STEM ANATOMY VARIATION IN COTTONWOOD GROWING UNDER NUTRIENT-DEFICIENT CONDITIONS

A. N. Foulger and J. Hacskaylo[1]

Investigations of mineral nutrient-tree growth relationships have dealt mainly with associations involving foliage composition, root formation, or volume production of wood. Few studies have been concerned with changes in wood anatomy associated with element deficiency. In 1949 Davis reported that calcium deficiency was accompanied by a reduction of primary tissue and an increase in secondary tissue in loblolly pine (*Pinus taeda* L.) stems. Murphey *et al.* (1962) found that nutrient defiencies affected the percentage of phloem formed and reduced both vessel diameter and the number of rays in sweetgum (*Liquidambar styraciflua* L.) seedlings. There was no apparent change in fibril angle.

The present study was a preliminary investigation into the degree of association between single mineral element deficiencies and anatomic characteristics in the stem of eastern cottonwood (*Populus deltoides* Bartr.) cuttings. All of the observations were made on first-year wood cuttings after 65 days of treatment. These data may, or may not, be applicable to mature plantation-grown trees. The hazards of extrapolating from data concerning rooted cuttings to considerably older stems are self-evident.

Material and Methods

First-year cuttings from a single eastern cottonwood, Wisconsin Clone No. 5, were rooted in quartz sand flats. When they were approximately 10 cm tall, they were planted in polyethylene-lined, 3-gal. crocks filled with HCl-washed silica quartz. Ten nutrient solutions, namely complete (table 1), -boron, -copper, -iron, -magnesium, -manganese, -nitrogen, -phosphrous, -potassium, and -suphur, were supplied to separate, continuously aerated crocks. Nutrient solutions were made from A.C.S. chemicals, with pure iron wire as a source of iron according to a modified Jacobson's method, and deionized water. If a compound supplied two elements to a solution, a sodium or chloride salt of the element not to be omitted was substituted. The pH of the solution was adjusted initially to 5.4 with 0.1 percent NaOH. The water level in the crocks was kept constant throughout the study, while pH was adjusted and nutrient solution changed every 2 weeks. Each treatment was randomly assigned to two trees in separate crocks. The plants were harvested after 65 days' treatment, height and stem diameters recorded on each, and the stems fixed in FAA.

Samples were taken from each stem at one-quarter, one-half, and three-quarters of the distance from the root collar to the stem apex. On a transverse section of the stem at each height, bark width, pith diameter, number and radial diameters of both vessels and fibers were recorded along 12 radii.

Macerations were prepared from stem portions immediately above each transverse section, allowing measurement of 50 fiber lengths and 30 vessel lengths and widths at each height in the stem.

Both the transverse section and maceration data were examined, using analysis of variance. Dunnett's procedure was employed to compare each treatment with the control, using a one-tailed test.

Results

Stem Height

The average stem heights after 65 days' treatment ranged from 138.5 cm for the complete to 38.5 cm for the S-deficient treatment as shown below.

Treatment	*Mean height (cm)*
Complete	138.5
-Boron	43.5*
-Copper	104.6

[1] *Respectively, Assistant Professor and Professor (now deceased) of Forestry at Ohio Agriculture Research and Development Center, Wooster, Ohio. The senior author is now Research Technologist, Forest Products Laboratory, Forest Service, U.S. Department of Agriculture.* *The authors wish to recognize the assistance rendered by Misses G. Kirchhofer and B. March, who made the observations, and by Dr. C. R. Weaver, Statistics Laboratory, The Ohio Agricultural Research and Development Center. The work was supported in large measure by a grant from Champion Papers Incorporated, Knightsbridge, Hamilton, Ohio.*

Table 1. — Compounds in the complete nutrient solution

Compound		Total parts per million of element
$Ca(NO_3)_2 - 4H_2O$	Calcium (Ca)	120.21
KNO_3	Nitrogen (N)	112.00
KH_2PO_4	Potassium (K)	156.36
	Phosphorus (P)	62.05
$MgSO_4 \cdot 7H_2O$	Magnesium (Mg)	48.65
	Sulfur (S)	64.12
Fe - EDTA	Iron (I)	5.00
H_3BO_3	Boron (B)	0.40
$MnCl_2 \cdot 4H_2O$	Manganese (Mn)	0.40
$ZnCl_2$	Zinc (Zn)	0.05
$CuCl_2 \cdot 2H_2O$	Copper (Cu)	0.02
MoO_3	Molybdenum (Mo)	0.03

Treatment	*Mean height (cm)*
-Iron	108.4
-Potassium	98.9
-Magnesium	123.1
-Manganese	124.1
-Nitrogen	49.7*
-Phosphorus	102.9
-Sulfur	38.5*

Deficiencies of B, N, and S resulted in height reductions which were significant at the 5-percent level (indicated by an asterisk in the tabulation).

