

The effects of growth and storage conditions on dislocations in hemp fibres

Lisbeth G. Thygesen · Mohammad R. Asgharipour

Received: 8 January 2008 / Accepted: 7 March 2008 / Published online: 23 March 2008
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Abstract Natural fibres contain structural distortions known as dislocations. Dislocations have been found to negatively affect the properties of plant fibre-based materials. The present study concerned the effect of the growth conditions of hemp plants (*Cannabis sativa* L.) on the amount of dislocations within the fibres retrieved from the stems. Dislocations were found in fibres from plants grown under absolutely wind-free conditions, but both windy and dry conditions introduced significantly more dislocations. Furthermore their maximum size was larger and they were situated closer together within the fibres. The average ultimate tensile stress of fibre bundles from the windy and the dry growth regimes was lower than that of fibre bundles from plants grown under wind-free conditions. In a separate study it was found that plant stems that were dried after harvest and remoistened prior to analysis contained a lower amount of dislocations than plants analysed directly after harvest.

Introduction

Within the study of natural fibres a dislocation is the term used to describe a region of the fibre cell wall where the angle of the cellulose microfibrils relative to the longitudinal direction of the fibre differs from that of the surrounding cell wall. The terms slip plane or micro compression are also used to describe this phenomenon. The effects of dislocations on the performance of natural fibres have been studied

in a number of papers regarding both fibre composites [1–6] and pulp and paper [7–9].

Dislocations may be induced by applying compressive stress in the longitudinal direction of the fibre [8]. Various post-harvest treatments such as defibration processes or high-consistency mixing or pumping of fibre suspensions may result in the formation of dislocations, but they have also been found in fibres carefully removed from living plants [3]. The occurrence of dislocations in living plants has been ascribed to wind damage [10], i.e. to compression stress in the lee side of the plant stem. Even though this explanation is widely recognized as a fact, no data from a dedicated experiment set up in a controlled environment seem hitherto to have been published. The purpose of the present study was primarily to test the hypothesis that wind introduces dislocations in living plants. A trial where plants were subjected to drought and less abundance of nutrients was also included for comparison.

The purpose was to study the possible effects of the growth conditions on dislocations, and it was consequently important to avoid inadvertently changing the amount of dislocations within the fibres between harvest and analysis. The possible effects of the storage conditions on the amount of dislocations were therefore studied, and fibres from stems subjected to three different treatments were analysed: (i) analysed directly after harvest, (ii) after drying and remoistening and (iii) after freezing and thawing.

Results diverge regarding the possible effect(s) of dislocations on the strength properties of plant fibres. Neither Baley [5] nor Thygesen et al. [6] found any relationship between single fibre tensile stress and the number or amount of dislocations for flax, respectively, hemp single fibres. However, others have recorded detrimental effects of dislocations. For high amounts of dislocations (20–60% of the cell wall), Davies and Bruce [1] recorded a weak

L. G. Thygesen (✉) · M. R. Asgharipour
Forest & Landscape Denmark, University of Copenhagen,
Rolighedsvej 23, Frederiksberg 1958, Denmark
e-mail: lgt@life.ku.dk

negative relationship between the amount of dislocations and the tensile strength of individual flax and nettle fibres. Terziev et al. [8] subjected wood samples to longitudinal compression stress. This treatment introduced dislocations in the fibres, and the paper produced from these stem sections had inferior strength properties compared to paper prepared from untreated wood. Hughes et al. [2] recorded increased stress in the interphase between fibres and the matrix near dislocations in hemp-epoxy composites when strained parallel to the fibre axis. In the present study the ultimate tensile stress of fibre bundles was determined in order to get an indication of the possible effect of growth conditions on the fibre tensile strength.

Materials and methods

Hemp plants (*Cannabis sativa* L.) were grown in a green house and subjected to three different growth conditions: wind free, windy or dry. Plants subjected to the wind free treatment were subjected to neither wind nor drought, and they were watered every day with water containing nutrients. Windy conditions were created by placing fans next to the plant bed from the time that the plant height reached about 300 mm and until harvest, otherwise the treatment was the same. Dry conditions implied that from the time the plants reached about 300 mm in height, they were watered only enough to keep them alive (1–2 times a week), and no nutrients were added to the water. At harvest (after about 3 months), the plants subjected to the wind-free treatment measured 3–4 m in height, while the other two treatments showed reduced plant height (windy: 2–3 m; dry: 1–2 m).

