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ORIGINAL

Measurements of fungal wood decay on Scots pine and beech by means of X-ray microdensitometry

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Abstract In this work, we present a fast and promising method to evaluate the natural durability of wood based on X-ray microdensitometry. Tested on beech and Scots pine wood samples, our findings show that this methodology and the traditional EN standards methodology based on mass loss are strongly correlated. X-ray methodology is less time consuming (we can detect the effectiveness of the attack within 5–6 weeks) and less expensive (very cheap plastic Petri dishes instead of the expensive glass Kolle flasks); moreover, the proposed method allows to thoroughly examine the phases and the kinetics of the fungal attack, and to investigate the spatial repartition of the attack within the samples due to the low thickness of the sample.

Introduction

The aim of this work is to measure fungal wood degradation by means of X-ray microdensitometry.

A reliable and fast determination of wood degradation by fungi is important to make rapid screening tests on the natural durability and on the performance of new wood preservatives. Recently, several non-destructive

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methodologies were utilized to investigate the wood resistance to fungal attack such as near infrared analysis (Bailleres et al. 2002; Bailleres and Durand 2000; Fackler et al. 2005; Flaete and Haartveit 2004; Gierlinger et al. 2003; Schwanninger et al. 2004), ultrasonic techniques (Troya et al. 1993), colorimetric technique (Gierlinger et al. 2004). Unfortunately, although most of those techniques are very fast during measuring, they are time consuming in calibration and setting up and up to now not reliable enough at industrial level.

Some accelerated methods for rapid screening of preservatives have been developed in the past: they were based on conventional agar-block using small dimensioned samples and a reduced exposure period (Bravery 1979; Brown et al. 1991) where the fungal degradation was still evaluated by means of mass loss.

In these methods the period of fungal exposure is related to the dimension of the sample. The mass loss is measured after a high decay grade on the reference samples.

The mass loss method is strictly correlated to the instrument sensibility and to the initial weight of the sample. It does not allow the use of very small dimension samples not even to investigate the fungal decay at an early stage.

For this reason a promising alternative for the evaluation of the fungal degradation is the measurement of wood density by means of X-ray microdensitometry.

Indirect X-ray microdensitometry was developed by Polge (1966) and constantly improved since then (Mothe et al. 1998) it measures wood density by means of X-ray attenuation of wood. The image on the X-ray plate is digitalized at a resolution of about 25×25 μm^2: grey level of each pixel on the digitalized image is related to cell-wall proportion. Digestion of wood by fungi will reduce the amount of cell-wall, thus density will correlatively decrease. An X-ray image of the decomposed wood sample will not only reveal the global density decrease, but also its spatial variation at a fine scale. Repeated X-ray images of the same wood sample exposed to fungal digestion should reveal the amount and the kinetic of wood density decrease.

Previous studies were carried out with this technique for the determination of the kinetics of wood degradation by fungi (Bucur et al. 1997).

Materials and methods

Wood species

Scots pine (*Pinus sylvestris* L.) sapwood and beech (*Fagus sylvatica* L.) were chosen. The beech samples were obtained from the external portion of a log in order to obtain only beech sapwood with a higher susceptibility to fungal decay.

Fungus

Coniophora puteana (Schumacher ex Fries) Karsten strain BAM 15, a brown rot occurring on softwoods and sometimes on heartwoods was used.

Preparation of wood samples

Wood samples were cut from sapwood of Scots pine (*Pinus sylvestris* L.) and beech (*Fagus sylvatica* L.), respectively, without knots and other wood defects, to obtain six blocks (A–F) dimensioned 300 mm long in longitudinal direction, 50 mm in tangential direction and 15 mm in radial direction. Each block was cut into six smaller blocks ($50 \times 25 \times 15$ mm^3, respectively), contiguously named A1...A6, B1...B6, C1...C6, D1...D6, E1...E6, F1...F6. Finally, from each block two sets of six wood slides 1.4 mm thick and variable cross section area were obtained through a twin blade circular saw adapted for this experiment. A flow chart of the preparation of wood samples is shown in Fig 1.

The progressive number 1, 2, 3, 4, 5, 6 represents the position of the sample in the wood block (A, B, C, D, E, F) with respect to the position as well as the weeks of exposure to the attack of fungus. The two sets of samples, conditioned in a climate controlled room (RH: 65%, *T*: 20°C) up to 12% moisture content (MC), A1–A6 and A1a–A6a were used, respectively, as samples and reference control in X-ray microdensitometry measurement of wood density.

