GENETIC VARIATION IN THE WOOD OF *FRAXINUS AMERICANA*[1]

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ABSTRACT

The wood structure of white ash seedlings representing seven populations ranging from New Brunswick to Arkansas was compared. All the seedlings were raised in a nursery in southern Illinois to reduce environmental sources of variation. Any variation observed was attributed to ecotypic adaptation to the environment at the seed source. The genetic variation in wood cell sizes was separated into two components, one related to the geographic origin of the seed source, and the other related to the ploidy level of the tree. Diploid trees from the southernmost seed source had slightly longer vessel elements and fibers than diploid trees from northern seed sources. The longest-celled trees studied were those that were found to have polyploid genomes. There was no correlation between the rate of cambial divisions and cambial derivative lengths. Variation related to ploidy and geographic source may prove useful in a tree improvement program designed to increase fiber length.

Keywords: Polyploidy, wood anatomy, genetic variation, white ash, fiber length.

The problem of variation in wood has been a concern of researchers studying both phylogeny and identification, and genetic improvement of tree species. Many studies have concerned various aspects of intra-tree and inter-tree variation; however, few studies of inter-tree variation have successfully separated environmental and genetic aspects of wood variation. A clinal variation in fiber-tracheid lengths of *Liquidambar styraciflua* grown under controlled environmental conditions was reported by Winstead (1972) and Randel and Winstead (1976). In a similar study, seedlings of *Acer negundo* from southern seed sources had longer tracheids than seedlings from northern seed sources (Winstead 1978). In these studies the longer cells found in more southern populations were found to represent genetic differentiation and not merely a response to differing environmental conditions. Other woody species with wide ranges, such as white ash, *Fraxinus americana,* which is found throughout the eastern deciduous forest, might be expected to show a similar clinal variation in wood cell sizes.

One of the first phases of any tree improvement program is to determine the extent to which phenotypic variation in important traits is determined by underlying genetic variation. We therefore took advantage of existing field research

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TABLE 1. *Origin of seven white ash populations.*

Location	Latitude deg. N	Elevation meters
NC 6804[a]—Northumlerland Co., New Brunswick	45.9	15
NC 6782—Addison Co., Vermont	43.9	457–610
NC 6793—Onondaga Co., New York	42.8	378–520
NC 6798—Warren Co., Ohio	38.9	275–370
NC 6795—Harrison-Washington-Jackson cos., Indiana	38.0	200
NC 6721—Jackson-Union-Williamson cos., Illinois	37.7	140–190
NC 6735—Marion-Boone cos., Arkansas	36.4	245–305

[a] NC Forest Experiment Station seed source accession number.

(Bey et al. 1977) to investigate variation in wood properties of young white ash trees.

METHODS AND MATERIALS

In 1974 white ash seed was collected and sent to the Forestry Sciences Laboratory at Carbondale, Illinois, by cooperators from thirty-five locations throughout the natural range of the species. Each collection was comprised of seed from several parent trees per location (average 5.8); the individual trees in each stand were located within a few kilometers of each other but separated by at least 150 m. Seed were sown in the Illinois Division of Forestry's Union Nursery near Jonesboro in a randomized complete block design with three replications. Two-year-old transplants were lifted from the nursery in February 1977 and used to establish field tests. Surplus seedlings were air-dried and stored at approximately 2 C until June 1977.

We selected five 2-year-old white ash seedlings from each of seven populations originating over a range of latitude of 36.4 to 45.9 degrees, from New Brunswick to Arkansas (Table 1). One tree was subsequently lost from both the Ohio and Indiana populations reducing the total number of trees studied to thirty-three.

A stem segment between the fourth and sixth nodes was removed from each tree (Richardson 1961), even though the small amount of intra-tree variation in fibers found in *Fraxinus excelsior* (Denne and Whitbread 1978) does not suggest the necessity of such sampling. Transections were microtomed from the basal end of each sample segment, stained, and mounted on slides for microscopic observation. The remainder of each sample segment was dissected, discarding all tissues external to the vascular cambium, and splitting the second-year wood away from the first-year wood and pith. The second-year wood was cut into small, uniform pieces and macerated in Jeffrey's fluid (Johansen 1940). The macerated tissue was stained, dehydrated, and mounted on slides. All cell size measurements were based on fifty randomly selected cells.

