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The effects of growth conditions and of processing into yarn on dislocations in hemp fibres

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Abstract Dislocations are irregular regions within the cell wall of natural fibres. Dislocations have also been called slip planes or nodes, and are important for the properties of natural fibres within a number of applications. This study compares the percentage of the cell wall consisting of dislocations at harvest in hemp fibres from plants grown in a green house under three different regimes (wind free, windy and dry) with the percentage found in commercial hemp yarn. As expected a higher percentage of the cell wall consisted of dislocations in the processed fibres, but the increase was only significant compared to two of the three growth regimes (wind free and windy). The dislocations were significantly larger in the yarn fibres than in the plants regardless of the growth conditions, even though both the windy and the dry conditions increased the sizes of the dislocations significantly compared to the wind-free regime. Interestingly, the typical longitudinal distances between neighbouring dislocations were found to be longer for the yarn fibres than for the fibres taken from the plants at harvest.

Introduction

Irregular regions within the cell wall are known as dislocations and are found in several different plant fibres and tracheids, for example, in flax [1–3], hemp [3, 4] and wood [5]. Dislocations have also been called slip planes, kink bands or nodes, but here the term dislocation is used in accordance with Nyholm et al. [6]. Fibres and tracheids are thick-walled cells that lent stiffness and strength to plant tissues. They vary in size both between and within species; typically, they have a length in the 10–30 mm range and a diameter in the 10–30 μm range. A polarized light transmission microscopy (PLM) image of a hemp fibre with dislocations is shown in Fig. 1. Using this technique, dislocations may be seen as bright stripes across the fibre.

Dislocations are present already in the living plant [7], but may also be introduced during processing by application of compression stress in the longitudinal direction of fibres [8, 9]. The reason for the occurrence of dislocations already in the living plant is unknown, but has been assumed also to be caused by mechanical action, i.e. wind leading to longitudinal compression within the lee side of the stem. However, since also drought induces dislocations, growth stress during biosynthesis of microfibrils may be another possible cause [7].

Dislocations are important for many properties of natural fibres, but their exact structure remains unknown. Cell walls of plant cells consist mainly of crystalline cellulose microfibrils embedded in amorphous lignin and hemicelluloses. Since many studies within pulp and paper science have shown dislocations to be the weak points in fibres [6], dislocations have long been assumed to contain amorphous cellulose. However, recent results question this assumption [10]. In any case, dislocations do not appear to be regions where cellulose microfibrils are discontinuous, as dislocations can be aligned with the surrounding cell wall by application of tensile load in the longitudinal direction of individual fibres [11]. This phenomenon could theoretically contribute to tensile stiffening of individual fibres. Tensile stiffening has been observed for flax [12], but not for hemp [11].

Results from studies attempting to link dislocations to fibre properties point both to the possibilities and to the

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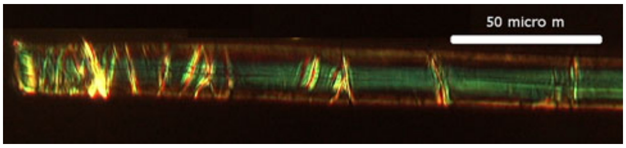

Fig. 1 Polarized light microscopy image of a hemp fibre containing dislocations (seen as white stripes across the fibre)

challenges connected with this idea. For example, dislocations are known to reduce the tensile strength of paper [8], whilst their effect on the tensile strength of individual fibres seems to be detrimental only in some cases [2, 9, 11, 13, 14]. As mentioned above, dislocations may be stretched and aligned with the microfibrils of the surrounding cell wall, at least under some testing conditions [11]. It is conceivable that low moisture contents and/or fast testing speeds do not allow this mechanism to distribute tensile stresses within the fibre cell wall, thus resulting in failure initiated in un-aligned dislocations. Consequently, it is not straight forward to link dislocations to the tensile strength of individual fibres; also the test conditions and the history/state of the fibres need to be considered. Other studies have shown that dislocations are more susceptible to hydrolysis than the bulk cell wall [10, 15, 16]. This characteristic points out the importance of dislocations within biorefining and the production of cellulosic bioethanol, as the resulting breaking of fibres into segments at dislocations is central to the initial liquefaction of the substrate [10]. A third example concerns the textile industry. In this industry, it has long been known that dislocations in bast fibres such as flax and hemp bind die better than the surrounding cell wall [17], which again points to the higher reactivity/susceptibility of dislocations compared to the surrounding cell wall material.