Fibers

A decrease in fiber length, significant at the 5-percent level, was found at two or more levels in the stem with deficiences of B, K, N, and S (fig. 1). Only in the top specimens did lack of Cu, Fe, Mg, Mn, and P cause significant reductions in fiber length. Both -Cu and -Mn treatments resulted in slight increases in fiber length at the lowest level in the stem.

In addition to a reduction in fiber length, deficiences of K, N, and S were associated with a significant reduction in fiber width at all levels in the stem (fig. 1). Mean fiber width was reduced in the top specimen from the Mg treatment, but no significant decrease or increase in fiber width was associated with a lack of the other elements.

The number of fibers formed was significantly reduced at all three stem levels in the absence of N and S while -B, -K, -Mg, -Mn, and -P were associated with significant reductions at the lower two levels (fig. 2).

Ring width due to fibers, shown in figure 3, has a pattern similar to figure 2. Deficiencies of Mg, N, and S caused a significant reduction at all three levels in the stem, while the absence of B, K, Mn, or P was accompanied by a reduction at the middle and bottom levels. However, a lack of Cu and Fe had no apparent effect on the amount of wood made up of fibers.

Vessels

No significant differences in vessel length were found with any of the treatments (fig. 4). Radial

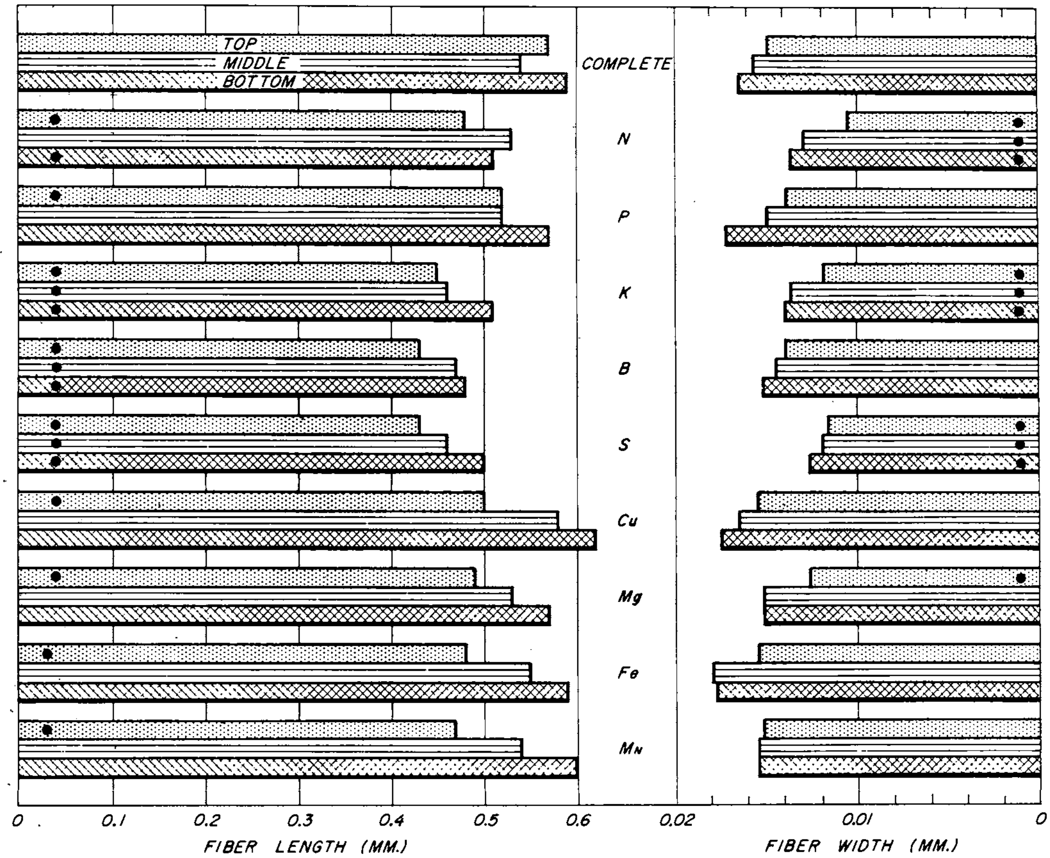

FIGURE 1. — Mean fiber length and width at each level in the stem is shown with the particular element which is absent. In this and following figures a dot indicates a value significantly different (0.05) from the complete treatment value at the corresponding stem position.

