

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Turk J Bot (2014) 38: 623-626 © TÜBİTAK doi:10.3906/bot-1308-25

Research Note

An alternative approach in sectioning of archaeological woods: the case of *Quercus pontica*

Bedri SERDAR*, Reha MAZLUM

Department of Forest Botany, Faculty of Forestry, Karadeniz Technical University, Trabzon, Turkey

Received: 15.08.2013	٠	Accepted: 19.02.2014	٠	Published Online: 31.03.2014	٠	Printed: 30.04.2014	
----------------------	---	----------------------	---	------------------------------	---	---------------------	--

Abstract: An alternative approach to the sectioning of archaeological woods is presented in this paper. The samples that we study were obtained through the excavations carried out in Yenikapı (Marmaray), İstanbul. Neolithic-aged wood samples were studied and identified using the frozen sliding microtome method. This method was utilized for the cross-sectioning of a leaf in 1984 for the first time. This work extends its usage by exploiting the method for the purpose of sectioning Neolithic-aged wood samples and providing a case of *Quercus pontica* C.Koch.

Key words: Quercus, Neolithic age, archaeology, wood, sectioning

1. Introduction

Anatomical studies require high-quality slides of sectioned plant parts. Although sectioning techniques have a long history, sectioning of plant parts that have both hard and soft tissues (like archaeological woods) is difficult. Rigid plant material has to be softened for sectioning. Some techniques use hydrofluoric acid to soften the material, infiltrating it with celloidin (Lodewick, 1924; Burn, 1964). Although this technique has been widely used (Zimmermann and Tomlinson, 1965, 1968), it has had only limited use for decades because of the toxicity of hydrofluoric acid, as well as being expensive, difficult, and time-consuming. Later, Kukachka (1977) recommended the use of a 4% ethylenediamine solution instead of hydrofluoric acid to soften rigid woods. Afterwards, Carlquist (1982) broadened the use of Kukachka's technique from the stem to include seeds and barks. Barbosa et al. (2010) also developed a new method to obtain good anatomical slides of heterogeneous plant parts. For this, they combined 3 of the prior techniques (Rupp, 1964; Kukachka, 1977; Carlquist, 1982). The advantage of this technique is that it can be used for a range of materials, from small to thick stems, always with satisfactory results. However, if all of the plant parts have suitable structures, we can mount plant materials to sliding microtome for sectioning with this technique. The main problem for waterlogged archaeological wood is that it

cannot be held by traditional sliding microtomes. First, we thus have to attach the waterlogged archaeological wood parts to the sliding microtome and, second, we must avoid tissue damage or tearing during sectioning. For this reason, we tried to section archaeological woods by frozen sliding microtome. A frozen sliding microtome (sliding microtome + carbon dioxide tube) was first used by Gerçek (1993) during his PhD studies on Camellia sinensis (L.) Kuntze leaf in Turkey. Gerçek (1993) used only this method for the cross-sectioning of the leaves. A sliding microtome is generally used for sectioning woods for anatomical studies. However, this method can only be used for nondegraded and hard woods that allow easy sectioning. Sectioning and identification of degraded or deteriorated wood (i.e. archaeological and waterlogged woods) is very difficult. Analysis of these types of wood is made superficially using scanning electron microscopy (Gonçalves et al., 2012) or episcopic microscopy (Schweingruber, 2012). Sectioning is hardly possible with woods logged with water and other materials dating back to prehistoric times. Under wet, near anaerobic conditions, the activity of aerobic wood-degrading fungi and bacteria are effectively suppressed. Waterlogged archaeological woods are generally obtained from archaeological excavations carried out underwater (Björdal, 2012). The goal of the present study is to introduce the frozen sliding microtome method for waterlogged archaeological and degraded wood sectioning in all directions.

^{*} Correspondence: bserdar@ktu.edu.tr

2. Materials and methods

The studied wood samples were taken from Yenikapı (Marmaray) excavations by the İstanbul Archaeological Museum and were supplied for the present study. To identify these wood samples, we obtained sections of 20-25 µm in 3 directions. For this, we used a sliding microtome with carbon dioxide to freeze wood samples, as first used by Gerçek (1993). A carbon dioxide tube is mounted to a sliding microtome with a pipe, turning it into a "freezing sliding microtome". These wood samples are first frozen and are then sectioned. After sectioning, they are immediately immersed in water for preservation, and then they are stained with safranin (Ives, 2001; Güvenc and Kendir, 2012; Vasic and Dubak, 2012). The permanent slides were examined and photographed with an Olympus BX 50 research microscope (Bs200Pro Image System Software). The terminology for anatomical descriptions follows the IAWA Committee Guidelines (1989).

3. Results

In the wood samples from the Neolithic Age obtained from the prehistoric layers of the Yenikapı excavation area, there were some difficulties because of the structural

features of the woods. The main difficulty was the fact that the 3-dimensional sections from the woods were not taken properly, because of the fact that the woods lost their firm structures due to fungi, bacteria, and long years spent under soil. The method proposed here makes wood sectioning possible in 3 directions (i.e. cross, radial, and tangential), and it is easy, practical, and time-saving in comparison with other methods. When sectioning this type of wood, a useful method developed by Schweingruber (2012) is usually used. However, this method allows only for crosssectioning, which is a disadvantage for identification as radial and tangential sections are also necessary for proper identification. In this new method, a wood sample is frozen with carbon dioxide and sectioning is carried out in 3 dimensions. A carbon dioxide tube is mounted to the sliding microtome with a pipe, turning it into a "freezing sliding microtome". The pipe not only freezes the wood sample but also bonds the wood sample onto the sliding microtome (Figures 1a and 1b), making sectioning easy in any direction (Figure 1c).