To obtain a more detailed knowledge of dislocations in plant fibres would be desirable for several applications. From a research perspective, the fact that dislocations in many ways behave differently than the rest of the cell wall draws attention to how structure–function relationships within plant biomass are best studied. It seems that in some cases there is much to be gained by striving to link behaviour to microstructure instead of handling the inherent heterogeneity of plant biomass by use of more or bigger samples.

The study reported here has a more limited perspective than outlined above, but the background given illustrates why dislocations in plant fibres are worth studying. This study compares the effects of growth conditions and of processing into yarn on dislocations in hemp fibres (*Cannabis sativa* L.). In addition to the effect on the percentage of the cell wall consisting of dislocations, the study also reports the effects on the sizes of the individual dislocations and on the distances between neighbouring dislocations in the longitudinal direction of the fibre.

Materials and methods

Hemp fibres

This study comprised data from four different hemp (*Cannabis sativa*, L.) fibre sets, each consisting of around 100 fibres (see Table 1 for exact numbers). Three of these fibre sets were extracted from Felina 34 hemp stems after harvest. Extraction of fibres by hand using precision tweezers was used [18]. The hemp plants were grown in a green house at the University of Copenhagen, Denmark during the summer of 2007 under three different growth conditions: ‘wind free’, ‘wind’ and ‘drought’. The ‘wind free’ regime implied no wind at all during the growth season of the plants, and no lack of water or nutrients. The ‘wind’ regime implied wind night and day throughout the growth season; the wind came from constantly changing directions. The supply of water and nutrients was the same as for the ‘wind free’ treatment. The ‘drought’ regime implied wind-free conditions, but minimum supplies of water and nutrients. The percentage of the cell wall consisting of dislocations found after the ‘wind free’ treatment may most likely be seen as a lower boundary for hemp at the time of harvest. Results for the two harsh treatments are probably close to the maximum percentages of the cell wall consisting of dislocations found at harvest in unmodified

Table 1 Data on dislocations in four different hemp fibre data sets

Fibre source	Relative dislocation areas			Area of dislocations			Longitudinal distances between dislocations		
	n	Mean (%)	SD (%)	*n*	Mean (μm^2)	SD (μm^2)	*n*	Mean (μm)	SD (μm)
Wind free	96	12.0^{a+}	8.5	1903	29^{a}	56	771	10.4^{a}	12.1
Wind	98	18.5^{b+}	12.8	2645	35^{b}	112	923	8.1^{b}	10.6
Drought	114	21.3^{bc+}	12.3	2783	41^{c}	116	959	8.0^{b}	9.4
Yarn	95	24.0^{c}	9.6	1253	80^{d}	132	798	15.1^{c}	12.7

Mean values in the same column marked with the same letter are not significantly different (5% level or better according to *t*-tests). Columns marked ‘*n*’ give the number of observations, columns marked ‘SD’ give the standard deviations. Results marked $^{+}$ are from [7] hemp plants grown in Europe. The fourth set comprised fibre segments extracted from commercial hemp yarn (product name: ‘Imperial’) obtained from the company Linificio e Canapificio (Italy). No information on variety, origin or processing methods are known for these fibres.

Part of the results regarding dislocations in the green house grown hemp has been published earlier [7]. Data for the hemp yarn fibre set were also used in [19].

Polarized light microscopy and image analysis

The dislocations in each of the fibre segments were identified by image analysis of micrographs obtained from PLM using the method described in [20]. This method implies the capture of two images per fibre segment under crossed polars; one image is taken with the longitudinal axis of the fibre parallel to the vibrational direction of one of the two filters, and one is taken with the fibre rotated 10° away from this position. Ideally, the first image then shows only the dislocations in the fibre whilst the second one shows the whole fibre. From these two images, two masks are produced using a series of image analysis tools: one which show the dislocations, and one which shows the fibre. Based on these masks three different parameters were calculated, all based on the 2D transmission images obtained from PLM: (a) the relative dislocation area, i.e. the area of the dislocations in percentage of the fibre segment area, (b) the absolute areas of the individual dislocations and (c) the absolute longitudinal distances between neighbouring dislocations. The relative dislocation area was calculated for each individual set of fibre segment images, whilst all values found for each of the other two parameters were pooled for all fibre segment images within each data set.

