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A Biometric Technique for Reaction Tissue Research

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A Biometric Technique for Reaction Tissue Research[1]

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Abstract. Images of sections of tissue taken from the upper side of leaning stems of *Populus deltoides* were projected on a paper grid system. Specimens were oriented so that the cambium and the last three annual increments were included in cross section. Counts of stimulated and non-stimulated cells in randomly selected grids were recorded. This procedure provides a means of subjecting the incidence of tension wood and other cytohistological phenomena to statistical analysis.

The measurement of specific cellular characteristics can be of fundamental value in the interpretation of cytological and histological phenomena such as the formation of the so-called tension wood of various angiosperms and compression wood of gymnosperms. The term reaction tissue is used to refer to these tissues which are found in leaning (or otherwise stimulated) stems and branches. The reaction tissue of angiosperms commonly occurs on the upper side of leaning stems, and the woody tissue from this region is both physically and chemically different from non-reaction tissue (Wardrop and Dadswell, 1948).

During the study of the reaction tissue of eastern cottonwood (*Populus deltoides* Bartr.), it became necessary to develop a system of micrometry that would be consistent with modern statistical computation and analysis. Recent developments in both computers and statistical theory open many new possibilities in biological research, and valuable information can be gained from otherwise obscure sources. These techniques can be valuable in phylogenetic and comparative studies as well as experimental botanical research (cf. Bailey, 1953).

MICROTECHNIQUE

Stem material was collected at breast height from the upper side of leaning cottonwood trees. Cores of tissue were extracted with a modified ½ inch plug cutter used in an ordinary brace and bit. Sections were cut on the sliding microtome without embedding. The specimens were oriented so that the cambium and the last three

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annual increments were included in cross section on the sections. The sections were then stained in chloroiodide of zinc. This temporary stain is specific for the cellulosic material of the so-called gelatinous layer of the cell wall of tension wood fibers. This gelatinous layer of the stimulated cells stains dark blue, and in eastern cottonwood frequently swells into the cell lumen in response to this stain (cf. Wahlgren, 1956). Coverslips were sealed with wax.

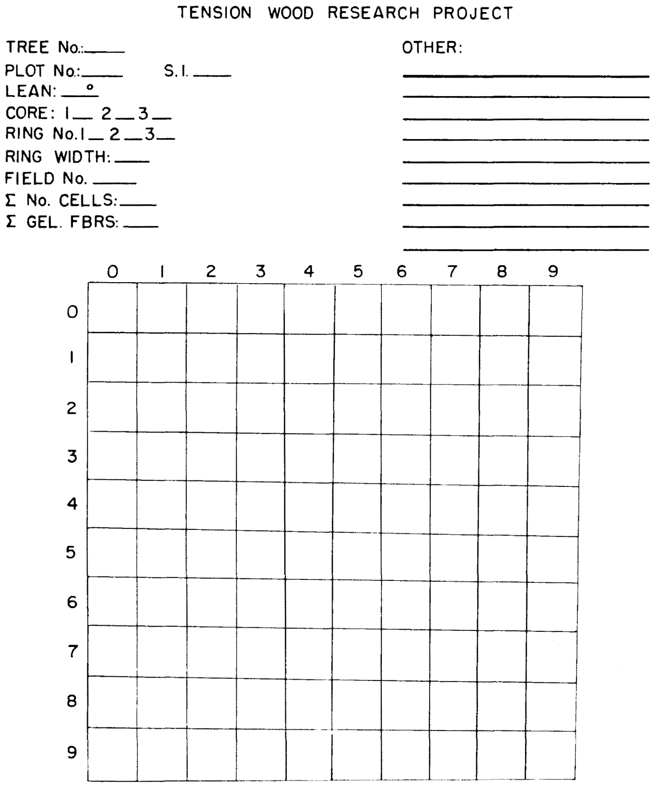

Figure 1. Sample grid sheet.

Micrometry

The slides were projected vertically at magnification 139 X with a microprojector using a ribbon filament lamp. Carbon arcs supply too much heat for these temporary mounts and are therefore unsuitable. The image of the tissue with its three included annual increments was projected on a paper grid system (Figure 1) which was taped to the desk surface. Observations for each microscopic field were recorded on separate grid sheets. These sheets serve as a permanent record. The multilithed 10 by 10 grid measures 12.5 cm by 12.5 cm. Each grid square is a 1.25 cm square. At 139 X the entire grid system covers an area of tissue 0.9 mm by 0.9 mm or

810,000 square microns. Each of the 100 individual grid squares then encloses 0.09 mm by 0.09 mm of tissue or 8100 square microns.

