Dendrochronologia 31 (2013) 175–178

Contents lists available at ScienceDirect

Dendrochronologia

journal homepage: www.elsevier.com/locate/dendro

Technical note

The advantage of using a starch based non-Newtonian fluid to prepare micro sections

Loïc Schneider, Holger Gärtner*

Swiss Federal Research Institute WSL, Landscape Dynamics/Dendroecology, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

ARTICLE INFO

Article history:
Received 7 February 2013
Accepted 25 April 2013

Keywords:
Wood anatomy
Cornstarch
Preparation techniques
Cell parameters
Sectioning

ABSTRACT

The breakage or distortion of cellular structures is one of the biggest problems in creating micro-sections for wood anatomical analyses in tree-ring as well as other branches of anatomical research. These broken or distorted structures cause artifacts in photomicrographs that require time consuming image manipulation or corrections prior to further analyses. The simple application of a cornstarch, water, and glycerol (CWG) solution (10:8:7 ratio), a so called non-Newtonian fluid to the surface of wooden specimen before sectioning improves the overall quality of the resulting micro-sections. In particular the problem of secondary cell walls splitting off the primary wall while sectioning is drastically reduced. The quality of the sections using this solution is comparable to that obtained from the more laborious and expensive paraffin embedding.

Background

In recent years, the analysis of anatomical variations within annual rings of trees has demonstrated a great potential for the reconstruction of past environmental conditions. When focusing on longer time-scales, most (paleo-)climatological attempts concentrated on intra-annual density fluctuations in conifers (De Micco et al., 2007), or vessel sizes in ring-porous broadleaved species (Matisons and Brumelis, 2012). The relatively large dimension of vessels enables the measurement of their size by preparing plain surfaces on cores without preparing micro-sections (Gärtner and Nievergelt, 2010). Smaller cell types, such as earlywood and latewood tracheids in conifers, however, cannot be accurately measured on a plane cores' surface. To overcome this problem, it is necessary to use manual measuring techniques that require binoculars or microscopic techniques. The effort is reasonable when analyzing a few rings, but becomes overwhelming when producing longer chronologies and/or highly replicated datasets. Furthermore, even for semi-automated cell measurements, the contrast of cell wall to lumen is not good enough to be automatically recognized by any cell-anatomical analysis software currently available (Gärtner and Nievergelt, 2010). Hence, to analyze variations in the structure of earlywood and latewood tracheids, it is indispensable to prepare micro-sections. Nevertheless, no common procedure is capable of producing high quality sections without embedding to realize an accurate measurement of tracheid dimensions. One of the biggest issues is the interaction of digital images showing the cell structures and related software measurement tools. If the images contain artifacts (defects in the structure as e.g., broken or bend cell walls), automated software measurement will result in erroneous data. The images therefore have to be based on perfectly prepared cell structures.

When cutting thin sections of the xylem of conifers, frequently the secondary wall, especially of earlywood cells, breaks or separates from the primary wall and bends into the cell lumen (Fig. 1). Other problems occur when sectioning the cambial zone for analyses of growing season duration and cellular developmental stages within the vegetation period (Thibeault-Martel et al., 2008; Moser et al., 2010). The cells of the cambial zone as well as the adjacent differentiating xylem cells (cell expansion and cell-wall thickening) are not stabilized by lignin (Wardrop, 1981). Consequently, these cells tend to be squeezed, torn, and distorted while cutting. All these potential artifacts are caused by low cell wall densities, sample size, microtome blade orientation, human dexterity/experience, or even from dull blades. Until now, the only way to overcome sample damage and structural artifacts was to embed the samples before sectioning (Feder and O'Brien, 1968) or to digitally edit images to correct measurements of the cell dimensions. Both techniques are time consuming, and are therefore not conducive to the development of millennium long chronologies of wood anatomical parameters. Moreover, digital image manipulation affects cell size, thus introducing an unknown amount of error or bias to the measurements.

* Corresponding author. Tel.: +41 044 739 24 25; fax: +41 044 739 22 15.
E-mail address: holger.gaertner@wsl.ch (H. Gärtner).

1125-7865/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.dendro.2013.04.002

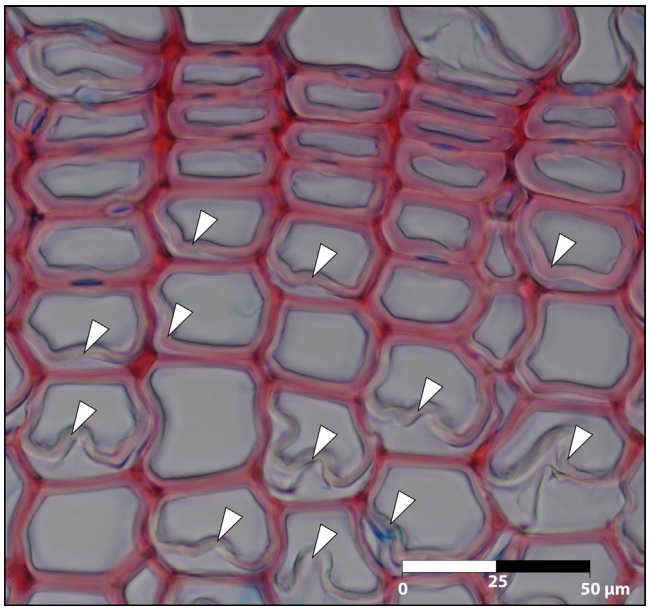

Fig. 1. Typical damage incurred when cutting earlywood cells of conifers (here: *Larix decidua* Mill.) without embedding: The secondary wall is stripped off the primary wall (arrows).