Samples that retain all anatomical properties go through chemical processes to prepare permanent slides. Others are used to prepare slides without any chemical



Figure 1. Freezing sliding microtome: a- sliding microtome + carbon dioxide tube, b and c- freezing archaeological wood samples and sectioning.

processes. Finally, microscopic photographs are taken from these slides with an image analysis system and identification is made using the photographs taken (Figure 2). After examining the permanent slides by microscope, we identified the wood of one of the samples from the Yenikapı excavations as *Quercus pontica* C.Koch (Figure 2).

3.1. Wood anatomical properties of Quercus pontica

Wood ring porous with indistinct growth rings, vessels of earlywood have bigger diameter than vessels of latewood. Earlywood vessels are found as one line at the beginning of growth rings and they separate from each other with the rays going all over growth rings, then they form a figure of islands at the growth ring boundaries. Vessel diameter does not get smaller instantly during the process of transition from earlywood to latewood. It is rather slow (Figures 2a and b). Perforation plate is simple. There is no helical thickening on the walls of vessels. Axial parenchyma is in the form of a chain, in the apotracheal tangential direction (diffuse in aggregates). Rays are uniseriate and multiseriate homocellular (homogeneous type I) (Figures 2c and 2d). Uniseriate rays are common. Multiseriate rays are decreased and yield their places to aggregate rays. Aggregate rays are common, contrary to multiseriate rays. Ground tissue is composed of libriform fibers, tracheid fibers, and vasicentric tracheids.



Figure 2. Microscopic photographs of *Quercus pontica* wood from Neolithic age. a- GRB, V. b- R, GT. c- HR. d- UR. Abbreviations: GRB: growth ring boundaries, V: vessels, R: rays, GT: ground tissue, HR: homocellular ray, UR: uniseriate rays.

4. Discussion

Different microtomes, such as sliding and rotary, can be used for the sectioning of modern woods. However, these kinds of microtomes are not able to section in 3 directions for degraded or deteriorated woods. Mounting degraded woody material to the microtome is impossible. Aforementioned techniques (Rupp, 1964; Kukachka, 1977; Carlquist, 1982; Barbosa et al., 2010) are only used for nondegraded woody materials that are mounted to sliding microtomes. The use of these methods for degraded woody material is impractical. Thus, another method (Schweingruber, 2012) is used to overcome this problem, which is not used for longitudinal sectioning of degraded wood. In this study, degraded wood samples were mounted to a microtome by freezing and were sectioned in 3 directions. This method is suitable for studying degraded wood and was used for the first time.

References

- Barbosa CFA, Marcelo RP, Luciana W, Veronica A (2010). A new method to obtain good anatomical slides of heterogeneous plant parts. IAWA J 31: 373–383.
- Björdal CG (2012). Microbial degradation of waterlogged archaeological wood. J Cult Herit 13: 118–122.
- Burn HF (1964). Acceleration of infiltration with low-viscosity nitrocellulose by alternated warming and evacuation. Stain Technol 39: 249–250.
- Carlquist S (1982). The use of ethylenediamine in softening hard plant structures for paraffin sectioning. Stain Technol 57: 311–317.
- Gerçek Z (1993). Anatomical characteristics of *Camellia sinensis* (L.) Kuntze. Research Bulletin of the Panjab University, Science 43: 69–72.
- Gonçalves TAP, Marcati CR, Scheel-Ybert R (2012). The effect of carbonization on wood structure of *Dalbergia violacea*, *Stryphnodendron polyphyllum*, *Tapiria guianensis*, *Vochysia tucanorum* and *Pouteria torta* from the Brazilian cerrado. IAWA J 33: 73–90.
- Güvenç A, Kendir G (2012). The leaf anatomy of some *Erica* taxa native to Turkey. Turk J Bot 36: 253–262.
- IAWA (1989). IAWA list of microscopic features for hardwood identification. IAWA Bull 10: 221–332.

The results of the present study show that this method is more practical and applicable than others. The main advantage of this technique is that it can be used for a range of materials, from herbaceous stems to soft wood portions (like waterlogged archaeological woods). This technique is cheap, uses no toxic chemicals, and can be applied easily and rapidly, and it is not time-consuming. Moreover, it allows for sectioning in 3 directions. We think that this technique will facilitate research on waterlogged archaeological woods, where studies have been limited for technical reasons.

Acknowledgment

We want to express our special thanks to Zeynep Kızıltan, the director of the İstanbul Archaeological Museums, for allow study of the archaeological woods, and to Prof Dr Ertuğrul Bilgili for his critical reading of the manuscript.

- Ives E (2001). A Guide to Wood Microtomy: Making Quality Micro Slides of Wood Sections. Suffolk, UK: Suffolk Offset.
- Kukachka BF (1977). Sectioning Refractory Woods for Anatomical Studies. Washington, DC, USA: United States Department of Agriculture Forest Service Forest Products Laboratory Research Note, FPL-0236: 1-9.
- Lodewick JE (1924). The shorter celloidin method. J Sci 60: 67-68.
- Rupp P (1964). Polyglykol als Einbettungsmedium zum Schneiden botanischer Praparate. J Microsc 53: 123–128 (article in German).
- Schweingruber FH (2012). Microtome sections of charcoal. IAWA J 33: 327–328.
- Vasic PS, Dubak DV (2012). Anatomical analysis of red Juniper leaf (*Juniperus oxycedrus*) taken from Kopaonik Mountain, Serbia. Turk J Bot 36: 473–479.
- Zimmermann MH, Tomlinson PB (1965). Anatomy of the palm *Rhapis excelsa*. I. Mature vegetative axis. J Arnold Arboretum. 46: 160–168.
- Zimmermann MH, Tomlinson PB (1968). Vascular construction and development in the aerial stem of *Prionium* (Juncaceae). Am J Bot. 55: 1100–1109.