vessel width, as measured on the cross sections, showed a significant decrease at all levels in the stem in the absence of N, and S, and in the upper two levels when B was absent. The other treatments showed no effect on vessel width. Vessel widths were slightly larger for the macerated material since the vessel segments had been flattened under the cover slip. These measurements indicated that -B, -N, and -S all were associated with reduced vessel width at all three levels in the stem. A deficiency of K caused a significant decrease in the top and middle of the stems. With the exception of -Mg, where vessel width increased slightly in the top of the stem, -Cu, -Fe, -Mn, and -P reduced vessel segment width significantly in the lower portion of the stem, the latter two also causing a reduction at the middle level.

Only -S was associated with a reduced number of vessels at all three levels in the stem (fig. 2), but all other element deficiencies caused a reduction in the middle and bottom of the stem.

Bark

The bark of young cottonwood has pronounced vertical ridges, which resulted in considerable variation in bark width for the same section. Mean bark thickness was reduced significantly at the top of the stem in the absence of Cu, Fe, K, Mg, Mn, N, and P, and at the bottom with a lack of K, Mg, Mn, N, or S (fig. 5). There was no significant reduction in bark width with the -B treatment.

Pith

Pith diameter was significantly less than the control at all three heights in the stem with B, Cu, K, Mn, N, P, and S deficiences (fig. 5). In the absence of Fe and Mg, pith diameter was reduced in the top and middle of the stem.

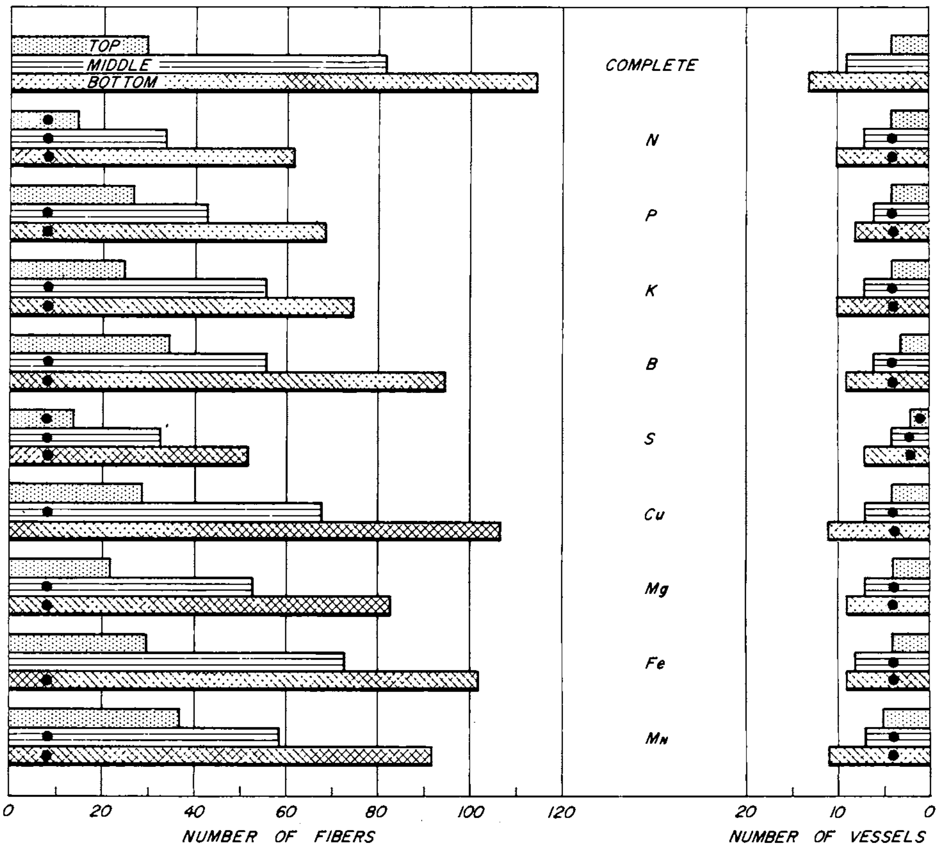

FIGURE 2. — Mean number of fibers and vessels per radius at each level in the stem is shown with the particular element which is absent.