Exposure to fungal decay

The samples sets A1–F1, A2–F2, A3–F3, A4–F4, A5–F5, A6–F6, were exposed to the attack of *C. puteana*, in a climate-controlled room (RH: 70% *T*: 22°C), on a malt (5%)–agar (2%) medium in Petri dishes (90 mm in diameter × 16 mm in depth). After the exposure (from 1 to 6 weeks), wood samples were cleaned, weighed, conditioned until a MC of 12% was reached.

Mass loss determination

The mass loss of wood samples exposed to fungal attacks was calculated with respect to initial masses conditioned to 12% MC with an accuracy of 0.0001 g.

Microdensitometry measurements

All the samples underwent the X-ray exposure according to the indirect methodology developed by Polge (1966) and adapted by INRA in Nancy and Orléans (Mothe et al. 1998; Rozenberg 2001).

The density measurement was obtained by means of the Windendro™ system.

Two complete microdensity profiles were obtained for each sample at each exposure stage: one for the control sample and one for the sample exposed to the fungi. The density profiles of the two samples in each pair were composed

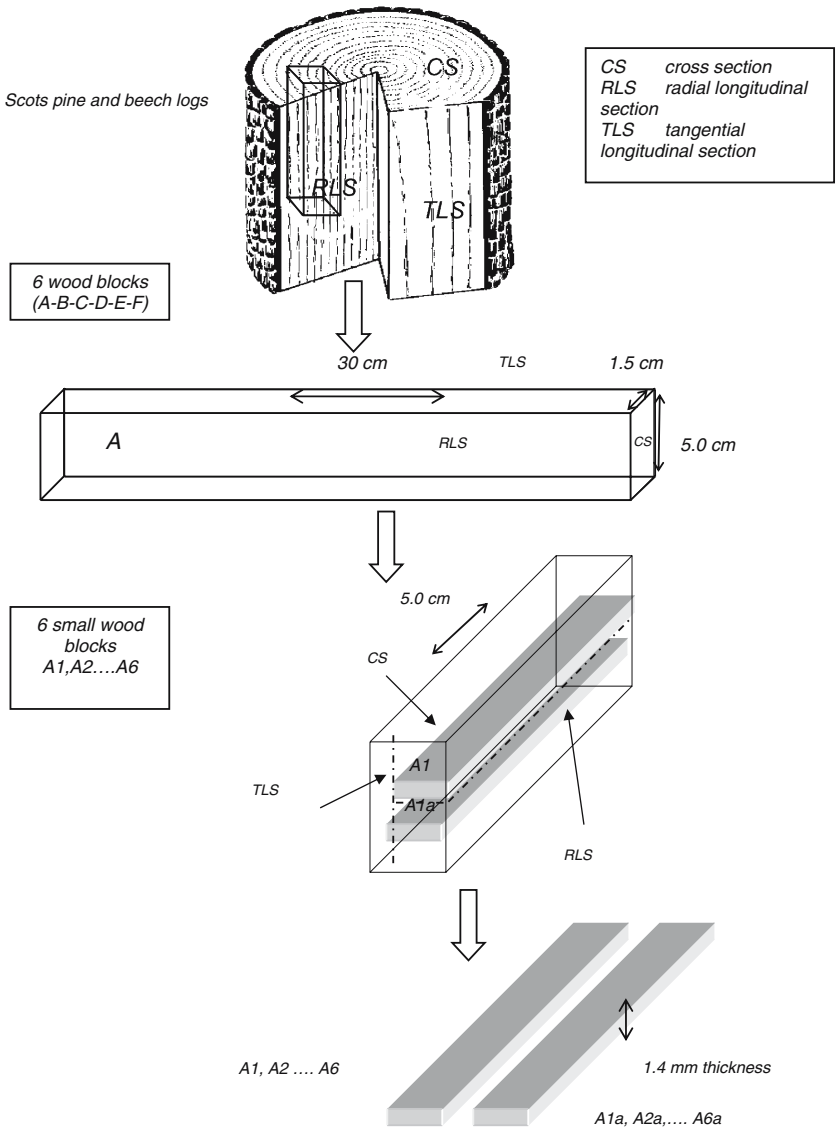

Fig. 1 Preparation of samples. Flow chart

of the same rings. However, the width of the corresponding rings did vary slightly as each sample was sawn a few mm apart from the other: the two profiles of each pair were thus standardized ring by ring to a common ring width (chosen as the width of the narrowest ring of the two associated rings) and reconstructed. A density-decrease profile was calculated by subtracting the profile of the decayed sample from the profile of the control sample. Figures 2 and 3 show examples of these paired profiles for beech sample B at

