POLYPLOIDY AND WOOD ANATOMY OF MATURE WHITE ASH, *FRAXINUS AMERICANA*[1]

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ABSTRACT

The ploidy levels of ten field-grown white ash trees from southern Illinois were determined using cytophotometric methods. A wood sample was removed from the bole of each tree and examined to determine wood cell sizes, increment widths, and specific gravity. Six of the trees examined were diploids, two were tetraploids, and two were hexaploids. There were no differences between the ten trees in wood specific gravity. The higher ploidy levels did show a statistically significant correlation with longer vessel elements and longer fibers. The increase in fiber length was attributable both to an increase in the average cambial initial length and a proportionate ontogenetic elongation.

Keywords: Polyploidy, wood anatomy, white ash, fiber length, specific gravity, *Fraxinus americana*.

In a prior study two components of genetic variation, one related to ecotypic variation and one related to the ploidy level of the tree, were identified in the wood of white ash seedlings (Armstrong and Funk 1980). The wood of polyploid white ash seedlings had significantly longer vessel elements, and therefore, longer fusiform cambial initials, and longer fibers than diploid seedlings. If the differences between the wood of polyploid and diploid individuals persisted until the trees attained commercial timber size, a breeding program to increase the fiber length by selecting polyploid white ash can be easily justified. The data from two-year-old seedlings do not provide any basis for assuming that a similar cell size differential exists in the wood of mature trees, especially since many juvenile wood characters change markedly with increasing maturity, particularly an increase in the average cambial initial length. Differences between wood cell sizes of diploid and triploid aspen were evident in young trees, but were not evident in more mature trees (Buijtenen et al. 1957, 1958; Einspahr et al. 1963, 1968), but the higher ploidy levels found in white ash, 4n and 6n, are not necessarily comparable to the 2n, 3n aspen. The fact that there is a genetic component influencing average cambial initial length suggests that individuals with longer cambial initials would be expected to maintain longer cambial initials throughout their life. Trees of *Fraxinus excelsior* that started with somewhat longer fibers retained a longer fiber length throughout 22 years of growth (Denne and Whitbread 1978).

Stomata size, bud morphology, bud and leaf scar morphology have been successfully used to distinguish diploid, tetraploid, and hexaploid nursery-grown ash trees, but with field-grown trees these anatomical/morphological characters could only be used to distinguish diploids from polyploids (Wright 1945; Santamour

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1962). Specific information on the distribution of ploidy levels throughout the range of white ash required cytophotometric methods. The distribution of tetraploids and hexaploids in the southern half of the range of white ash was confirmed using seed embryos, the progeny of open-pollinated native populations (Schaefer and Miksche 1977). The ploidy levels of white ash seedlings have also been successfully determined using cytophotometric methods (Armstrong and Funk 1980). Since the later study used sectioned material and measured the DNA content of cells located in the phellogen and phelloderm, the same method could be used on tissue removed from the twigs of mature trees. Identifying the ploidy level of individual trees in populations growing under natural field conditions still presents a problem because cytophotometric methods are laborious and do not yield immediate results.

Since identification of tetraploid and hexaploid field-grown white ash trees has to be based either on cytophotometric study of the progeny or limited because of the inherent variation of anatomical/morphological characters used to identify polyploids, the precise ploidy levels of mature field-grown trees remain largely unknown. As a result it has been impossible to study wood anatomy in relation to ploidy level, and no attempt has been made to determine if there are qualitative and quantitative differences in the wood of mature trees of different ploidy levels. Wright (1944) suggested that the presence of polyploids in the central portion of the range of white ash might help explain some seemingly inexplicable differences observed in the wood strength of white ash trees in the same region. To these ends it is the purpose of this study to select mature white ash trees, which represent a seed-source population that has produced progeny of different ploidy levels, and to determine the ploidy level of these mature trees by cytophotometric methods. The wood will be examined for qualitative and quantitative differences that might result in differences in wood quality.

METHODS AND MATERIALS

The seedling and seed embryo progeny of the USDA Forest Service seed collection source location NC-6721 from Jackson, Union and Williamson Cos. in Illinois had been found to have diploid, tetraploid, and hexaploid chromosome compliments (Schaefer and Miksche 1977; Armstrong and Funk 1980). Since these trees were convenient and easily located (each tree is individually identified), ten trees were selected from this population for study. Five of the trees were pistillate from which seed had been previously collected, and the remaining five were staminate. The ten trees were from three locations (Table 1). All of the trees studied were mature and of a similar size, 27–35 cm dbh, except Tree 23, which was about 40% smaller in dbh (Table 1). The individual trees were located within a few kilometers of each other but were separated by at least 150 m. In December 1978, a large-diameter core was removed from the south side of each tree at a height of 1 m. The cores contained several increments and the five most recent increments were chosen for study (1974–1978). Scions were collected in April 1979.