Relationship Between Anatomic Characteristics

Data from all 10 treatments were grouped together by in-stem position (i.e., top, middle, bottom), and analysis of variance was used to determine the amount of correlation between certain of the anatomic characteristics measured. Those pairs of characteristics with a significant correlation are shown in table 2. Both fiber width and vessel width, as measured on the cross sections, showed a significant association with ring width at all three levels in the stem, but the correlation between ring width and macerated vessel width was significant only in the two lower positions. Ring width and fiber length were correlated significantly only at the bottom of the stems, while fiber length varied linearly with change in fiber width in the middle and bottom of the stems. Only vessel segment length at mid-height in the stem showed a significant relationship, at P equals 0.95, with vessel width and with ring width.

Discussion

The sampling method used in the study may be questioned in that comparison is made between points nominally similar in the stem, but which practically may be at quite different distances from the stem apex. From one standpoint, it might have been more desirable to compare points on the stem at a specific distance below the apex. However, the shortest specimen was 34.0 cm tall at the end of the study, and since the specimens were approximately 10 cm tall at the start, sampling would have been limited to the topmost 20 cm of each stem. Because an estimate of overall stem development was desired, it was decided that the sampling system used would give the most meaningful results for this particular study.

Of the various observations, possibly the most noteworthy is the thoroughly debilitating effect of N and S deficiences, which were associated with

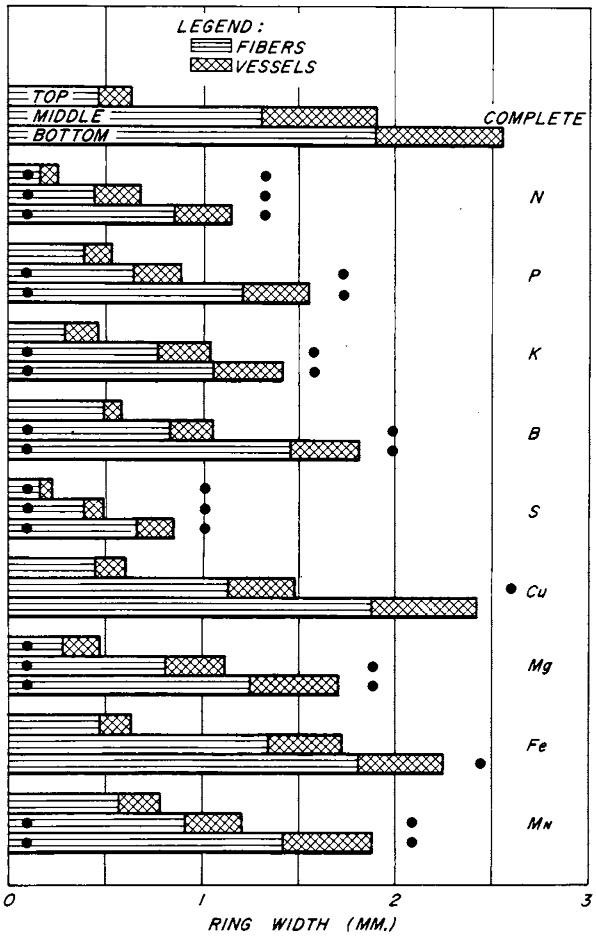

FIGURE 3. — Mean radial ring width due to fibers (lined) and to vessels (hatched). Together the two comprise total ring width at each level in the stem by treatment. Dots on the lined area indicate significant differences in width due to fibers, while dots to the right of the graphs show differences in total ring width.

significant reductions in fiber length and width, vessel width, ring width, bark width, and pith diameter. In addition, the lack of B, K, and, to a lesser extent, P resulted in marked reductions in size and number of fibers and vessels. Not only is there a reduction in the amount of wood formed, but there is a change in fiber and vessel anatomy. On calculating the ratio of vessel-formed wood to fiber-formed wood, the -N treatment showed an increase in the ratio of vessel wood to fiber wood. While these five elements, i.e., N, S, B, K, and P, have an overall effect on stem anatomy, each of the other treatments affected one or more of the facets studied. Significant reductions in both wood and bark width occurred with K, N, P, and S deficiencies while only wood formation was reduced in the absence of B.