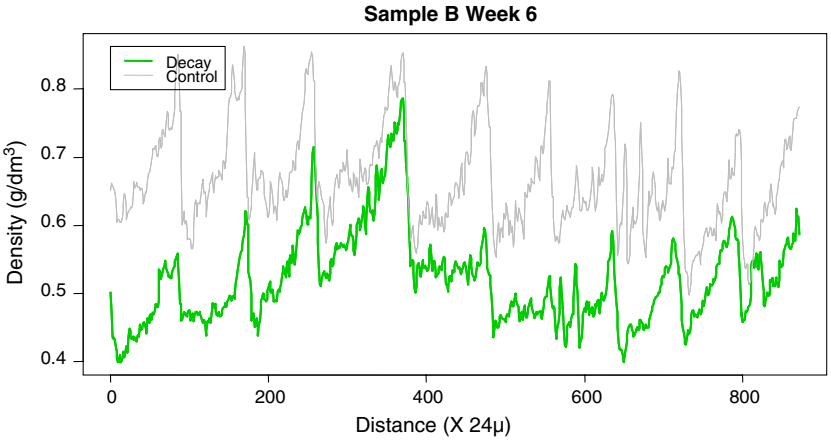

Fig. 2 Two superimposed density profiles of a controlled and decayed beech sample (sample B) after 6 weeks of exposure to fungi

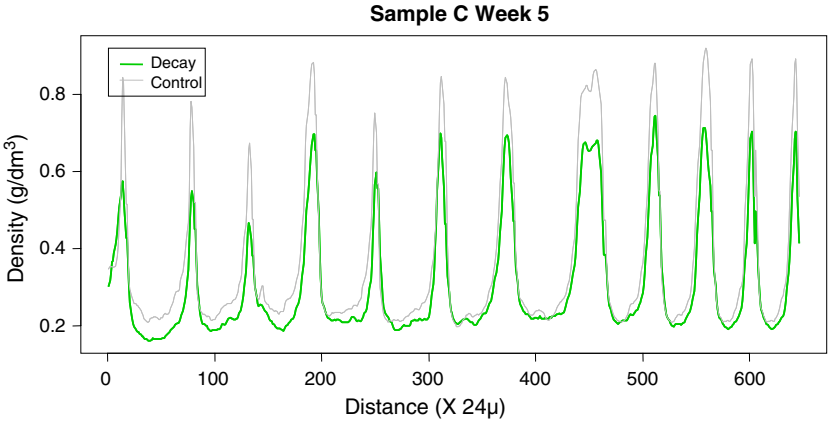

Fig. 3 Two superimposed density profiles of a controlled and decayed Scots pine sample (sample C) after 5 weeks of exposure to fungi

week 6 and Scots pine sample C at week 5 of exposure. Then the density-decrease profiles were used to compare mass loss with density-decrease, to study the kinetics of the fungal attack and to examine its localisation within the wood samples.

Results and discussion

Mass loss/density decrease

Tables 1 and 2 show the mass loss of Scots pine and beech after exposure to *C. puteana*. The mass loss of Scots pine after 5 weeks is greater than 20%. For

Table 1 Average mass loss of scots pine set samples exposed to *C. puteana* attack for different weeks

Scots pine set samples	Weeks of exposure to *C. puteana*	Average mass loss (%)
A1-F1	1	0
A2-F2	2	0
A3-F3	3	0
A4-F4	4	8.25 (10.47)
A5-F5	5	24.27 (8.55)
A6-F6	6	22.75 (7.70)

Standard deviations are in brackets

Table 2 Average mass loss of beech sets samples exposed to *C. puteana* attack for different weeks

Beech set samples	Weeks of exposure to *C. puteana*	Average mass loss (%)
A1-F1	1	0
A2-F2	2	0
A3-F3	3	1.37 (2.00)
A4-F4	4	3.19 (3.04)
A5-F5	5	8.52 (7.65)
A6-F6	6	15.95 (9.72)

Standard deviations are in brackets

beech after 6 weeks the value is lower (near 16%) because the chosen fungus is specialized in softwood and thus less active on hardwood.

Figure 4 shows the relationship between mean mass loss and mean density decrease. It is very strong, showing that density decrease is an efficient way to indirectly estimate mass loss.

According to Fig. 4, mass loss and density decrease change with time (weeks): for beech there is an increase of the mass loss and of the density decrease from 2nd week. For pine the same global trend starts only in week 4 with an inversion in week 6.

