or *E. grandis* × *nitens*, which agrees well with the results displayed in Fig. 3a, which showed a higher surface lignin content for *E. grandis* clone than for *E. grandis* × *camaldulensis*.

The adhesive forces determined on fibres from the same species, but from different growth sites, are presented in Fig. 3.

The adhesive forces determined on fibres from *E. grandis* × *nitens* hybrids across a MAT/MAP gradient are displayed in Fig. 3a. Most fibres showed a higher sensitivity towards the COOH-coated tip than towards the CH_3-coated tip, which means that the cellulose content on the surface was higher than the lignin content in these samples. The only exception was the second set of fibres originating from a hot and dry site, where the results were contradictory. The highest cellulose content was found for cool and moist growth conditions, and it decreased with increasing MAT and decreasing MAP. At the same time, the lignin content at the fibre surface increased slightly with increasing MAT and decreasing MAP.

The adhesive forces determined on fibres from *E. dunnii* across an MAP gradient are displayed in Fig. 3b. Fibres originating from the warm/dry site showed comparable sensitivity to the CH_3- and COOH-coated tips, whereas the fibres from the warm/wet site had a considerably higher affinity to the COOH-coated tip. This means that the cellulose content on the surface of fibres originating from moist sites is significantly higher than that from dry sites, whereas the surface content of lignin remains the same.

The results show that the chemical composition on the fibre surface differs in some cases from the bulk and that differences in composition between genotypes and growth

site can be more pronounced on the fibre surface than in the bulk. This affects further processing of the fibres such as pulping and paper formation. Statistically significant differences with regard to genotype were found in *E. grandis* × *nitens*, which showed a lower lignin content on the fibre surface and *E. dunnii*, which had a considerably lower lignin and cellulose content on the fibre surface compared to *E. grandis* and *grandis* × *camaldulensis*.

The differences due to growth site were less significant, but the trend showed that the cellulose content on the fibre surface decreased with increasing temperature and decreasing moisture, whereas the lignin content on the fibre surface increased.

If a blend of fibres is pulped together, a comparable fibre composition is desirable so that the amount of pulping chemicals can be adjusted accordingly and evenly distributed. Large differences in the fibre stock could result in uneven pulping and consequently in a yield with variable end-product quality.

Conclusion

In conclusion, we could show that the surface composition of the fibres followed the trend of the bulk composition in some, but not all, cases. The cellulose content of fibres from different species but various growth sites was comparable within the standard deviations in the fibre bulk, as well as on the fibre surface. The lignin content, on the other hand, showed considerably more variation on the fibre surface than in the fibre bulk. While *E. grandis*, *E. grandis* clone and *E. grandis* × *camaldulensis* showed comparable values, *E. grandis* × *nitens* and *E. dunnii* had a significantly lower surface lignin content than that suggested by the bulk composition.

The effect of MAT and MAP on the surface composition on pulpwood fibres was recognisable. The surface cellulose content of *E. grandis* × *nitens* hybrids decreased with increasing MAT and decreasing MAP, while in turn the surface lignin content increased with increasing MAT and decreasing MAP. This could be confirmed by increasing surface cellulose content on fibres from *E. dunnii* with increasing MAP.

These results could prove useful for the pulp and paper industry as they indicate why some species produce pulp of lower quality and also why some species cannot be pulped well together.

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