Chapter **2**

Tracheid Length Measurement In Selected Conifer Species

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INTRODUCTION

Most of the secondary xylem of conifers consists of longitudinally-arranged tracheids (sometimes called fibres). White pine wood, for example, is 95% by volume longitudinal tracheids (Panshin and de Zeeuw, 1980). Tracheids are long cells with rounded or tapered ends which overlap each other. They allow conduction of fluids and provide support (see Meylan and Butterfield, 1972, and Papermaking Fibres. *A* Photomicrographic Atlas, 1980).

The length of tracheids in wood is an important determinant of the use to which that wood is put (Hocker, 1979). The quality of both lumber (Daniel, Helms, and Baker, 1979) and pulp (Clark, 1978) is determined, in part, by the length of tracheids in the wood. Long fibres give lumber greater strength and paper produced from pulp more strength and fold-resistance. Tracheid length is also being investigated as a predictive factor in tree breeding programs (Daniel, Helms and Baker, 1979) because of observations that tracheid length is correlated with tree height (Echols, 1958) and appears to be an inherited characteristic (Zobel, 1961).

Tracheids are easily separated and can be measured using compound light microscopes or stereomicroscopes. They are therefore well suited for a laboratory practical teaching measurement using calibrated microscopes or for a project intended to draw students into an area of active research.

This paper describes an undergraduate project based on these concepts. Some of the factors which influence tracheid length are also discussed because each factor is potentially a source of new laboratory exercises. Though the project described in this paper used three conifers which are abundant in Canada similar techniques have been used with other conifer species (Echols, 1958, and Jagels, Gardner and Brann, 1982).

METHOD

The method described here is easy to manage in an open laboratory setting, but many refinements and alternatives are available. Jagels, Gardner and Brann (1982) is a good source of current information.

Small blocks of wood were cut from similar positions on similarly-aged local trees of three conifer species: **Pinus strobus** L. (Eastern White Pine), **Picea glauca** (Moench) Voss. (White spruce), and **Abies balsamea** (L.) MIll. (Balsam Fir). Each student cut two or three thin sections of approximately 20 mm x 1 mm from a block. Students were instructed to confine the cuts to one quadrant and within a few growth rings and this was supervised whenever possible.

Each thin section was placed in a small, brown bottle filled with a macerating solution of 1:1 (v:v) 30% hydrogen peroxide: glacial acetic acid. The bottle cap, lined with aluminum foil, was placed loosely on the bottle to allow gases to escape. (Students were warned that the solution is corrosive and taught appropriate safety procedures). The section was left in macerating solution at 60°C for 48-72 hours, with adequate ventilation to disperse the escaping gases. During this time the section began to break into fragments and the tracheids became silvery-white. After cooling to room temperature, the macerating solution was removed and the tracheids were gently rinsed with several changes of distilled water. With the last change of distilled water, the container was covered and shaken 5-15 minutes with a regular motion. This motion which causes the tracheids to separate is a critical step in this method. The distilled water was replaced with 1% (w:v) active formaldehyde. Appropriate safety precautions with this chemical fixative were stressed. At this stage, the tracheids could be stored for periods of at least one month, a definite asset for laboratory management.

For microscopic examination, the tracheids were placed on a slide in either distilled water or 1% (w:v) basic fuchsin. After the technique of using and calibrating compound and stereomicroscopes was learned by the students, each student measured the length of fifty tracheids.

THE CLASS PROJECT

The project was conducted during the second month of an 'Experimental Laboratory Techniques' course for 60 undergraduate students, chiefly second-year biology majors. The advance preparation in light microscopy, including calibration, was given in this course in a series of mastery-based modules of laboratory work. For reasons related to our course objectives, this project was preceded by two additional exercises. A series of computer drills allowed students to become able to use the computer to handle data. An advance, individualized, mini-project gave each student the opportunity to apply information received in lectures about experimental design and execution and about data analysis.

