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TECHNICAL REPORT

TECHNIQUE TO IMPROVE VISUALIZATION OF ELUSIVE TREE-RING BOUNDARIES IN ASPEN (*POPULUS TREMULOIDES*)

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ABSTRACT

A simple, quick, and inexpensive technique to improve visualization of aspen (*Populus tremuloides*) tree rings under the microscope, the 'shadow technique', is described. The technique assumes appropriate preparation of increment cores or cross-sections and works well on the lighter portions of the sample with fungus- and bacteria-free wood. The shadow technique was used successfully to elucidate tree-ring boundaries in small diameter (<5 cm DBH) aspen from northern Utah that commonly had >100 annual rings. Crossdating verified whether the elusive rings were missing or false rings. Aspen tree-ring measurement will be greatly enhanced with the shadow technique and preliminary investigation suggests it could be used on other species such as curlleaf mountain mahogany (*Cercocarpus ledifolius*).

Keywords: Shadow technique, tree rings, visualization enhancement, wood anatomy.

INTRODUCTION

Many fast growing, diffuse-porous species (*e.g. Populus* spp.) from temperate forest regions have annual tree-ring boundaries that are difficult to see, even when viewed under a dissecting microscope (Deflorio *et al.* 2005). This is caused by the obscure latewood/earlywood boundary between growing seasons that results from a seemingly continuous pattern of large vessels. Indeed, many studies use aspen (*Populus tremuloides* Michx.) tree-ring data that are not crossdated, and instead only perform ring counts (*e.g.* Ripple and Larsen 2000; Halofsky *et al.* 2008), which might be prone to error as a result of false (Maini and Coupland 1964) or missing rings, especially in understory and slow growing individuals. The minute axial parenchyma cells that make up the ring boundary in aspen are difficult to see under conditions of imperfect preparation (*e.g.* improper or inadequate sanding, mounting at some angle other than perpendicular), and sometimes for no apparent reason, which may be ascribed to vagaries of growth. The 'shadow technique' can enhance identification of annual ring boundaries for light-colored (fungus- or bacteria-free) rings of aspen in all of these situations.

Previously existing techniques to help discern growing season boundaries in diffuse porous hardwoods abound in the literature and include (1) repeated sanding with progressively finer grits of sandpaper (Asherin and Mata 2001), (2) application of powdered zinc oxide (personal experience), (3) the application of a small bead of water or solution (Campbell 1981), (4) staining with chemicals (Rose 1957; Campbell 1981), (5) inoculation of decay fungi (Deflorio *et al.* 2005), (6) impregnation of sample with wax (primarily to maintain the integrity of the sample) (Ghent 1954), and (7) cutting or shaving with a razor (Maini and Coupland 1964). Each of these methods has shortcomings. For example, machine sanding can flatten the ends of the cells, potentially degrading the viewing surface. Zinc oxide

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powder works well in diffuse porous species that characteristically begin spring growth with very large vessels but does not work when vessels are of uniform size throughout the growing season, such as in aspen. Using drops or beads of water or chemical solution directly over aspen rings creates a convex surface that, when viewed under the microscope can often enhance magnification and allow ring characteristics that are otherwise not visible (Campbell 1981). However, the water quickly infiltrates the wood and makes it difficult to see ring boundaries again until the wood dries. Chemical staining and inoculation with fungi have both been shown to greatly improve ring boundaries in aspen (Rose 1957; Patterson 1959; Trujillo 1975; Campbell 1981; Asherin and Mata 2001; Deflorio *et al.* 2005), but the primary shortcoming with these approaches is the large increase in preparation time. Shaving or cutting with a razor provides an excellent surface for visualization under a microscope. However, in our experience razor blades are good for only one cut, after which they tend to crush the cells, rather than cut them. Using one blade per core can become expensive. The 'shadow technique' is quick, preparation requires only sanding, does not degrade the sample, and uses common dendrochronological laboratory equipment.

THE SHADOW TECHNIQUE

When using the technique we assume the cores or cross-sections have been mounted properly (*i.e.* so that vessel cross-sections are perpendicular to the stage) and have been sanded with progressively finer grades of sandpaper to at least 1200 grit (Stokes and Smiley 1968; Phipps 1985). For our samples a belt sander, mounted upside-down, was used to sand through 120, 220, 320, 400 and 600 grit belts, followed by hand sanding with 9, 15, and 30 grade micron sandpaper.