Producing a perfect thin section showing the earlywood portion or the cambial zone of any tree, shrub or herb without damage is a challenge. This is due to the high pressure exerted on the sample even when using extremely sharp blades. While thin-section quality is improved by using new, sharp blades and by oblique orientation (i.e., using the full length of the blade for cutting the sample), this still does not guarantee a good thin-section without cutting artifacts.

Although the potential problems presented so far have been described for conifer tracheids, the most frequent cell type and plant group in tree-ring research, these challenges apply for all specimens of trees, shrubs or other plants to be sectioned for further analysis. Artifacts or damage to samples while cutting extends beyond broken or stripped-off cell walls, and also includes simple distortions of the cell lumen. The problem of creating artifacts while sectioning hinders diverse research topics such as analyzing white rings in aspen wood (Sutton and Tardif, 2005), frost rings (Rixen et al., 2012), traumatic resin ducts (Gärtner and Heinrich, 2009), tension wood (Heinrich and Gärtner, 2008) or even ring properties in tropical trees (Fichtler and Worbes, 2012).

Common tissue stabilization techniques

One of the oldest techniques to overcome the problem of soft or brittle tissue is the application of carbowax (Stamm, 1956), for example, to stabilize charcoal prior to cutting (Cousins, 1975). Carbowax application is helpful for certain analysis, yet its application also has several drawbacks. Apart from the fact that carbowax is quite expensive, it requires heating to be liquefied and successfully applied to the surface of the specimen. Moreover, this treatment in most cases is only superficial because carbowax cools down rapidly and for this does not penetrate deeply enough into the wood.

The most effective way to stabilize cellular structures is embedding the specimen in paraffin (Rossi et al., 2006; Rathgeber et al., 2010). During the embedding process, all cavities within the wooden sample are filled with paraffin and are therefore stabilized after hardening. The cutting process almost always results in sections without artifacts as broken cell walls. Although special tissue processors (Čufar et al., 2011) exist for automated embedding, this procedure is still time consuming. The embedding process itself takes at least 20 h (Rossi et al., 2006). In addition, the paraffin has to be dissolved from the sections after cutting, before the structures can be further treated (e.g., stained) and analyzed. A faster procedure of paraffin embedding was presented by Xing et al. (2010). They were able to reduce the time needed for embedding to 2 h, but this technique only applies to unlignified plant tissue smaller than 3 mm in diameter.

Here we present an alternative to produce high quality thin sections that (i) minimizes the occurrence of artifacts (like cell distortions, broken or bend cell walls) and (ii) does not require time consuming and/or expensive techniques such as embedding and (iii) is easy to apply and perform.

Introducing a non-Newtonian fluid for thin sectioning

Non-Newtonian fluids (Jeffrey and Acrivos, 1976; Larson, 1999) are solutions where particle to particle interactions within the liquid phase can produce effects such as shear thinning (e.g., ketchup) or shear thickening (e.g., cornstarch) (Bischoff White et al., 2010). The shear thickening effect of cornstarch solutions is expressed by a fast thickening, and therefore stabilization, of the liquid when exposed to pressure. For all solutions showing a shear thinning effect the opposite is true, which is familiar to everyone who have hit the bottom of an open ketchup bottle.

Employing a cornstarch solution in micro-sectioning is a novel application of the unique properties of non-Newtonian fluids. The basic principle is to fill the cells of the specimen with a liquid that stabilizes as soon as pressure is exerted to the cells while cutting. The application procedure is easy and straightforward. Since the solution is nontoxic it is perhaps easiest to apply by hand (a stiff spatula or any other object might be used if preferred). A small quantity of the fluid is dropped on the surface of the sample. If the sample is stable enough, the solution can also be gently rubbed or spread to facilitate penetration into the sample. For very small or delicate samples gravity alone will fill the cells. Because the fluid flows into the open cells, it effectively plugs the cell lumen with starch grains (Fig. 2). These starch grains are responsible for the stabilizing effect by turning the solution into a solid phase as soon as the blade exerts pressure on the cell wall. This shear thickening effect of the starch solution enables cutting the wooden material as if it would have no pores, comparable to embedded specimens. The starch solution stabilizes the cell and prevents the cells from being distorted and the secondary wall from breaking or separating from the primary wall. An example illustrating the effect of applying the non-Newtonian fluid to a root sample is presented in Fig. 3. The two micro-sections were cut in succession. Neither the blade nor any of the other settings of the microtome were changed between the two cuts. The only difference is that before the second cut the starch solution was added to the sample. As a result, the second section does not show any distortions such as broken or bent secondary cell walls. Images taken from the latter section can easily be analyzed using automated image analysis software.

There are a few advantages of the cornstarch based non-Newtonian fluid compared to other agents as carbowax or paraffin. Firstly this solution easily fills up the cells when placed on the cut surface of a sample; heating or vacuum techniques are not required. Moreover the starch solution is nontoxic, inexpensive, easy to obtain, and does not physically or chemically damage the cutting blade or the wood specimen. Lastly the cornstarch solution