This inversion is clearly visible for both methods and is uniform for all the small samples in the same Petri dish. We attribute it to a virulence decrease of the fungal strain.

Analysis of variance (week and decay effect on microdensity decrease) demonstrates that microdensity significantly decreases between control and attacked samples and among weeks for both species.

The results clarify the kinetics of fungal attack: the start of the decay (week 3 for pine and week 2 for beech) and the shape of the density-decrease curve is different between species. There is a more rapid increase of the fungal attack after week 3 in pine than in beech. Figure 5 plots the speed of density decrease against time: both curves are parallel from weeks 1 to 4. At week 5 the speed of density-decrease is maximum for Scots pine while it slightly drops off for beech. Then at week 6 there is a dramatic reduction for Scots pine, while beech raises again to the same high level as Scots pine in week 5.

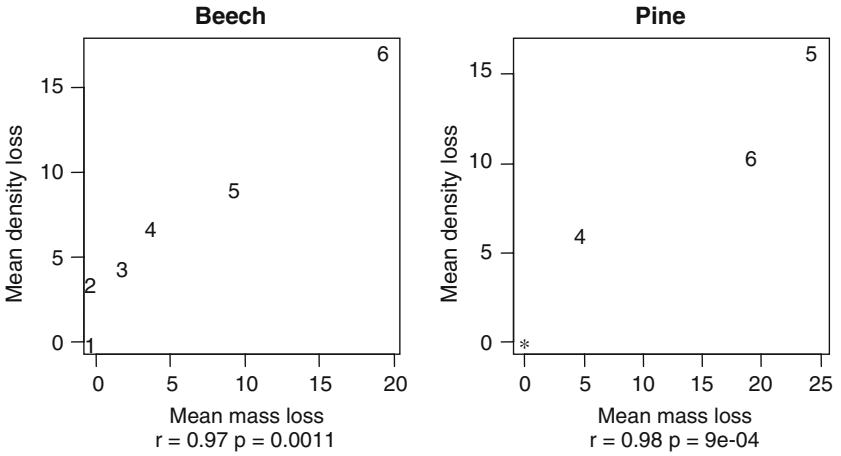

Fig. 4 Relationship between mean mass loss and mean density decrease in beech and pine samples. Numbers in the graph are number of weeks, symbol * corresponds to 1st, 2nd, 3rd week; *p* probability

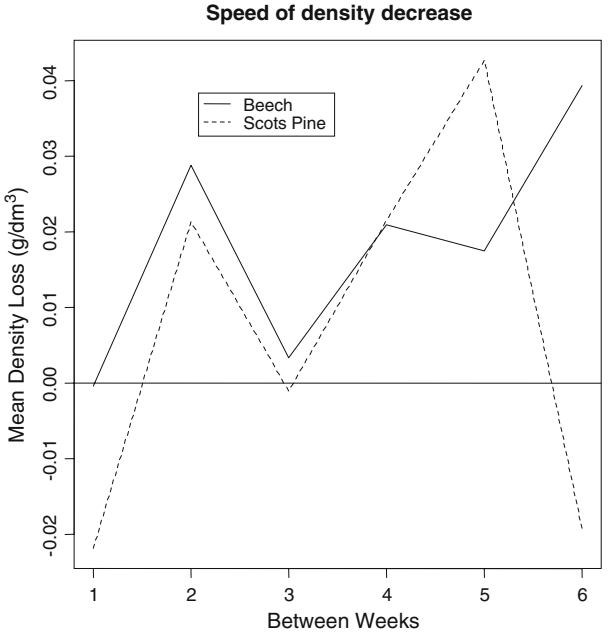

Fig. 5 Speed of average density decrease plotted against time (beech and Scots pine)

In pine, after 5 weeks, samples become to be too fragile for measurements: some parts of the samples are digested; attacked samples cannot be compared to the control samples.

Beech is different: density-decrease values of week 6 are consistent, samples are complete and can be compared with control samples. The test could last longer.