One-half-cm bark sections were removed from 1- and 2-year-old twigs and fixed in Carnoy's #2 (Berlyn and Miksche 1976) for 4 h. The tissue was then dehydrated in an ethanol series, transferred to t-butanol, and infiltrated with a paraffin-based

TABLE 1. *Field data of study trees.*

Tree ID[a] number	Location	Flower type	dbh (cm) 1978
10	Shawnee National Forest, Jackson County	Pistillate	28
11	Shawnee National Forest, Jackson County	Pistillate	30
12	Shawnee National Forest, Jackson County	Pistillate	31
13	Evergreen Park, Jackson County	Pistillate	30
14	Evergreen Park, Jackson County	Pistillate	31
23	Evergreen Park, Jackson County	Staminate	18
24	Evergreen Park, Jackson County	Staminate	35
25	Evergreen Park, Jackson County	Staminate	27
26	Evergreen Park, Jackson County	Staminate	29
27	Hickam Woods, Jackson County	Staminate	28

[a] USDA Forest Service, North Central Experiment Station, Seed Source Location NC-6721.

embedding medium. The bark was sectioned radially at 10 μm. The sections were mounted on slides with a chrome-alum gelatine adhesive. Hydrolysis and Feulgen staining were done following the method outlined by Schaefer and Miksche (1977) and Berlyn and Miksche (1976). Nuclei were readily located in the phellogen and phelloderm. Chicken erythrocyte nuclei were not used to compute actual DNA content per nucleus, since for the purposes of this study it was only necessary to compute relative values of DNA per nucleus to determine the ploidy levels of the trees. The cytophotometry was done on a Leitz research microscope in conjunction with a Leitz MPV microspectrophotometer. The instrumentation was described in detail by McGinnes and Melcarek (1976). It was determined that the Feulgen stain absorbency was near maximum at 560 nm. The absorbancy of each nucleus at 560 nm was measured and multiplied by the area of the nucleus to calculate the relative DNA content per nucleus. A minimum of twenty-five nuclei were measured from each tree. The average DNA content per nucleus of the ten trees was compared by a Tukey-B Multiple Range Test. Sections from one tree were used as a slide-to-slide control. The slide-to-slide variation in average DNA content per nucleus of the control tree was found to be statistically negligible.

The specific gravity of the five study increments was determined by the maximum moisture content method (Smith 1954). The wood was macerated in Jeffrey's fluid (Johansen 1940). The macerated tissue was stained, dehydrated, and mounted on slides. All cell size measurements are based on fifty randomly selected cells and reported as means +/− one standard deviation. All fiber measurements were made on non-pore zone fibers. Pore zone fibers are considerably shorter than the fibers in the rest of the increment and can be easily distinguished by their abruptly constricted cell ends (Chalk 1970). Cell-wall thickness and fiber diameter were measured in the median portion of the fibers. The orientation of cellulose microfibrils in the S2 layer of the secondary walls of the fibers was determined by measuring the angle of deviation of pit aperatures and wall striations from the vertical cell axis.

RESULTS

Differences in the average DNA content per nucleus between trees demonstrated that there were six diploids, two tetraploids, and two hexaploids in the

TABLE 2. *Average DNA content per nucleus and corresponding tree ploidy level.*

Tree ID number	DNA content/nucleus[a]	Ploidy level
24	251 ± 30 *[b]	2n
14	260 ± 66 *	2n
26	280 ± 98 *	2n
25	282 ± 49 *	2n
13	283 ± 67 *	2n
27	325 ± 44 *	2n
11	517 ± 66 ***	4n (1.9×)[c]
23	568 ± 112 ***	4n
10	792 ± 109 ******	6n (2.9×)
12	820 ± 117 ******	6n

[a] In relative units, ±1 standard deviation.
[b] Multiple Range Test, Tukey-B procedure. (*) denotes groups significantly different at the 0.050 level.
[c] 4n and 6n values are 1.9 and 2.9 times the average 2n value, respectively.

ten tree samples (Table 2). Although confirmatory chromosome counts could not be made[2], these findings agree with those of Schaefer and Miksche (1977) and Armstrong and Funk (1980). In partial confirmation, Schaefer and Miksche (1977) found that embryonic offspring of Tree NC 6721-12 were hexaploid.