Of these five elements, N, S, and probably K are directly involved in protein synthesis. N is essential to all amino acids, and is found in some vitamins. In addition, S is a constituent of the plant hormones biotin and thiamine, while a prolonged lack of K results in a reduction of carbohydrates. The role of B in plants is not known precisely, but this element appears to be necessary in carbohydrate transport, while a deficiency of it reduces the rate of protein synthesis. The nucleic acids contain P, and a lack of P also limits protein production since it plays an important role in energy transfer.

The primary function of the study was to determine which elements were associated with ma-

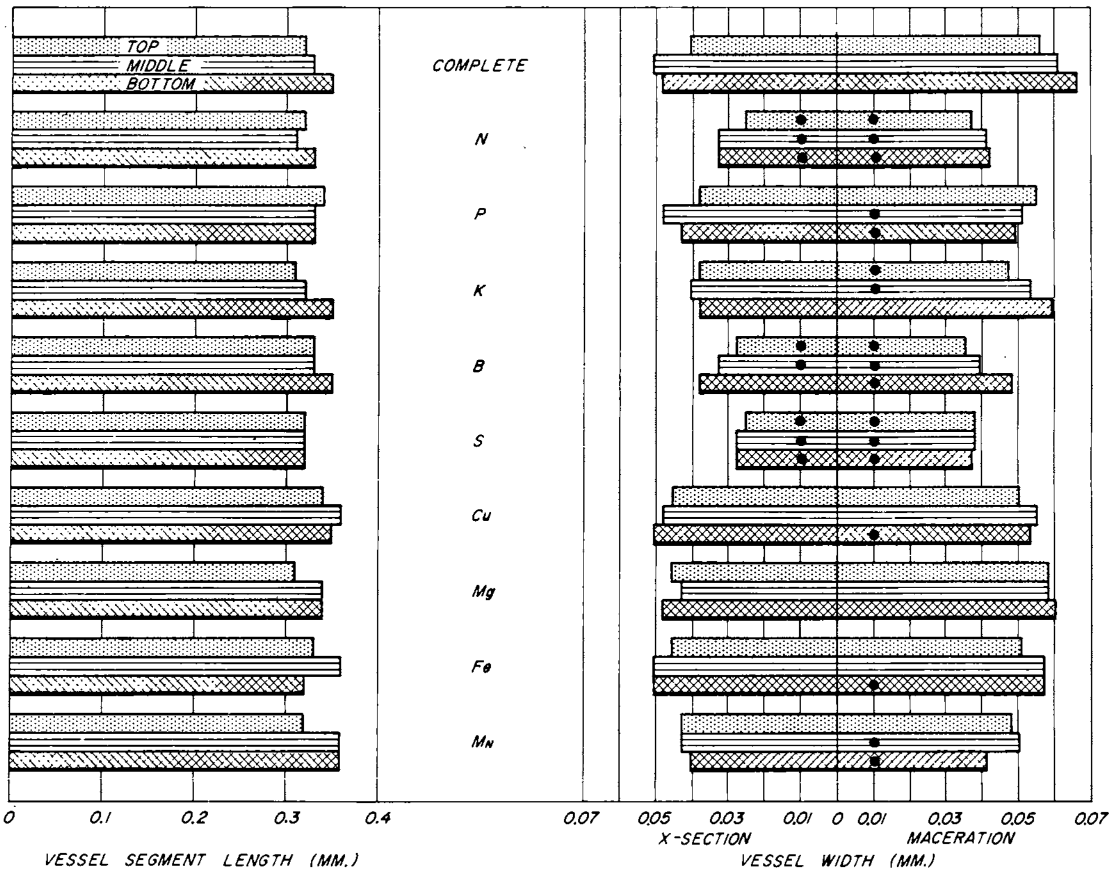

FIGURE 4. — Mean vessel segment length and width at each level in the stem is shown with the particular element which is absent. Two vessel widths are shown; those on the left are cross-section measurements while those on the right are from macerated material.

jor changes in stem anatomy, and with those anatomical aspects of economic importance to the pulp and paper industry. With this in mind, it seems that N, S, B, or K deficiency would cause most embarrassment to the woodland manager in reduced volume production, and, possibly, to the mill production engineer due to altered wood anatomy. Other elements do affect various facets of growth, but absence of the four mentioned above was accompanied by significant departures from "normal" in most of the anatomic characteristics examined.