In each of the individual grid squares selected for observation, actual counts were made of stimulated and nonstimulated cells. The data were recorded directly in the individual grid squares. In the binomial system used, the first number in a particular grid square refers to the number of cells with a gelatinous layer and the second number refers to nonstimulated cells.

It is generally not necessary or practical to take data for every square of the grid system. In this study 8 grid squares were randomly selected for observation on each grid system. This was conveniently done with the aid of a random number table (Snedecor, 1956). Three grid systems were analyzed for each of three annual increments, making a total of 9 grid systems per slide. Since 8 grid squares were analyzed per grid system, data were recorded for 72 grid squares. If each grid square contained about 20 cells there would be data on 1,440 cells for every slide. For more detailed analysis 10 to 15 grid squares per grid system could be analyzed without excessively increasing the time of analysis. Data taken in this manner fit nicely into a wide variety of statistical analyses.

Table 1

Data from Plot 5 site index 97

Tree No.	Year	Lean (degrees) X	Ring Width (mm.)	Proportion Gelatinous fibers in sample Y
31	1956	3	2.14	0.000
	1955	3	1.75	0.000
	1954	3	1.54	0.000
30	1956	5	0.60	0.000
	1955	5	1.00	0.000
	1954	5	1.09	0.000
34	1956	6	2.96	0.201
	1955	6	2.36	0.0196
	1954	6	2.90	0.0775
29	1956	9	1.42	0.341
	1955	9	1.25	0.291
	1954	9	1.57	0.390
33	1956	12	2.33	0.945
	1955	12	1.80	0.860
	1954	12	1.21	0.591
32	1956	15	2.57	0.786
	1955	15	0.96	0.795
	1954	15	1.14	0.876

Regression analysis of Y on X:

$\hat{Y}=0.343+0.8114(X-\bar{X})$

$r=0.946^{**}$

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Results

The foregoing method is being used in connection with a study on the effect of environmental and genetic factors on the reaction tissue of eastern cottonwood. For purposes of illustration some raw data (Table 1) were taken from a cottonwood grove along the Missouri River in western Iowa. A simple linear regression of proportion of gelatinous fibers on lean was calculated. In this particular plot lean accounted for 89.4 per cent of the variation as measured by the sums of squares. The plot of the raw data has not been subjected to any smoothing function and merely represents the data as recorded (Figure 2). The graph shows three points for each lean class sampled. These three points above a specific lean value represent three annual increments from the same tree. Each point is identified as to year and the corresponding ring width for that year is recorded to the left of the point.

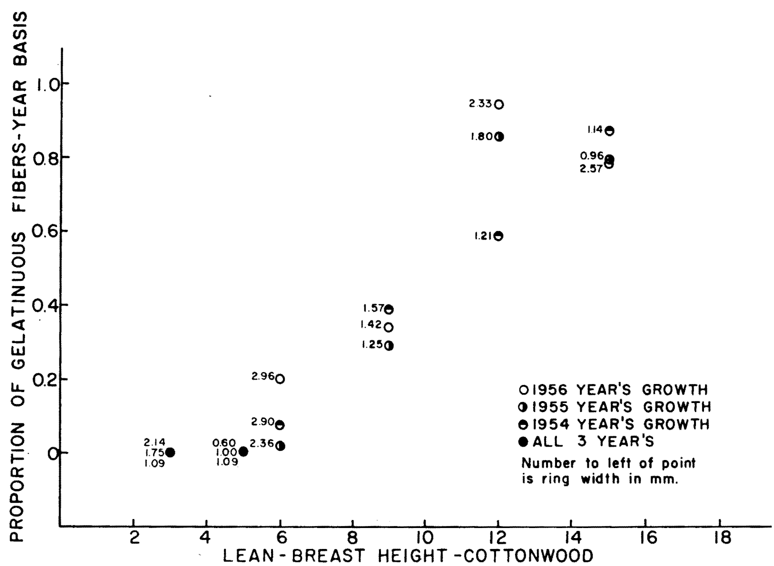

Figure 2. Sample data from a stand of eastern cottonwood showing relationships between lean ring width, year, and proportion of gelatinous fibers.

Summary

The procedures described herein provide a means of subjecting the incidence of tension wood and other cytohistological phenomena to statistical analysis in relation to various environmental and genetic factors.

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