Results and discussion

An overview of the results is given in Table 1. Table 1 shows that harsh growth conditions increase the mean relative dislocation area significantly compared to fibres from plants grown without wind and with no lack of water or nutrients (wind free), as reported earlier [7]. It is also seen that a larger percentage of the cell wall consists of dislocations in the processed fibres than in the fibres isolated from the plants at harvest. However, the difference is only significant when comparing to the ‘wind free’ and the ‘wind’ regimes, not when comparing to the ‘drought’ regime. Table 1 also shows that the average size of the dislocations as seen in PLM images increases significantly with both variants of harsh growth conditions compared to the ‘wind free’ conditions. A dramatic increase in the average dislocation size of about 100% or more is seen upon processing into yarn, no matter which growth conditions the value is compared to. The last part of Table 1 shows the average longitudinal distances between dislocations. It is seen that as expected, the harsh growth conditions decrease the average distance significantly between dislocations compared to the ‘wind free’ regime. However, surprisingly the mean distance between dislocations is seen to be about 50% *longer* in the processed fibres than at harvest. Since the yarn fibres came from a different batch than the other three fibres sets, the reason for this cannot be identified. One possibility is that the fibres used for the yarn contained fewer dislocations at harvest than fibres from the ‘wind free’ regime. This is not likely if the percentage of the cell wall consisting of dislocations at harvest is determined mostly by growth conditions and not by variety, as is presently assumed. Another possible explanation is that some of the smaller dislocations disappear during processing. Processing into yarn comprises a number of steps involving the use of mechanical force, so exposure to both compressive and tensile stresses is likely to take place. Tensile stress can remove dislocations as reported earlier [11]. In that study it was however also found that dislocations re-appeared two months after failure. Alternatively, one can speculate that small neighbouring dislocations present at harvest merge into larger dislocations during processing, thus increasing both the average dislocation size and the mean longitudinal distance between them.

Figure 2 gives more detailed information regarding the sizes of dislocations and the distances between them for the four data sets at hand. The figure shows the effects of different growth conditions and of processing on the number of dislocations per mm fibre, and these results are shown separately for different ranges of dislocation sizes. Baley [2] reported up to 15–20 defects per mm for flax, whilst Ander et al. [16] found approximately 10 cleavages per fibre for soft wood pulp after enzymatic hydrolysis. When comparing these numbers to Fig. 2, it is clear that the automatic method of Thygesen and Ander [20] find many small dislocations not counted as individual dislocations by the methods used in [2] and [16]. Figure 2 shows that for the three data sets extracted from the plants at harvest, the vast majority of dislocations found are smaller than 25 μm^2. It also shows that the harsh growth conditions generated more dislocations per mm practically within all ranges of sizes, and that except for dislocations smaller than 25 μm^2, drought generated more dislocations per mm than the windy conditions. These two regimes, thus, had different effects, even though the relative dislocation areas were not significantly different. The figure furthermore confirms what can be suspected from Table 1, namely, that the processed fibres contained far fewer of the small dislocations than the fibres extracted from hemp plants at harvest. The number of the larger dislocations is