Microspectrophotometry was used to determine relative amounts of DNA per nucleus, since material was unavailable for chromosome counts. One centimeter of bark distally adjacent to the sample segment was removed from each stem, infiltrated, and embedded in Paraplast using standard histological techniques. The bark segments were sectioned tangentially at 10 μm and mounted on slides with chrom-alum adhesive. Nuclei were readily located in phellogen, phelloderm, and cortical cells. Each slide had bark sections from a tree of unknown ploidy level

and from a tree from a known diploid population of white ash, as determined by Schaefer and Miksche (1978). Chicken erythrocyte nuclei were not used as an internal standard, since for the purposes of this study it was only necessary to compute relative values for the DNA content per nucleus to sort polyploids from diploids. The hydrolysis and staining procedure of Schaefer and Miksche (1978) was followed exactly. The instrumentation used was described in detail by McGinnes and Melcarek (1976) and consisted of a Leitz research microscope in conjunction with a Leitz MPV microspectrophotometer. The absorbancy at 560 nm was determined and multiplied by the area of the nucleus being measured to calculate the relative DNA content per nucleus. A minimum of twenty-five nuclei were measured for each unknown tree, and a minimum of ten nuclei were measured per slide of the control tree. The average DNA content per nucleus of each unknown tree was compared by t-test to the DNA content per nucleus of the control diploid tree. Since the slide-to-slide variation between the sections of the diploid control was statistically negligible, the measurements of the control, a minimum of fifty nuclei, were pooled for each geographic area tested.

RESULTS

Intra- and inter-population variation in wood cell sizes was evident. The wood cell sizes of the four northernmost populations were very homogeneous (Table 2, Fig. 1), while those of the three southernmost populations were more variable. Two of the three southernmost populations, Indiana and Illinois, were known to have hexaploid individuals (Schaefer and Miksche 1978), so it was necessary to compare the relative DNA content of the nuclei with the average wood cell size, since increased cell size is the most common result of polyploidy (Stebbins 1950).

Seven trees, four from the Indiana seed source, two from Illinois, and one from Arkansas, had nuclei that contained significantly greater amounts of DNA (Table 3). Six of the same seven trees had the longest vessel elements, and four of the seven trees had the longest fibers (Fig. 1). The average amount of DNA per nucleus varied considerably from tree to tree. Arkansas 3 had three times as much DNA as the diploid control. Four trees, Indiana 1 and 2, Illinois 3 and 4, had slightly less than 2 times as much DNA as the control. Indiana 4 had 1.4 times and Indiana 3 had 2.3 times as much DNA as the diploid control. Since the sample size is rather small and confirmatory chromosome counts could not be made from the stem specimens, the level of ploidy cannot be established. Throughout the remainder of this report, those seven trees will be referred to as polyploids, since the actual ploidy level is not crucial to the interpretation of the results.

Intrapopulation genotypic variation was not as great as the interpopulation variation between polyploids and diploids. The average cambial initial length, as reflected by the lengths of the vessel elements and fibers, did not show a statistically significant change from north to south; however, the diploid trees with the longest vessel elements and fibers were from the southernmost seed source. The polyploid trees had significantly longer and larger diameter vessel elements, and also had longer fibers (Table 2, Fig. 1). The vessel elements of the seven polyploid trees averaged 0.36 mm in length by 0.042 mm in diameter compared to 0.26 mm by 0.033 mm for the diploid trees. The fibers of the polyploid trees measured 0.80

