MEASURING MICROFIBRILLAR ANGLES USING LIGHT MICROSCOPY[1]

John F. Senft

Associate Professor of Wood Technology
Department of Forestry and Natural Resources
Purdue University, West Lafayette, IN 47907

and

B. Alan Bendtsen

Head, Engineering and Economics Research Section
USDA Forest Service, Forest Products Lab
Madison, WI 53705

(Received June 1984)

ABSTRACT

A rapid, reliable method of enhancing microfibrillar angles for viewing in wood cell walls, a modification of I. W. Bailey's technique, is reviewed. Dried microtomed sections are treated with an iodine-potassium iodide solution and nitric acid to produce crystals in cell walls of softwood tracheids or hardwood fibers. The crystals are aligned with the predominant microfibrillar angle and may be easily viewed and measured.

Keywords: Microfibril angle, microscopy, cell walls.

INTRODUCTION

Microfibrillar angle measurements in wood provide valuable information as an index of mechanical properties and wood quality. In general, the lower the microfibrillar angle, as measured from the longitudinal axis of the cell, the stronger the wood. This is particularly true for strength properties such as bending and compression and tensile strength parallel-to-grain. Microfibrillar angle is also a useful indicator of the presence of compression wood in conifers or tension wood in hardwoods. However, measuring microfibrillar angles in wood cells is a tedious and somewhat haphazard process, difficult at best in conifers and even more difficult in fine-grained hardwoods.

A common procedure for measuring microfibrillar angles under a light microscope is to dry wood specimens rapidly. The rapid shrinkage and high drying stresses cause the cell walls to check and split along the direction of the microfibrils in the S-2 layer of the cell wall. Subsequent staining enhances the cracks for measurement by light microscopy. Another technique used when cell-wall cracks are not present is to measure the angle of elongated pit apertures, which are regarded as being closely aligned with the microfibrillar angle of the S-2 cell-wall layer.

These methods have serious disadvantages because cracks and pit apertures can not always be found in sufficient quantity to meet a researcher's needs or in specific locations where measurements are desired. They tend to be observable

[1] Paper Number 9617, Agricultural Experiment Station, Purdue University, West Lafayette, IN.

Wood and Fiber Science, 17(4), 1985, pp. 564–567

in relatively large numbers in some areas on a slide, sporadic or absent elsewhere, and in some species not visible at all. Thus, a rather lengthy staining and mounting procedure may not produce reliable data.

In a current study of the properties of loblolly pine (*Pinus taeda* L.) and cottonwood (*Populus deltoides* Bartr.) microfibrillar angle measurements of tracheids and fiber cells were desired. Fiber cells in cottonwood are quite small in diameter relative to softwood tracheid diameters and efforts to produce visible cracks in the cell walls were not successful. The following method, a modification of one reported by Kobayashi (1952) in the literature, is a variation of I. W. Bailey's technique (Bailey and Vestal 1937). One purpose of reporting on this aspect of research is that Bailey's technique is, apparently, little used today, but was found to be valid and efficient. It and the one used by Kobayashi were somewhat cumbersome as originally described. Furthermore, the technique is described in rather obscure literature; consequently, we felt that the modified technique should be presented so as to be readily available to those involved in anatomical research.

Several steps originally presented by Bailey may be omitted to save considerable time; chlorination, alcohol rinses, and dilute ammonia treatments were not found to be necessary. Using the modified technique, the entire process, beginning with an unstained, microtomed section in water or stored in 50% alcohol, can be completed and measurements taken in a matter of a few moments. The method was found easy to use and quite reliable. Experimentation with times and solution concentrations may be required to get good results with species other than pine and cottonwood. Kobayashi delignified the wood sections before treatment, although this was not necessary for the species evaluated.

The section may also contain many randomly oriented crystals. These randomly oriented crystals may be differentiated from crystals deposited within cell-wall layers by their obviously nonparallel arrangement and the overlapping of crystals and walls between two cells. The section is usable for several hours. Too long a period of immersion in the iodine-potassium iodide solution will produce a section that is very dark brown in color; shorter immersion times produce lighter colored sections in which the iodine crystals are more discernible.

PROCEDURE

Oven-dry small green specimens of wood at 100 C to induce checking in the cell walls. Resoaking to saturation and redrying may be advantageous for some species to induce further checking. Microtome radial sections 15 microns thick and store in 50% alcohol. Dehydrate sections in absolute alcohol for 5 min, twice. Immerse the sections in a 2% solution of iodine-potassium iodide for 2–10 seconds. Place the section on a slide and blot the excess solution with a paper towel. Gently add one or two drops of 60% nitric acid to the section and apply a coverslip. The microfibrillar angles will be immediately visible as elongated, dark crystals of iodine filling the cracks in parallel lines.

Once the sections are microtomed, viewing is accomplished in just a few moments without the need of lengthy staining schedules. Careful focusing of the microscope shows the microfibrillar angles in the walls of contiguous cells. Depth of focus can be used to show change in fibril angle across the thickness of a cell wall; fibril orientation within the S-1 and S-3 cell-wall layers was discernible at times.

FIG. 1. Radial section of cottonwood (*Populus deltoides* Bartr.) showing iodine crystals deposited in the cell walls (about 700× enlarged).

APPLICATION

Serially cut radial sections of one annual ring of loblolly pine approximately ¼ square centimeter in size were examined by two methods. Microfibrillar angle measurements were recorded by measuring cracks and/or pit aperture angles and by measuring iodine crystal angles. When cell-wall cracks and pit apertures were examined on a safranin-stained section, 57 measurements yielded an average microfibrillar angle of 15 degrees, with a standard deviation of 4.4 degrees. Using the iodine crystal technique, 79 measurements yielded an average angle of 16 degrees, with a standard deviation of 4.2 degrees. A statistical comparison indicates that the two means are not significantly different. Any real differences are probably attributable to randomness of choice of cells to be measured. The cracks were evenly distributed throughout the section. The iodine crystal technique produced results similar to those produced by the safranin staining technique, but the iodine crystal technique was much faster and yielded many more measurements. Because the measurements were more uniformly distributed over the section, a much truer profile of angles from earlywood through late summerwood was produced. A comparison of the average microfibrillar angle as measured by two different investigators, both using the iodine crystal technique, yielded average angles of 16 degrees ($n = 79$) and 17 degrees ($n = 148$). This small difference was, however, significantly different statistically.

The iodine crystal technique was also successfully used on cottonwood (Fig. 1). Even though the cottonwood fibers are much smaller than pine tracheids, the microfibrillar angles were readily discernible throughout a section. Safranin-stained

sections of cottonwood did not produce satisfactory microfibrillar angle data because visible cracks suitable for observation could not be induced in the cell walls.

In summary, this technique provides an efficient, easily used method to rapidly evaluate microfibrillar orientation in the cell walls of hardwoods and softwoods.

REFERENCES

BAILEY, I. W., AND M. R. VESTAL. 1937. The orientation of cellulose in the secondary wall of tracheary cells. J. Arnold Arboretum 18(3):185–195.

KOBAYASHI, Y. 1952. A simple method for the preparation of samples for measurement of inclination of micelle in the wood cell wall. J. Jap. For. Society 34(12).