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► **To cite this version:**

Jana Dlouha, Tancrede Alméras, Bruno Clair. Representativeness of wood biomechanical properties measured after storage in different conditions. *Trees - Structure and Function*, 2012, 26 (3), pp.695-703. 10.1007/s00468-011-0636-9 . hal-00641281

**HAL Id: hal-00641281**

**<https://hal.science/hal-00641281>**

Submitted on 15 Nov 2011

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**Type of article: Original Article**

**Title: Representativeness of wood biomechanical properties measured after storage in different conditions**

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#### ABSTRACT

Obtaining representative values of green wood properties is essential for studies investigating the biomechanical aspects of tree development and ecology. Here we compare the biomechanical properties of wood stored in various conditions between their collection in the field and their measurement. The study was performed on a large sample of wood specimens from different tropical species and different location in the trees, representing a wide diversity in wood structures. Elastic and viscoelastic properties are measured on green wood, and measured again after storage in different conditions: immersion in cold water during various durations, storage in an ethanol solution with or without washing in water, and air-drying with or without re-hydration. The systematic and random errors induced by these storage methods are quantified. Storage in cold water is the best way to preserve wood native properties. Soaking in ethanol is a fair alternative regarding elastic properties, but induces a significant change in viscoelastic properties. Air-drying causes important and partly irreversible changes in mechanical properties. However, regarding elastic properties, this change is a systematic bias so that the air-dried elastic modulus provides a good basis for comparative studies of green wood stiffness.

**KEY WORDS:** biomechanics - green wood - elastic modulus – damping coefficient

## INTRODUCTION

The biomechanics of woody plants is increasingly taken into account in different fields of plant biology and forest ecology (Baraloto et al., 2010; Chave et al., 2009; Moulia et al., 2006; Niklas and Spatz, 2010). In the context of a decreasing biodiversity and increasing atmospheric carbon dioxide, the rationale for this growing interest can be briefly summarised as follows: mechanical support is the primary function of wood, wood contains the largest part of forest biomass, and forest ecosystems host the largest part of terrestrial biodiversity and biomass. Evaluating functional mechanical properties of green wood is a prerequisite to many studies involving plant biomechanics. This evaluation is the basis of either quantitative estimation of composite functional traits at the tree level, such as its critical height (Greenhill, 1881; Niklas and Spatz, 2004), gravitropic performance (Alméras et al., 2009; Alméras and Fournier, 2009; Alméras et al., 2005; Coutand et al., 2007; Fournier et al., 2006), safety factors against buckling (Jaouen et al., 2007; Niklas, 1994) or breaking (Sterck et al., 2006; Van Gelder et al., 2006), or comparative studies assessing e.g. trade-offs between the mechanical function and other functions of wood (Alvarez-Clare and Kitajima, 2007; Russo et al., 2010; Sterck et al., 2006; Van Gelder et al., 2006), variations in mechanical design between lifeforms (Rowe et al., 2006), during ontogeny (Isnard et al., 2005) or during evolution (Ménard et al., 2009).

A huge amount of data regarding mechanical properties of different wood species and types has been collected to characterize wood as a building material (Bergman et al., 2010). Wood in this context is mostly used and studied in air-dried conditions yielding a moisture content of approximately 12%. It is therefore generally submitted to kiln drying, and mechanical properties are measured after a given stabilisation period in room conditions. However the drying process is known to induce irreversible changes in wood structure and properties (Kifetew et al., 1998; Muller et al., 2003; Sakagami et al., 2009a; Thuvander et al., 2002). Moreover, the amplitude of the change in mechanical properties of wood induced by drying can be affected by occurrence of some particular microstructures such as G-layer (Yamamoto et al., 2010) resulting from high stresses generated during the tree growth (Archer, 1986). Therefore, it is questionable to extrapolate mechanical properties measured in dried or rewetted state to the properties of wood in native conditions in the living tree *i.e.* with fully saturated cell walls. The straightforward way for obtaining such data is to perform mechanical tests in the forest immediately after harvesting, but the accuracy of experimental equipment usable in field work conditions is generally limited. Moreover, some properties such as viscoelastic parameters require longer times of measurement or finer experimental equipment and cannot be obtained out of a laboratory. Therefore the wood generally needs to be stored between harvesting and laboratory testing. However, one may wonder if the parameters measured after a given storage period are still representative of the native state and what is the relation between the native properties and those measured after storage.