TABLE 2. *Selected characteristics of second-year wood.*

Population	Vessel element length	Fiber length	Vessel element diameter	Growth[a]	Pore arrangement	Polyploid[b]
New Brunswick						
Tree 1	0.23 ± 0.06 mm[c]	0.53 ± 0.13 mm	0.032 ± 0.012 mm	1.65 mm	Ring-porous	–
Tree 2	0.24 ± 0.05 mm	0.64 ± 0.12 mm	0.034 ± 0.015 mm	0.81 mm	Ring-porous	–
Tree 3	0.26 ± 0.07 mm	0.71 ± 0.15 mm	0.031 ± 0.010 mm	1.70 mm	Semiring-porous	–
Tree 4	0.24 ± 0.03 mm	0.65 ± 0.13 mm	0.034 ± 0.014 mm	1.68 mm	Diffuse-porous	–
Tree 5	0.25 ± 0.04 mm	0.62 ± 0.10 mm	0.028 ± 0.007 mm	1.90 mm	Diffuse-porous	–
Vermont						
Tree 1	0.25 ± 0.04 mm	0.73 ± 0.11 mm	0.030 ± 0.008 mm	1.49 mm	Ring-porous	–
Tree 2	0.25 ± 0.05 mm	0.69 ± 0.12 mm	0.032 ± 0.008 mm	1.20 mm	Semiring-porous	–
Tree 3	0.24 ± 0.04 mm	0.69 ± 0.13 mm	0.032 ± 0.011 mm	0.93 mm	Semiring-porous	–
Tree 4	0.26 ± 0.06 mm	0.68 ± 0.13 mm	0.030 ± 0.009 mm	1.24 mm	Diffuse-porous	–
Tree 5	0.27 ± 0.06 mm	0.65 ± 0.11 mm	0.026 ± 0.009 mm	1.35 mm	Semiring-porous	–
New York						
Tree 1	0.25 ± 0.04 mm	0.71 ± 0.10 mm	0.037 ± 0.012 mm	0.94 mm	Semiring-porous	–
Tree 2	0.27 ± 0.06 mm	0.70 ± 0.13 mm	0.028 ± 0.008 mm	0.54 mm	Diffuse-porous	–
Tree 3	0.27 ± 0.05 mm	0.75 ± 0.09 mm	0.032 ± 0.009 mm	0.97 mm	Ring-porous	–
Tree 4	0.24 ± 0.04 mm	0.70 ± 0.07 mm	0.032 ± 0.009 mm	0.64 mm	Diffuse-porous	–
Tree 5	0.27 ± 0.06 mm	0.68 ± 0.11 mm	0.037 ± 0.006 mm	0.62 mm	Diffuse-porous	–
Ohio						
Tree 1	0.26 ± 0.04 mm	0.74 ± 0.08 mm	0.029 ± 0.010 mm	1.48 mm	Diffuse-porous	–
Tree 2	0.25 ± 0.04 mm	0.68 ± 0.07 mm	0.033 ± 0.009 mm	1.65 mm	Diffuse-porous	–
Tree 3	0.26 ± 0.03 mm	0.67 ± 0.10 mm	0.034 ± 0.010 mm	1.09 mm	Ring-porous	–
Tree 4	0.26 ± 0.05 mm	0.72 ± 0.08 mm	0.033 ± 0.010 mm	0.94 mm	Diffuse-porous	–

(Table 2 continued on page 116)

TABLE 2. *Continued.*

Population	Vessel element length	Fiber length	Vessel element diameter	Growth[a]	Pore arrangement	Polyploid[b]
Indiana						
Tree 1	0.42 ± 0.12 mm	0.89 ± 0.14 mm	0.046 ± 0.013 mm	1.17 mm	Diffuse-porous	+
Tree 2	0.34 ± 0.05 mm	0.68 ± 0.08 mm	0.046 ± 0.009 mm	0.52 mm	Diffuse-porous	+
Tree 3	0.34 ± 0.05 mm	0.86 ± 0.13 mm	0.051 ± 0.014 mm	2.03 mm	Diffuse-porous	+
Tree 4	0.29 ± 0.05 mm	0.66 ± 0.09 mm	0.046 ± 0.010 mm	1.76 mm	Diffuse-porous	+
Illinois						
Tree 1	0.26 ± 0.04 mm	0.60 ± 0.12 mm	0.034 ± 0.012 mm	1.07 mm	Diffuse-porous	−
Tree 2	0.25 ± 0.05 mm	0.58 ± 0.10 mm	0.030 ± 0.014 mm	1.09 mm	Diffuse-porous	−
Tree 3	0.39 ± 0.08 mm	0.75 ± 0.19 mm	0.026 ± 0.005 mm	1.50 mm	Diffuse-porous	+
Tree 4	0.34 ± 0.06 mm	0.86 ± 0.10 mm	0.035 ± 0.012 mm	1.36 mm	Diffuse-porous	+
Tree 5	0.28 ± 0.04 mm	0.70 ± 0.09 mm	0.029 ± 0.009 mm	1.45 mm	Diffuse-porous	−
Arkansas						
Tree 1	0.30 ± 0.04 mm	0.83 ± 0.14 mm	0.040 ± 0.010 mm	0.93 mm	Diffuse-porous	−
Tree 2	0.26 ± 0.05 mm	0.79 ± 0.16 mm	0.034 ± 0.012 mm	2.41 mm	Diffuse-porous	−
Tree 3	0.40 ± 0.07 mm	0.89 ± 0.17 mm	0.042 + 0.014 mm	2.84 mm	Diffuse-porous	+
Tree 4	0.31 ± 0.05 mm	0.78 ± 0.12 mm	0.039 ± 0.012 mm	0.92 mm	Diffuse-porous	−
Tree 5	0.23 ± 0.04 mm	0.65 ± 0.09 mm	0.039 ± 0.011 mm	1.09 mm	Diffuse-porous	−

[a] Average radius of second growth ring.
[b] See Table 3 and text for explanation.
[c] All measurement data expressed as mean ± 1 standard deviation.