The average DNA content per nucleus of the ten trees fell into three discrete groups that are very close to the predicted intervals for diploid, tetraploid, and hexaploid chromosome compliments. The tetraploids have 1.9× as much DNA per nucleus as the diploids, and the hexaploids have 2.9× as much. The slight depression below the expected 2× and 3× values had been observed and noted previously (Schaefer and Miksche 1977) but not explained.

Two of the six diploid trees are pistillate, the remaining four are staminate. One tetraploid tree is pistillate; the other is staminate. Both hexaploid trees are pistillate. Both hexaploid trees and one of the tetraploids are from the Shawnee National Forest location. The Hickam Woods tree is diploid. Five of the six trees located in Evergreen Park are diploids; the remaining tree is tetraploid. It should be pointed out that five of the six trees in Evergreen Park, all but number 28 (2n), represent plantings of unknown origin. The remaining five trees in the study, by all appearances and by their locations, are certain natives.

All of the trees studied were mature and of a similar size, 27–35 cm dbh (Table 1), except Tree 23, which was about 40% smaller in dbh. The average increment width varied from 1.5 mm (Tree 23) to 7.6 mm (Tree 26) (Table 3). Both were located in Evergreen Park. The average increment width showed a weak positive correlation with the specific gravity ($r = +0.58$), but there was no correlation with the ploidy levels. Gross observations of the wood prior to maceration showed that the smaller increments had porportionately larger amounts of spring wood, which is identified by larger diameter vessels.

The mean vessel element length of the mature wood ranged from 0.18 mm (Tree 14) to 0.32 mm (Tree 12) (Table 3). The mean fiber length of the wood

[2] All attempts to root scions failed.

TABLE 3. *Specific gravity and wood cell data.*

Tree ID number	Ploidy level	Specific gravity	*Fiber length* (mm)	*Vessel element* length (mm)	F/V[a] ratio	Fiber diameter (10^{-3} mm)	Cell-wall[b] thickness (10^{-3} mm)	Helix[c] angle	Increment width (mm)
13	2n	0.59	0.76 ± .10	0.19 ± .04	4.0	16 ± 2	3.1 ± 0.4	30 ± 6	4.1 (3.6, 4.2, 4.7, 4.0, 3.8)[d]
14	2n	0.52	0.93 ± .13	0.18 ± .03	5.2	16 ± 2	2.6 ± 0.4	34 ± 5	5.4 (3.5, 8.9, 5.8, 4.0, 4.7)
24	2n	0.55	0.86 ± .14	0.20 ± .04	4.3	19 ± 2	2.6 ± 0.4	35 ± 5	5.1 (4.6, 4.1, 4.4, 6.3, 5.9)
26	2n	0.57	0.89 ± .23	0.23 ± .04	3.9	16 ± 2	2.7 ± 0.4	29 ± 6	7.6 (6.8, 6.2, 8.5, 8.0, 8.5)
27	2n	0.52	0.87 ± .16	0.26 ± .04	3.3	21 ± 2	2.6 ± 0.7	27 ± 9	2.8 (2.0, 2.0, 2.5, 2.7, 4.8)
25	2n	0.47	0.89 ± .20	0.24 ± .04	3.7	16 ± 2	3.0 ± 0.5	38 ± 3	4.8 (3.6, 4.2, 5.5, 5.9, 5.0)
11	4n	0.58	1.11 ± .30	0.30 ± .06	3.7	21 ± 3	3.3 ± 0.6	28 ± 3	4.3 (5.1, 5.2, 3.5, 2.7, 5.0)
23	4n	0.51	0.93 ± .11	0.25 ± .06	3.7	18 ± 2	2.8 ± 0.4	32 ± 4	1.5 (1.7, 1.2, 1.0, 1.3, 2.5)
10	6n	0.58	1.20 ± .28	0.30 ± .06	4.0	20 ± 3	3.2 ± 0.4	24 ± 7	5.2 (6.1, 6.8, 5.6, 3.2, 4.1)
12	6n	0.54	1.25 ± .29	0.32 ± .05	3.9	24 ± 3	3.4 ± 0.6	22 ± 3	7.3 (5.2, 6.2, 6.0, 8.5, 10.8)

[a] Fiber length/vessel element length.
[b] Measured in median portion of non-pore zone fibers.
[c] Cellulose microfibril orientation measured in degrees from vertical cell axis.
[d] Average increment width 1974–1978 (increment width 1978, 1977, 1976, 1975, 1974).

ranged from 0.76 mm (Tree 13) to 1.25 mm (Tree 12) (Table 3). Those trees with longer vessel elements, and therefore longer cambial initials, also had longer fibers, and the ratios of fiber to vessel element length were not affected by ploidy levels (F/V ratio, Table 3).