Change in mechanical properties of wood during its storage may result from the attack of fungi and/or from the removal of extractives (Brémaud, 2006; Matsunaga et al., 1999; Matsunaga et al., 1996; Yano, 1994). Fungi need an optimal temperature together with appropriate moisture and oxygen content for their development. Drying removes from the material the moisture necessary for fungi development. Another way to preserve wood is to keep it in water-saturated conditions thus depriving the fungi of the oxygen (Richardson, 1993). Low temperature is generally preferred during storage, but the possibility of storage in an anoxic aquarium with water at room temperature was also reported for archaeological wood (Björndal et al., 2007). Alternatively, samples can be immersed in alcohol, method frequently used for preserving organic materials in historic and medicine collections and occasionally used for fresh wood samples (Coutand et al., 2004).

The aim of present study is to assess the effect of storage procedures on the mechanical properties of green wood, in order to suggest the most appropriate storage method to preserve the green wood properties. Three storage methods were studied: air-drying, storage in water at low temperature and storage in ethanol solution. In order to get rather general results, two dynamic mechanical properties (elastic modulus and damping coefficient) were measured on various wood samples taken from six tropical tree species having diverse microstructure and quality.

## MATERIALS AND METHODS

### *Plant material*

Wood material of six angiosperm tree species was collected in the tropical rainforest of French Guyana. The sampling was designed to be representative of a large diversity of wood qualities in order to draw conclusions valid for most angiosperm woods. The species were chosen to cover a wide range of wood specific gravities and mechanical properties and exhibited different extractive contents as illustrated in Table 1. Positioning of the selected species among the more than 1,000 species recorded in database of CIRAD (International Centre of Agronomic Research for Development) from the viewpoint of mechanical properties is displayed in Fig. 1.

Straight trees as well as tilted trees were sampled in order to account for a wider range of wood microstructures, including normal wood, tension wood and opposite wood. Tree diameter at the breast height ranged between 4 and 7 cm.

#### *Wood specimens preparation*

After tree felling, 50 cm-long logs were sawn in the field and its ends were covered with wet paper and plastic bags to limit the drying. In each log, 12 specimens were prepared at different location along a diameter and at two different vertical positions, in order to include specimens of varying age and quality. A total of 18 trees and 216 wood specimens were used for the study. Specimens were cut along the fibre direction (150 x 12 x 2mm, L x R x T) within a week after felling the trees, wrapped in a wet paper, sealed in plastic bags and stored in the fridge. Specimens were then transported to the laboratory in Montpellier (France) for measurements and kept in cold water until further testing.

#### *Measurement of mechanical properties*

Mechanical properties were first measured 1 month after felling the trees accounting for the time necessary for processing and transport of the specimens. This first measurement is taken as a reference and designated by index 0 in the following text and figures. The specific modulus  $E/\rho$  was measured by forced flexural vibrations of free-free beams at the first resonant frequency ranging from 200 to 650Hz. Used experimental device is pictured in Fig. 2, details about the method are given elsewhere (Brémaud, 2006; Brémaud et al., 2010). The specific gravity  $\rho$  was estimated from measurements of the sample volume and mass. The dynamic elastic modulus  $E$  was calculated as a product of specific modulus and specific gravity. Volume of the specimen was estimated by double weighting to obtain maximal accuracy (especially in the case of dried specimens that can be more or less curved). The damping coefficient  $\tan\delta$  was calculated by the half-power bandwidth method defined as the ratio of the frequency range between the two half power points to the natural frequency at this mode.

#### *Storage treatments*

The different samples and storage treatments are summarised in Fig. 3. For investigating the effect of long-term storage in cold water ( $4\pm 0.5^\circ\text{C}$ ), a sample of 144 specimens (6 species\*2 trees\*12 specimens) was measured repeatedly after 3, 8 and 12 months storage in cold water. Afterwards, half of the sample was soaked during 40 days in 40% ethanol, measured, and then washed in water during 80 days at  $20^\circ\text{C}$  before being measured again. In parallel, a group of 72 specimens was let drying in room conditions ( $20^\circ$ , 65% relative humidity) during 14 days and measured in air-dry state. Afterwards, they were re-saturated in water (at  $20^\circ$ ) during 14 days and measured in rewetted state.

## RESULTS

#### *Effect of air-drying and rewetting*

Sets of measurements were performed on specimens in different conditions: in never dried state (taken as a reference), after air-drying and in rewetted condition. As we can see from Fig. 4 and Fig. 5, air-drying has significantly increased the elastic modulus ( $+12.7\pm 7.8\%$ ) and strongly decreased the damping coefficient ( $-16.9\pm 10\%$ ). The relationship between the elastic modulus in air-dried and never-dried state is linear with some dispersion ( $R^2=0.97$ ). The change in damping coefficient due to air-drying also induced some dispersion ( $R^2=90$ ), and is clearly non-linear: the reduction in damping due to drying is disproportionately larger for wood specimens with higher damping coefficients. These changes in mechanical properties between green and air-dry states are expected and well-documented (Bergman et al., 2010). They are due to the stiffening of the matrix components of wood (lignin and hemicelluloses) associated to water desorption. Average values of measured parameters for each species together with the density and moisture content in air dry conditions are summarized in Table 2.