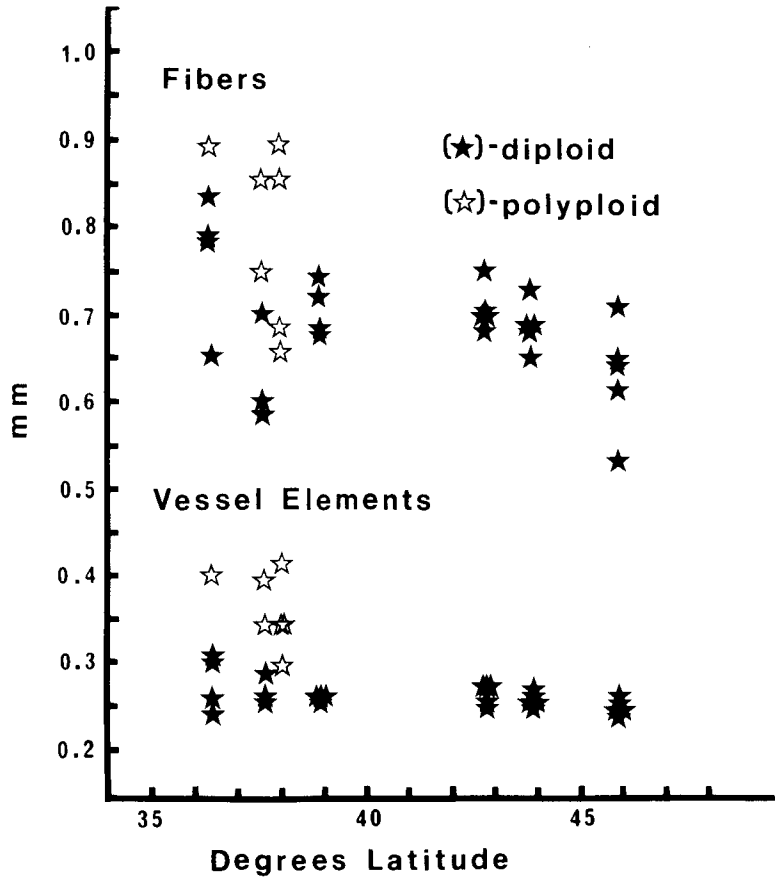

FIG. 1. Latitudinal variation in mean fiber and vessel element length of diploid and polyploid white ash trees.

mm in length compared to 0.69 mm for the diploid trees. Three of the polyploid trees had fibers whose length fell well within the range of the diploid trees, even though two of the same trees had longer vessel elements than the diploid trees, indicating longer cambial initials.

The growth rates, as expressed by the amount of secondary xylem produced, showed a great deal of variation, from about 0.5 mm to as much as 2.8 mm (Table 2), and did not correlate with the cell size data or geographic location of the seed source. There was a slight tendency for the pore arrangement to be ring-porous or semiring-porous in the more northern populations, but the majority of the trees were diffuse-porous. There was no correlation between the elevation of the seed source and the cell size data.

DISCUSSION

These data show two genetic components to the variation in wood cell sizes in white ash. One component is related to geographic origin of the seed source; the other is related to the ploidy level. The two sources of genetic variation will be considered separately for purposes of discussion.

TABLE 3. *Relative amounts of DNA per nucleus.*

	Ohio	Indiana	Illinois	Arkansas
Control[a]	687	760	746	764
Tree 1	833 n.s.[b]	1,420**[c]	679 n.s.	685 n.s.
Tree 2	603 n.s.	1,340**	675 n.s.	582 n.s.
Tree 3	802 n.s.	1,710**	1,462**	2,433**
Tree 4	754 n.s.	1,005**	1,385**	587 n.s.
Tree 5	—	—	936 n.s.	763 n.s.

[a] Tree from known diploid population.
[b] Not significantly different from diploid control.
[c] Significant at greater than 0.01 level.

The vessel elements and fibers of diploid trees of southern origin are slightly longer than those from more northerly locations. The final length of any imperforate tracheary element is determined by two components, the length of the cambial initial from which it was derived, and the extent of intrusive growth during development. The increase in fiber length may be related to an increase in either or both of these components. In white ash the increase in the length of fibers is equal to or slightly greater than the increase in the length of the vessel elements (Fig. 1). Since vessel elements do not elongate during their ontogeny, vessel element length is directly related to cambial initial length; therefore, the increase in fiber length of white ash may be attributed to an increase in cambial initial length.