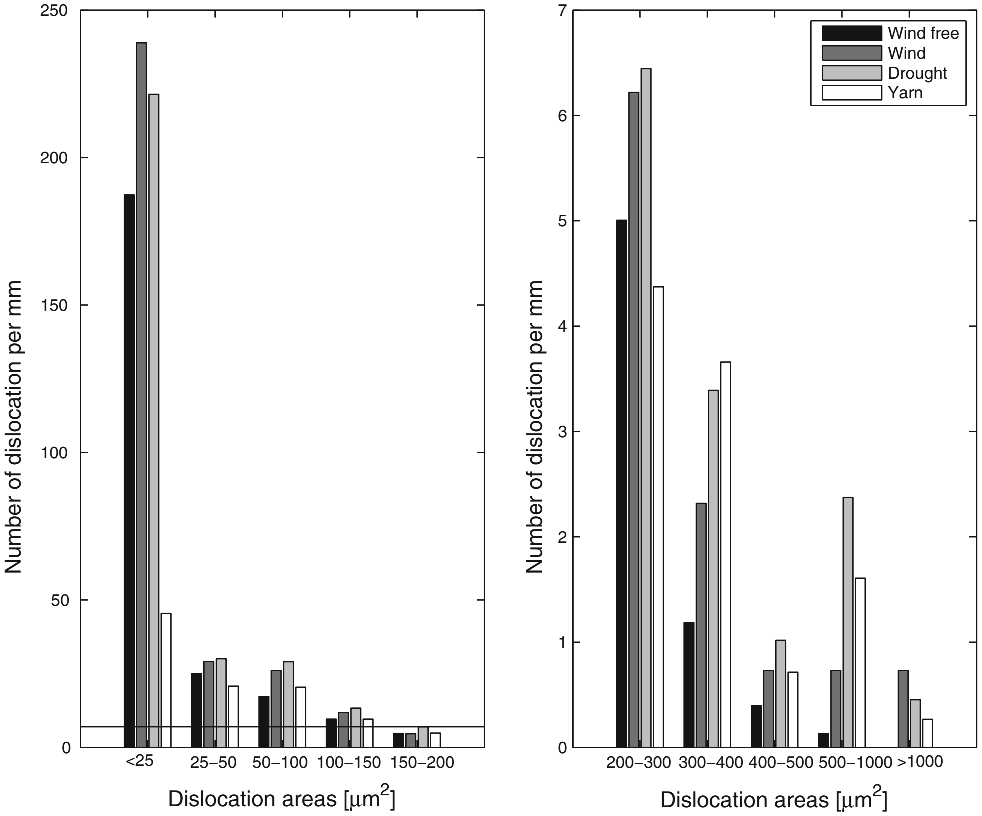

Fig. 2 The frequency of dislocations and their sizes. The number of dislocations per mm fibre within ten different dislocation size ranges. The horizontal line at 7 mm^{-1} in the *left panel* marks the upper limit of the scale in the *right panel*

seen to be similar for processed fibres and for the two variants of harsh growth conditions. These results show that the reason why the relative dislocation area increases when going from the wind-free growth conditions to the windy or dry conditions is an increase in the number of dislocations within all size ranges, i.e. it is not due to an increase in the sizes of already existing dislocations. Contrary to this, the increase in the relative dislocation area seen for the processed fibres seems to be due to a ‘clustering’ of the dislocations into fewer but larger areas. It has earlier been shown that hemp fibres break more often in large dislocations than in small dislocations during acid hydrolysis [19], so the ‘clustering’ makes the processed fibres more susceptible to fibre shortening even though the number of dislocations per mm is reduced. Likewise, the result that drought generated larger dislocations than wind means that fibres extracted from plants exposed to drought can be expected to be more susceptible to fibre shortening than fibres extracted from plants grown under windy conditions. These results are interesting from the perspective of biorefining, as they suggest that cell wall recalcitrance may be affected both during the growth phase of the plant and during mechanical processing before the hydrolysis step. Furthermore, the results illustrate that quantification of dislocations in the form of a percentage of the cell wall is not sufficient to describe how prone a given fibre source is to fibre shortening. Also the size distribution of the dislocations or a direct measure of the fibre segment length distribution after/during hydrolysis is needed.

Conclusions

The percentage of the cell wall consisting of dislocations, their sizes and the distances between them in hemp fibres were studied for four different data sets. Three sets comprised fibres isolated from plants grown in a green house under three different regimes (wind free, windy and dry), whilst the last set comprised fibres isolated from commercial hemp yarn. As expected a higher percentage of the cell wall consisted of dislocations in the fibres processed into yarn, but the increase was only significant compared to two of the three growth regimes (wind free and windy). The average dislocation size was significantly larger in the yarn fibres than in the fibres isolated at harvest regardless of the growth conditions, even though both the windy and the dry conditions increased the mean size of the dislocations significantly compared to the wind-free regime.

Interestingly, the typical longitudinal distances between neighbouring dislocations were found to be longer for the yarn fibres than for the fibres taken from the plants at harvest.

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