The comparison between the rewetted state and the never-dried state shows that dried wood does not recover its initial properties after re-saturation. The elastic modulus measured in rewetted state was slightly lowered ( $-5.8\pm 2.1\%$ ) but a very good linear correlation was observed with the never-dried state ( $R^2>0.99$ ). The damping coefficient was higher in rewetted state ( $15.9\pm 2.1\%$ ) than in the never-dried state and the relation between these

parameters was very scattered ( $R^2=0.82$ ). Observation of Fig. 4 shows that the drying-rewetting sequence actually induced more scattering than the drying event alone. The scatter of this relation is probably due to variations in the response to the drying-rewetting treatment between specimens having different micro-structural and chemical characteristics, for example due to differences in wood type, e.g. tension wood *vs.* normal wood (Clair et al., 2003; Coutand et al., 2004; Yamamoto et al., 2010). The decrease in elastic modulus and increase in damping coefficient can be explained by irreversible damages occurring during drying process such as formation of micro-cracks (Sakagami et al., 2009a), that may be accentuated by the swelling of wood during re-saturation. Drying/rewetting treatment is known to reduce mechanical properties in particular wood strength of oven-dried (Kifetew et al., 1998) as well as air-dried samples (Muller et al., 2003). Microcracks are in generally initiated in the middle lamella and follow the ray parenchyma (Sakagami et al., 2009b). Location of microcracks in the middle lamella can explain why damping coefficient is highly affected by drying/rewetting treatment while the effect on elastic modulus is limited. Sensitivity to cracks formation is also species dependant which can explain great scatter of values (Sakagami et al., 2009b).

#### *Effect of long-term storage in cold water*

The effect of storage of never-dried wood specimens in water at low temperature ( $4\pm 0.5^\circ\text{C}$ ) was investigated. The elastic modulus and damping coefficient were measured on the sample after 3, 8 and 12 months of storage, and compared to the initial measurement, taken as a reference state. The relation between the reference elastic modulus and that measured after different storage durations was linear with low dispersion ( $R^2>99$ , Fig. 6). Mean relative changes in elastic modulus are displayed in Fig. 7. A slight decrease ( $-2.1\pm 0.3\%$ ) was observed after 3 months storage. A further decrease ( $-4.2\pm 0.3\%$ ) was observed after 8 months, but no further significant change was noticed after 12 months ( $-3.4\pm 0.3\%$ ). No conclusion can be drawn on this change compare the initial state just after tree felling, but because the changes are very low after one year, one can suppose the difference to be low.

The relation between the damping coefficient in reference state and after different storage durations was linear with some dispersion ( $R^2=0.98, 0.97, 0.96$  for 3, 8 and 12 months; Fig. 6). Slight but significant variations in damping coefficient were noticed ( $-1.4\pm 0.6\%$ ,  $-2.7\pm 0.9\%$ ,  $-0.7\pm 0.9\%$  after 3, 8 and 12 month, see Fig. 7). We hypothesised that the observed increase of the damping coefficient after 8 months storage compared to 3 months storage may be an artefact due to the experimental conditions because this measurement was done during the summer period. Thus, slightly higher ambient temperature during this measurement might be responsible for the observed increase of the damping coefficient.

Moisture content during the first measurement ranged from 43 to 160% depending on the species ensuring that all sample was kept above the saturation point. Therefore, in the computation of the elastic modulus dimensions of the specimen were assumed to be stable while the variations in specimen weight were taken into account. The weight slightly increased between the first and the second measurements and remained stable afterwards. This was ascribed to the completion of the saturation process of the specimens that was not homogeneous between species as we can see from the Table 3. Weight variations did not affect the damping coefficient and were not correlated with the elastic modulus variations indicating that slight changes in the free water content do not affect mechanical properties.

The removal of water-soluble extractives possibly occurred during storage and is known to induce some modifications of wood mechanical properties for some species (Brémaud, 2006; Matsunaga et al., 1996; Obataya et al., 1999). Its effect on the elastic modulus could not be detected because of overlapping with the uptake of water. However, as the damping coefficient is known to be sensitive to the extractive content, and no significant change in damping coefficient was observed during the storage, it is likely that the removal of water soluble extractives was inhibited by the low temperature (extraction procedures are in general using hot water).