The method is implemented in the following sequence: (1) place the core under the scope, focusing on the area of interest; (2) using a standard fiber-optic microscope light (we used a Dolan-Jenner Fiber-lite M1-152), adjust the beam of light toward the core at a ~30 degree angle from only one side of the microscope; (3) hold an object, such as the tip of a mechanical pencil or the end of a paper clip, just above the sample between the light and the area where a ring boundary is questionable (Figure 1). The object creates a shadow on the core surface, but allows a 'backlight' effect, presumably from light passing by the small object and entering the large vessels in the core. This causes the illumination of the area of interest and an enhancement of the ring boundary. The result is visibility of the latewood/earlywood boundary, which indicates annual growth cessation. Using the shadow technique, the ring boundary will appear illuminated compared to the surrounding cells in the same annual growth ring (Figure 1).

APPLICATION

We discovered this technique while trying to measure very small ring widths on aspen increment cores from northern Utah (41°59′N, 111°31′W). As a result of the sampling design, it was not uncommon to have aspen smaller than 5-cm diam. at breast height with more than 120 annual rings. The shadow technique greatly facilitated our ability to discern tree rings that were not readily visible after appropriate increment core preparation. It was successful in locating elusive rings that could be either missing or false rings indicated during cross-dating. Besides being useful for finding missing and false rings, the shadow technique was particularly useful in general for facilitating the rapid measurement of aspen tree rings. Since discovering this technique, it has been successfully used to elucidate annual ring boundaries in aspen from central and southern Utah as well. Furthermore, we have found the technique useful in limited situations with a much higher-density ring-porous species, curlleaf mountain mahogany (*Cercocarpus ledifolius* Nutt).

SHORTCOMINGS

The major shortcoming with this approach is its inability to discern ring boundaries in discolored wood of aspen. Aspen often becomes infected with any of a whole suite of decay fungi at some point during stand development (Tainter and Baker 1996). It is likely the shadow technique does not

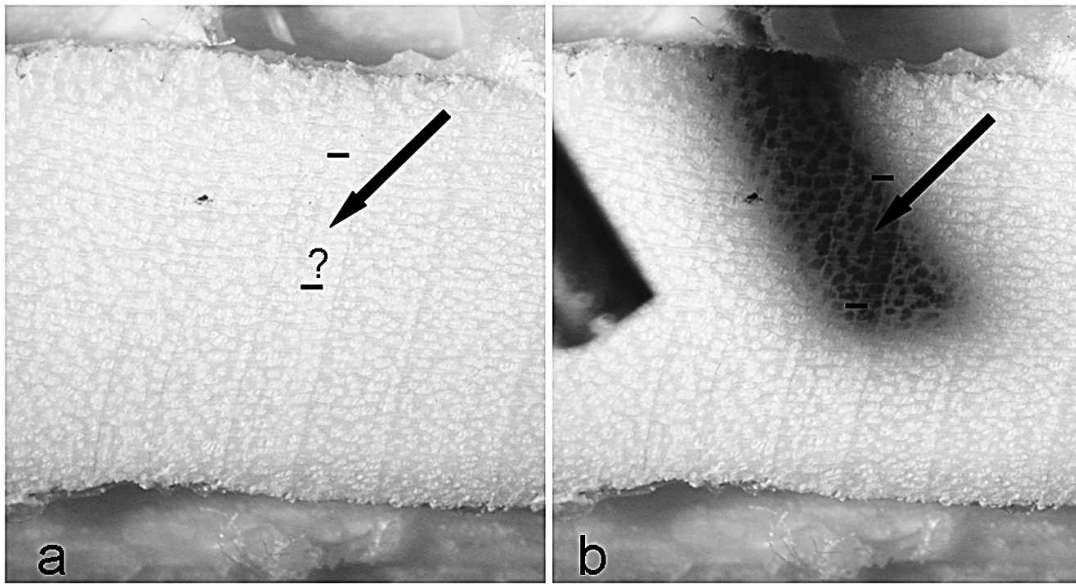

Figure 1. Identical pair of photographs taken under a trinocular microscope showing an aspen increment core without a shadow (a) next to one with the shadow technique (b) created using a paper clip revealing clear lines associated with growing season boundaries. Arrows show approximate location of elusive ring and short horizontal dashes cross it almost perpendicularly. Questionable ring boundary (?) in panel (a) is revealed using the shadow technique (b), as are the surrounding ring boundaries.

work in the infected wood because light from the illumination source does not penetrate the darkened cells and therefore can not create the backlight effect. Fortunately however, ring boundaries in fungus stained wood are typically much easier to see than light colored wood (Deflorio *et al.* 2005).

If the elusive ring found in Figure 1 is actually a 'white ring' (*sensu* Hogg *et al.* 2002), a result of early growing season defoliation, then the shadow technique would appear to be effective in elucidating these generally lighter rings. However, we were unable to positively identify any historical severe defoliation events in our study area, and are therefore unsure whether the Figure 1 example ring is a 'white ring' or not. Additional investigation is needed to determine whether the shadow technique is effective for 'white rings' in aspen. Given the usefulness of the technique in light colored wood, we speculate it would successfully highlight the lighter color of 'white rings'.

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