With this advanced preparation, the project was introduced to the students. While they received all details of the method, all that was said about the project was that it was intended to determine whether the average tracheid length differed among **Pinus strobus**, **Picea glauca** and **Abies balsamea**. Students measured fifty tracheids each at their convenience over two weeks. The measurements were shown to the instructor who verified correct calibration technique then they were entered by the student into the class computer workspace. The importance of accurate data entry into the computer workspace was stressed. After all the data was entered, each student obtained a print-out of the computerized t-test analysis of the class project data. Provided with some information about factors which are known to influence tracheid length, each student prepared a scientific report for evaluation by the instructor.

PROJECT RESULTS AND DISCUSSION

Table 1 summarizes the class project data. Even with no direct supervision of the data entry into the computer workspace and no editing of the data once entered, there are no apparent data entry mistakes such as double digit entry or forgotten decimals. Since the project lasted only two weeks and involved sixty students, a few students measured less than the requested fifty lengths. The total number of observations for the three species was 2920. Though using the computer for this purpose was one of our course objectives, data can be analyzed by any available means.

We elected to manage this project in an open laboratory format and encountered no difficulties. We were limited to one fumehood so the students were "paced" by the availability of that facility. We feel that availability of fumehoods or other means of ventilation and microscopes are most likely to determine the format. Provided the facilities are available, this method and class projects based on it should also fit easily into more traditional laboratory time slots.

For this particular project, the students were asked to determine whether average tracheid length is characteristic of the species. The first step was to analyze the samples obtained by the class using a t-test analysis. Table 2 shows the result of the t-test analyses. The differences between sample means are all highly significant.

How this information should be interpreted was addressed in the discussion section of each student's scientific report of this project. With appropriate advance information about data analysis and evaluation, it should be clear to them that, while the sample means appear different, the differences are not necessarily attributable to species. In this particular project, some care was taken to control for factors known to influence tracheid length (Table 3), however not all of these factors could be controlled.

The information provided in Table 3 can also serve as the starting point for other projects of this type. Among many factors lumped under growth conditions are latitude, altitude, longitude, and amount of rain and sun (Panshin and deZeeuw, 1980). The fact that enhanced growth rate often results in shorter tracheids has implications for woodlot managers who thin and fertilize their lots (Daniel, Helm and Baker, 1979). The assumption often made in tree breeding programs that longer tracheid length is advantageous still needs more testing in properly controlled experiments (Keith and Kellogg, 1981).

There is also the possibility of relating results like these to the pulp and paper industry. Clark (1978) describes in detail the type of measurement and reporting procedures used in the industry and provides formulae for calculating the weighted average length values which are used.

CONCLUSION

Tracheids in conifers are easy to separate and measure. The method is flexible enough to be used in fixed-hour laboratory periods or in open-laboratory project formats.

Conifer wood is an inexpensive source of material for laboratory practicals or microscope use. Both chemicals and equipment used in this exercise are readily available in most laboratories. Since there is a fair amount of information available about factors influencing tracheid length, a class can select and design their own project. The information, however, is not available for all conifer species so the project will be current. Tracheid lengths characteristic of the species make these measurements particularly suitable for classroom discussions of data analysis and evaluation.

Table 1. Lengths of tracheids in three conifer species. Class projec: data.

Species	Number of Observations	Numerical Average Length (%)
Pinus strobus	1057	3.08 ± 0.80*
Picea glauca	1074	3.23 ± 0.95
Abies balsamea	789	1.97 ± 0.81

Key: *, mean ± standard deviation

Table 2. Statistical comparison of numerical average tracheid lengths.

Comparison	T in TTest*	Probability T
Pinus vs Picea	3.91**	< 0.0001
Pinus vs Abies	29.50***	< 0.0001
Picea vs Abies	30.90**	< 0.0001

Key: *Using "PROC TTEST", Statistical Analysis System
**The hypothesis that variances are equal was rejected in a F' test ($p < 0.001$) so the T test assumes unequal variances.
***The hypothesis that variances are equal was accepted in a F' test ($p < 0.5$) so the T test asumes equal variances.

Table 3. Some factors influencing conifer tracheid length.

A. Within a single tree
maturity of the Layer of wood
age of wood across a single layer
vertical position within a single Layer
growth rate
B. Within trees of the same species
growth conditions
genetic make-up
C. Between species
genetic (?)

References:

Keith and Kellogg (1981), Daniel, Helms and Baker **(1979),** and Panshin and de Zeeuw(1 980).

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