#### *Effect of soaking in ethanol and washing in water*

Ethanol was used as an alternative way to protect specimens from the biological attack. Measurements of the specimens were performed after soaking in 40% ethanol during 40 days, and after washing in water at ambient temperature during 80 days (Fig. 8). The elastic modulus after soaking in ethanol and after washing were linearly related to the reference values, with very low dispersion ( $R^2>99$ , not shown). Compared to the reference value, the elastic modulus was slightly higher after soaking in ethanol ( $+1.8\pm 0.9\%$ ) and slightly decreased after washing ( $-0.9\pm 0.8\%$ ). The damping coefficient was more affected by the ethanol treatment. Values after soaking and after washing were linearly related to reference values, but with some dispersion ( $R^2=0.98$ , not shown). The damping coefficient was significantly increased with ethanol ( $+18.4\pm 1.4\%$ ), and this increase was not completely reversed after washing in water ( $+6.2\pm 1.0\%$ ).

Some weight variations were observed after the soaking in ethanol ( $-1.5\pm 0.1\%$ ), likely due to the lower density of ethanol compared to water. Part of this weight variations remained after the washing in water ( $-0.2\pm 0.1\%$ ) indicating residual ethanol content and/or removal of some extractives.

## DISCUSSION

### *Comparison of storage methods for comparative and quantitative biomechanical studies*

We studied different methods of wood storage to determine which is more suitable to obtain representative values of the mechanical properties of green wood when measurements cannot be performed in the field immediately after sampling. To assess the representativeness of measurements after storage, it is important to distinguish between two possible applications of these measurements: quantitative estimations of the mechanical parameter or comparative studies between species.

Quantitative estimations can be useful for example to provide input for biomechanical models and evaluate composite biomechanical traits at the tree level, *e.g.* a safety factor against buckling (Jaouen et al., 2007), or the efficiency of gravitropic reaction (Alméras and Fournier, 2009). For this kind of application, it is important to have minimal bias on the estimation of the mechanical parameter. The relevant parameter to quantify this effect is the mean relative difference between the value after storage and the reference value. Our results show that drying is prohibited in this context, regarding both the elastic modulus and the damping coefficient, since it modifies mechanical properties in a non-negligible and partly irreversible way (Fig. 5). Storage in cold water is advisable for both parameters, since their value is not much affected by this storage method (Fig. 7). For the modulus of elasticity, soaking in ethanol can be used as an alternative method *e.g.* if no fridge is available. This storage method does however induce a significant change in viscoelastic properties (Fig. 9).

For comparative purpose, for example in studies of diversity (Alvarez-Clare and Kitajima, 2007; Russo et al., 2010; Sterck et al., 2006; Van Gelder et al., 2006) or comparative biology (Rowe et al., 2006), the precise estimation of the value of the parameter is not that much important and some bias can be acceptable provided its effect is systematic and does not change the ranking of individuals or species, *i.e.* it does not induce additional dispersion. The relevant parameter in this context is the coefficient of determination ( $R^2$ ) between the values measured after storage and the reference values. Our results show that storage in cold water is appropriate regarding that for both elastic and viscoelastic properties. Storage in ethanol (with or without washing in water) is a fairly good alternative, since it does not lead to a substantially larger dispersion. Drying is prohibited for the study of viscoelastic properties, because it induces a large dispersion (Fig. 5). However, it can to some extent be used for comparative studies on the elastic modulus: although it induces an important bias, this bias is almost systematic and linear so that it does not change the comparison between species. For example, analysis of variance and post-hoc analysis applied to our dataset (not shown) showed that the ranking and discrimination between species elastic modulus was statistically unchanged by drying. This means that databases about elastic properties of dry wood produced by wood technologists can be used to compare biomechanical properties of different species.

### *Conclusions*

Storage of fresh wood specimens in water at low temperature has revealed to be the most appropriate way to preserve its elastic and viscoelastic properties. Besides a slight decrease of elastic modulus (approx.  $-4\%$ ), no further change in mechanical properties was detected during one year. Soaking in ethanol is a satisfying alternative method regarding elastic properties, but it induces a significant change in viscoelastic properties. Drying causes important and partly irreversible changes in mechanical properties. However, in the case of elastic properties, this change is a systematic bias so that the air-dried elastic modulus provides a good basis for comparative studies on the elastic properties of green wood.

## ACKNOWLEDGMENTS

This work was carried out in the framework of the *Woodiversity* project, funded by the French National Research Agency [ANR05-BDIV-012-04]. Authors would like to thank Iris Brémaud for useful discussions about the manuscript and Shanshan Chang for help with experimental work.

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