The latitudinal range studied was only 9.5° and the related change in vessel element and fiber lengths was not statistically significant at the 0.05 level; however, vessel elements and fibers were greater in length, by 13% and 21% respectively, in the population originating at the lowest latitude (36.4°N) compared to the population originating at the highest latitude (45.9°N). This trend agrees with the studies by Winstead (1972, 1978) and Randel and Winstead (1976) and indicates that an increase in cambial initial length is an ecotypic response to conditions at a lower latitude. Higher elevation ecotypes have been shown to be similar to higher latitude ecotypes (Baas 1973, 1976), but the elevation of the seed source was not related to wood cell lengths in our white ash seedlings. The maximum range of elevation of origin of the collections was only 600 m and the lack of effect is hardly surprising. Similarly only slight changes were noted by Van der Graff and Baas (1974) between specimens collected as much as 2,000 m apart in elevation.

These data indicate that polyploid trees can be expected to have cells significantly longer than diploids. Six of the seven polyploid trees had longer vessel elements, indicating longer cambial initials than any diploid trees. It is impossible to speculate why the other two polyploid trees did not have longer fibers as well, but since they did have longer cambial initials, it must be related to less ontogenetic elongation.

From these results it is impossible to make any certain correlation between levels of polyploidy and wood cell size. The amounts of DNA observed in these trees are very close to ploidy levels of 2N, 3N, 4N, 5N, and 6N, as calculated using the average value of DNA/nucleus of the diploid control tree as 2N, but the possibility of variation caused by experimental treatment cannot be eliminat-

ed. The accuracy of the microspectrophotometric method of determining DNA content per nucleus could be adversely affected by the aldehydes in the fixative and the handling and storage of the specimens prior to fixation. However, Indiana 4 had both the shortest cells and the lowest average DNA content per nucleus of the seven polyploid trees.

The possibility remains that the longer wood cell characteristics of young polyploid trees may not be evident in mature wood. For instance, young triploid aspen contained longer fibers than diploids, but the differential in wood cell sizes between diploids and triploids was not apparent in more mature wood (Buijtenen et al. 1957, 1958; Einspahr et al. 1963; Einspahr et al. 1968). During maturation the average cambial initial length increases such that the initial difference between the cell lengths of diploids and polyploids may not be maintained. Nevertheless, of the trees of *Fraxinus excelsior* examined by Denne and Whitbread (1978), the trees that started with somewhat longer fibers retained longer fibers through twenty-two years of growth. Additional studies are required to determine whether polyploid white ash trees in natural populations have longer wood cells and different wood quality than diploids.

Trees with longer fibers could be important for pulp-paper industries and longer cells might improve overall wood quality. Of the two sources of genetic variation found in this study, the variation related to the ploidy level was greater and would seem to offer more potential for a program to increase fiber length in white ash. This could prove to be futile if polyploid trees had lower wood quality and/or were significantly slower growing and generally less fit than the diploids. Early results from the study of field performance of white ash originating throughout the natural range (Bey et al. 1977) suggest that the opposite may be true with regard to vigor. Ploidy level has not been determined for many families, but among those that have been identified, tetraploid ash trees have consistently outgrown diploids at several locations in the southeastern United States.[2] As Wright (1944) pointed out, the relationship between wood quality and polyploidy in white ash is not known and "might help to explain some seemingly inexplicable differences in strength of wood."

The lack of correlation between the amount of wood produced and cell size indicates that faster growing trees might be selected without reducing wood cell length. The lack of correlation between the rate of cambial division and length of cambial initials was also noted for *Fraxinus excelsior* (Denne and Whitbread 1978), even though the correlation has been well established in conifers (Bannan 1967) and reported in at least one hardwood species, *Liquidambar styraciflua* (Randel and Winstead 1976). There was no significant difference in amount of secondary xylem produced between the diploid and polyploid trees, although the seven polyploid trees averaged slightly less radial growth. The slowest and fastest wood producers in the study were both polyploids.

If differences in fiber length among seedlings persist until the trees attain commercial timber size, and if the early relationships between ploidy level and both fiber length and growth are maintained in mature trees, a breeding program for

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[2] Clausen, Knud E. Unpublished data from study NC-1151, CG-428 on file at Forestry Sciences Laboratory, Southern Illinois University, Carbondale, Illinois.

polyploidy white ash can be easily justified. On the basis of these very early results, tetraploid white ash appears especially promising for further testing in the southern United States.

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