Within-sample variation of the intensity of the attack

The microdensitometry system for decay measurement allows to explore the pattern of the fungal attack: no trend in the localization of the attack in the samples can be detected in the raw density profiles (see example for beech in Fig. 2 and for Scots pine in Fig. 3). However, the study of the development with time of the general relationship between density-decrease and control density showed a global trend (example in Fig. 6). The complete results (correlation coefficients and slope of the linear relationships) are shown in Table 3. They show a significant positive relationship between density decrease and control density. The strength of this relationship is increasing with time in Scots pine (from $r = 0.18$*** at week 1 to $r = 0.53$*** at week 6), while it is uniform in beech (r varies between 0.44*** and 0.55*** with no perceptible trend). The slope of this relationship quantifies the rate of density decrease. It is increasing with time in Scots pine, from a low value of 0.06 at week 1 to a moderate value of 0.26 at week 6. It is higher and more uniform in beech (from 0.24 to 0.45), with no perceptible trend with time. In other words, latewood is more attacked than earlywood in both species; in Scots pine this trend is weak at the beginning of the exposure to the fungi, but it is increasing with time. In beech this trend is quite strong from the beginning of the attack and is rather uniform with time. This is probably due to the higher content of cellulose in latewood, where cell wall is thicker because the S_2 layer of the secondary wall being richer in cellulose (Wilson and White 1986) is more

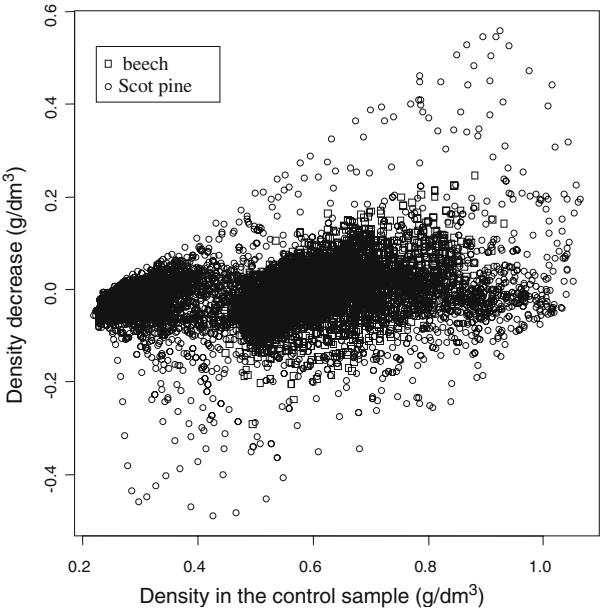

Fig. 6 Localization of density decrease at week 5 for beech and Scots pine samples: relationship between density decrease and control density

Table 3 Change with time of the correlation coefficients and slopes of the relationships between densities decrease and control density in the beech and Scots pine samples

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Correlation coefficient						
Scots pine	0.18***	0.28***	0.2***	0.31***	0.6***	0.53***
Beech	0.44***	0.46***	0.46***	0.45***	0.55***	0.46***
Slope						
Scots pine	0.06	0.11	0.08	0.15	0.22	0.26
Beech	0.24	0.23	0.29	0.25	0.45	0.39

***$P < 0.001$, all slopes are significantly different from 0

developed. In some cases, the intensity of the fungal attack is very different between Petri dishes: beech samples C, D week 5 and E, F week 5 are examples with large between-dishes and low within-dishes variation. On the other hand, Petri dishes with samples A, B for weeks 5 and 6 are very heterogeneous. This could be an illustration of the within-dishes variation of fungal activity.

Conclusions

The comparison of the density decrease with mass loss shows that both are strictly correlated; compared to the EN standards the proposed method is less time consuming (it is possible to detect the fungal decay within 5–6 weeks) and less expensive (it uses very cheap plastic Petri dishes instead of the expensive glass Kolle flasks); it allows to finely examine the phases and the kinetics of the fungal attack. Finally, it is the first time that it is shown that fungal attack is globally more intense in latewood than in earlywood and that this trend is different between Scots pine and beech.

Nevertheless, this article also shows that the method can take advantage of many improvements: the effect of the fungal strain virulence and the possible influence of the fungal tissue in the X-ray microdensitometry method have to be better controlled.

One way to improve the methodology is to use all the strains of fungi utilized in EN 113 (1996) standard with respect to the susceptibility of the different wood species (e.g. brown rot for softwood and white rot for hardwood), and inside the two categories the fungus strain most useful to obtain more visible results in as little time as possible.

The improved methodology could be very effective for geneticists and tree breeders, for early evaluation of natural durability on standing trees using increment cores, e.g. in genetic field tests where trees are valuable plant material that cannot be felled.

It could also be used to test the efficiency of wood preservatives: the Scots pine samples can be soaked with the wood preservative and then put in contact with fungi. Comparing the microdensity profiles of soaked samples

with respect to untreated samples will determine the efficacy of wood preservatives in a time lag not longer than 6 weeks. Microdensity can be used to investigate the localization of the attack within the samples as a function of the preservative repartition and of the local density.

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