Stem sections were stored at $-18\text{ }^{\circ}\text{C}$ for up to about 3 months after harvest. For determination of the amount of dislocations, fibres were removed from the stem sections using precision tweezers according to the method of Burgert et al. [11]. Mechanical isolation of fibre samples from plant material may introduce dislocations, especially in the case of microtoming, as discussed by Hoffmeyer [12]. However, Burgert et al. found that their isolation technique did not introduce defects, and that the strength and stiffness of fibres isolated using tweezers was larger than fibres isolated using a soft chemical treatment with

Jeffrey solution. About 100 fibre segments per treatment were isolated and analysed using polarized light microscopy (PLM) and image analysis according to the method of Thygesen and Ander [13]. Three different parameters were calculated based on this approach. *The relative dislocation area*, which is the area of the dislocations in percent of the fibre segment area as seen using PLM, *the relative area of the largest dislocation*, which is the area of the largest dislocation found in a given fibre segment in percent of the total area of that segment, and *the normalised mean distance* between dislocations, which is the average distance between dislocations in the fibre longitudinal direction, relative to the corresponding mean value found for the wind-free treatment.

In order to obtain an indication of possible differences between the three different growth conditions with regard to ultimate tensile stress, 20 fibre bundles per trial were tested to failure using an Instron testing machine at zero span. The cross-sectional area of each bundle was estimated from the weight and the length (3 mm) of the bundle and assuming a cell wall density of $1,500\text{ kg m}^{-3}$.

For the study of the possible effects of storage conditions, stem sections from a few extra plants grown separately were used. Fibres from three different treatments were analysed: *Fresh*, i.e. taken from stem sections directly after harvest or after 1–2 days of storage at $+5\text{ }^{\circ}\text{C}$, *Dried*, i.e. dried until constant weight at $40\text{ }^{\circ}\text{C}$, and then remoistened in liquid water for 24 h, or *Frozen*, i.e. stored at $-18\text{ }^{\circ}\text{C}$ for 48 h and then thawed at room temperature.

Results and discussion

Growth conditions

The amount of dislocations quantified as the percentage of the cell wall is given in Table 1 for all three growth conditions included in the study.

Table 1 shows that windy conditions result in more dislocations within the hemp stems than wind-free conditions, but that dislocations also were found in fibres from plants grown under wind-free conditions. In the extreme case studied here with wind exposure throughout the growth phase of the plants, the increase was about 50%

Table 1 Effect of growth conditions on dislocations in hemp

Growth conditions	No. of fibres	Relative dislocation area (%)	Largest dislocation area (%)	Normalized mean distance between dislocations (wind free = 1)
Wind free	96	12.0 ^a	3.8 ^a	1 ^a
Wind	98	18.5 ^b	5.7 ^b	0.75 ^b
Drought	114	21.3 ^b	6.7 ^b	0.70 ^b

Note: Mean values marked by different letters are significantly different from each other on the 5% level or better

compared to the wind-free case. Furthermore the maximum size of the dislocations increased and they were located closer together. The study thus convincingly confirms the hypothesis that wind introduces dislocations into fibres from the stems of hemp plants.

More surprising and more interestingly, the dry conditions introduced just as many dislocations as the windy conditions. None of the three parameters calculated (the amount, the maximum size and the distance between neighbouring dislocations) differ significantly between the windy and the dry growth conditions. As mentioned in the “Introduction” section, the mechanism behind the increase in the amount of dislocations in plants subjected to wind is thought to be longitudinal compression stress in the lee side of the stem. The result that wind and drought in the present experiment introduced the same amount of extra dislocations within the hemp stems compared to wind-free conditions may be explained in two different ways: either the same mechanism was responsible for both results or two different mechanisms gave similar results. We find it more likely that the same mechanism was responsible for the results from both regimes, as it appears unlikely that two different mechanisms would result in the same amount of extra dislocations, albeit we cannot dismiss this possibility. As the dry regime was also wind free, the mechanical mechanism may obviously not explain both results. We hypothesize that if a single mechanism is behind both results, it could be related to plant stress during cellulose biogenesis. The idea is that if formation of cellulose crystals is stopped and restarted during the growth of the secondary cell wall, it might result in more frequent changes (and possibly some kind of disorder?) in the direction in which the microfibrils leaves the cellulose synthesizing complexes within the plasma membrane. That is, we propose that such incidents might be responsible for initiating the formation of dislocations. Such a mechanism would also agree with the fact that dislocations were found also in the plants grown under totally wind-free conditions. Both the windy and the dry conditions were present throughout the growth phase of the plants and both affected the macroscopic phenotype of the plants. This shows that both regimes disturbed the growth of the plants throughout the experiment, which could at least qualitatively explain that the same amount of extra dislocations was formed in both cases. However, it should be emphasized that the present study merely showed an effect of wind and drought on the amount of dislocations formed within the plant, it did not reveal anything about possible mechanisms responsible for the effect seen.