The effects of the several element deficiencies on foliage appearance and the chemical composition of the roots, stem, and leaves of cottonwood seedlings have been reported elsewhere by Hacskaylo (1966). While the anatomic changes associated with particular element deficiencies were less marked than those of leaf form and color, the variation from "normal" was significant in several instances. It is doubtful if these data can be extrapolated to apply to stands 10 years of age or older.

A further complication lies in the fact that nutrient-deficient soils frequently, though not invariably, are lacking in more than one element, and are most unlikely to have one element completely absent.

The data presented herein are applicable to young cottonwood, possibly up to 5 years of age, with some degree of confidence. Additional work is required to determine the effects of varying two or more elements simultaneously, and the effect of nutrient deficiencies on the stem anatomy of older trees.

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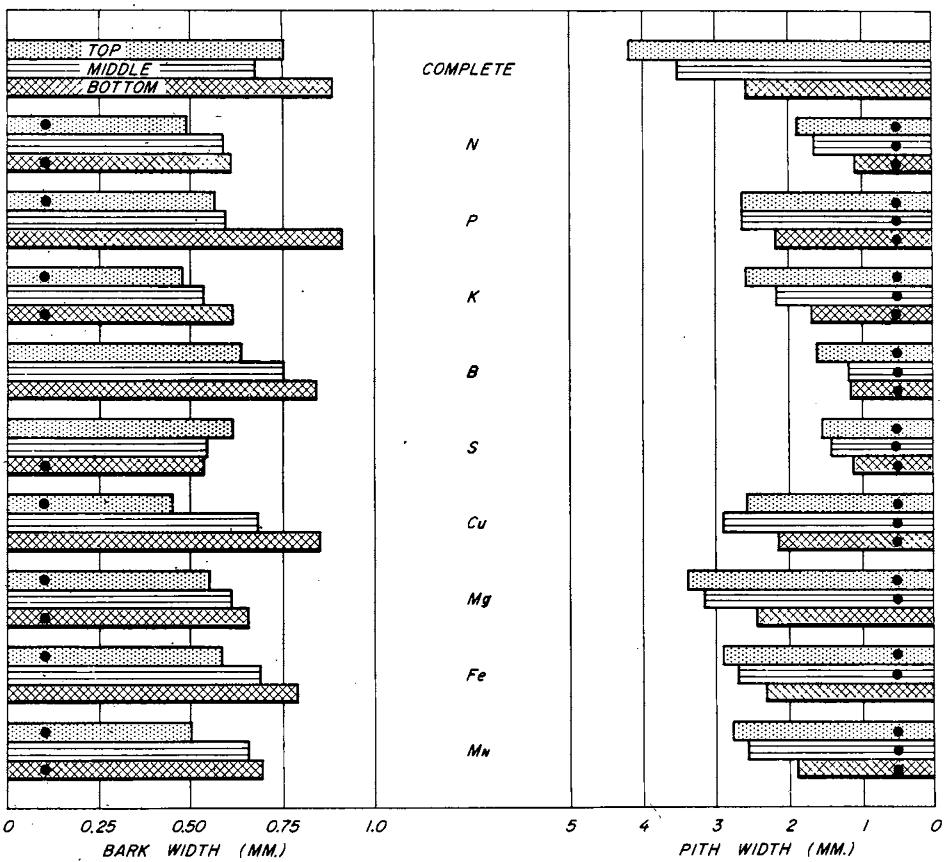

FIGURE 5. — Mean bark width and pith diameter at each level in the stem is shown with the particular element which is absent.

Table 2. — Correlation coefficient, mean and standard error of estimate for pairs of anatomic features by stem position for all specimens taken together

X	Y	Stem position[1/]	Correlation coefficient, all positive	Mean Y (mm.)	Standard error of estimate of Y
Fiber length	Fiber width	T	0.511	0.014	0.001
		M	.681*	.015	.001
		B	.716**	.016	.001
Ring width	Fiber width	T	.877**	.014	.001
		M	.894**	.015	.001
		B	.778**	.016	.001
Ring width	Fiber length	T	.364	.487	.039
		M	.562	.519	.032
		B	.738*	.553	.029
Ring width	Vessel width (cross-section)	T	.703*	.038	.006
		M	.854**	.042	.004
		B	.883**	.043	.004
Ring width	Vessel width (maceration)	T	.525	.049	.007
		M	.771**	.051	.005
		B	.668*	.052	.007

1/ T = top; M = middle; B = bottom.
* Significant at the 5-percent level.
** Significant at the 1-percent level.