The tensile strength measurements are given in Table 2. As expected, the ultimate tensile stress values for the bundles are generally lower than the values recorded earlier for individual hemp fibres [6]. The table shows that the average fibre bundle ultimate tensile stress was higher for

Table 2 Effect of growth conditions on the fibre bundle ultimate tensile stress in hemp

Growth conditions	No. of fibre bundles	Ultimate tensile stress (MPa)
Wind free	20	594 ^a
Wind	20	532 ^b
Drought	20	547 ^b

Note: Mean values marked by different letters are significantly different from each other on the 5% level

the wind-free condition than for the windy and the dry regimes, which were not significantly different from each other. That the results from these two treatments were indistinguishable was also seen in Table 1 regarding the amount of dislocations. This result supports the hypothesis that an increased amount of dislocations reduces the fibre tensile strength, a relationship that has earlier been found for paper [8], but not for individual fibres [5–6], except in extreme cases [1]. This result might indicate that dislocations negatively affect the tensile strength of natural fibres more when bundles or networks of such fibres are tested. It could be that while dislocations are free to stretch and realign during tensile testing of individual fibres and thus do not affect their ultimate tensile stress significantly except maybe in extreme cases, the dislocations are ‘locked’ in fibres bound to each other in bundles or networks, which implies that the dislocations negatively affect the tensile strength because they cannot contribute to a relaxation of the stresses within the cell wall when prevented from movement. Alternatively it may be that the dislocations *do* realign also within these systems, and that it is this movement that weakens or even breaks interfibrillar bonds when the sample is strained. One can also imagine that both mechanisms exist and that the former dominates at low moisture contents, while the latter is more likely to occur at high moisture contents. However, the present study merely showed an effect of the growth conditions on the fibre bundle ultimate tensile stress. The experiment does not say anything about the possible mechanisms behind this result.

Storage conditions

The results from the experiment regarding the storage conditions are shown in Table 3. The table shows that freezing and thawing of hemp stems prior to isolation of individual fibres did not significantly affect the amount of dislocations. However, drying and remoistening of hemp stems prior to isolation of fibres was found to significantly reduce the amount of dislocations. This result indicates that the process of shrinkage and subsequent swelling of the fibres allows part of the dislocations to realign with the

Table 3 Effect of storage conditions on the amount of dislocations in hemp

Storage	No. of fibres	Relative dislocation area (%)
Fresh	112	15.8 ^a
Dried	104	12.7 ^b
Frozen	120	16.2 ^a

Note: Mean values marked by different letters are significantly different from each other on the 1% level

surrounding cell wall, at least temporarily, as the fibres were analysed immediately after remoistening. In an earlier study [6] it was found that tensile testing of individual fibres removed dislocations from individual hemp fibres (i.e. the dislocations were no longer visible in PLM), but that the dislocations reappeared in the fibres after a few weeks of storage at ambient laboratory conditions. It is conceivable that the dislocations in the dried and remoistened samples would also have reformed after some time. These results mean that the history of a natural fibre from harvest to usage affects the amount of dislocations present more than hitherto acknowledged, as the amount may be affected not only by the tensile and compressive stresses it has experienced, but also by the humidity levels it has been exposed to. The results also suggest that the amount of dislocations may fluctuate even during service life, as one can hypothesize that the process of disappearance and reforming of dislocations may be an ongoing and repetitive process within natural fibres when subjected to varying humidity levels due to, e.g., diurnal or seasonal climatic fluctuations.

With regard to research studies concerned with the effect of growth conditions on the amount of dislocations, the results imply that care should be taken to store samples in a way that affects the moisture content of the fibres as little as possible. Freezing of whole stem sections appears to be a feasible approach, if analysis cannot be performed within a few days after harvest.

Conclusions

The study showed that dislocations were formed also in hemp plants grown under absolutely wind-free conditions,

but that both windy and dry growth conditions introduced extra dislocations. Under these conditions also the maximum size of the dislocations increased, while the average longitudinal distance between them decreased. Both the windy and the dry regime reduced the ultimate tensile stress of fibre bundles.

In a separate study of the possible effect on the amount of dislocations of the type of storage after harvest, it was found that freezing of whole hemp stem sections did not affect the amount of dislocations afterwards found in fibres isolated from the thawed sections. This was in contrast to the results for dried and remoistened stem sections, as fibres from these were found to contain less dislocations than fibres isolated from fresh stems directly after harvest.

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