Increases in the vessel element length and the fiber length of the bole wood were statistically correlated with the increases in the ploidy level found in these trees ($r = +0.84$, $P < .05$; $r = +0.90$, $P < .01$, respectively). Neither the vessel element nor the fiber length showed any correlation to the increment width or the specific gravity. Fibers from hexaploid and tetraploid trees averaged 41% and 18% longer, respectively, than fibers from diploid trees. Similarly, vessel elements from hexaploid and tetraploid trees average 43% and 27% longer, respectively, than vessel elements from the diploid trees. An increase in the average fiber length had a statistically significant correlation ($r = -0.69$, $P < .05$) with a decrease in the average angle of the cellulose microfibral orientation. The six diploid and two tetraploid trees all had similar microfibril orientations, with mean angles ranging from 27° to 38° (Table 3). The two hexaploid trees showed microfibril orientations averaging 23°.

The specific gravity of the bole wood varied from 0.47 to 0.58, all very close to published values for white ash wood. These was no significant correlation with ploidy level or cell size. While an increase in mean fiber diameter was significantly correlated to the increases in ploidy level ($r = +.68$, $P < .05$), there was a concomitant increase in mean cell-wall thickness that was highly correlated to the increase in fiber diameter ($r = +0.75$, $P < .01$) (Table 3). The fibers of the two hexaploid trees were about 29% larger in median diameter than those of the diploids. Similarly, the fiber cell walls of the hexaploids were about 18% thicker than those of the diploids.

DISCUSSION

Seed source NC-6721 does contain diploid, tetraploid, and hexaploid individuals. While the cytophotometric methods allowed identification between tetraploid and hexaploid field-grown trees, which is not possible using only anatomical/morphological features, the technique is laborious. Since chromosome counts could not be made, it was impossible to confirm the ploidy levels determined by this indirect technique; however, in partial confirmation, a progeny of tree number 12 (6n) was determined to be a hexaploid by Schaefer and Miksche (1977). While this seed source (NC-6721) has individuals of all three ploidy levels known for white ash, it is unfortunate that this seed source does not represent a totally native population. At least five of the six Evergreen Park trees were in locations that suggested that they were plantings and not naturalized. Since only one of these five trees was a polyploid (Tree 23), the native population in this area might have a higher frequency of polyploid individuals than this study, or similar studies, would suggest. Also, Tree 24, which was similar to 2n white ash in all characters studied, was subsequently identified as green ash.

The tetraploids and hexaploids averaged 1.9 and 2.9 times as much DNA/nucleus as the diploids. This slight reduction below the expected DNA content values was also observed by Schaefer and Miksche (1977). While offering no

explanation for this phenomenon, the similarity of results further verifies the accuracy of the cytophotometric method when adapted to paraffin-embedded, sectioned material.

The environmental differences between trees and between locations seem to have been contributing a very small component to the differences in the wood of these trees. The largest differences, cell sizes, were directly related to the increased ploidy levels. This is not surprising since cell length seems to be largely affected by environmental differences associated with latitudinal changes (Winstead 1972, 1978; Randel and Winstead 1976). The only suggestion of environmental influence concerned Tree 23, which was located 3 m from a small brick building and adjacent to a driveway. Tree 23 had the smallest dbh, 18 cm, the smallest average increments, and the smallest vessel elements and fibers of the four polyploid trees. The absolute ages of the trees are unknown, although Trees 23 and 24 could have been planted at the same time since they are in the same roadside row. In this case slow growth and somewhat more xeric conditions, perhaps as a result of soil compaction associated with relatively heavy human and automobile traffic, and a corresponding reduced availability of moisture, may be responsible for the shorter wood cells.

The final length of any imperforate tracheary element is determined by the length of the cambial initial from which it was derived and the extent of intrusive growth during the cell's ontogeny. Both the vessel elements and fibers of the tetraploid and hexaploid trees were longer than their diploid counterparts to about the same degree. The 4n and 6n vessel elements were 27% and 43% longer, respectively, than the diploids. The 4n and 6n fibers were 18% and 41% longer, respectively, than the diploids. This indicates that the intrusive growth of the fibers was in proportion to the vessel element length, and therefore, in proportion to the cambial initial length. While there was some variation, the fibers in all the trees averaged about 4 times as long as the vessel elements (F/V ratio, Table 3); therefore, differences in the ploidy level had little influence on the proportionate amount of intrusive growth of the fibers, and the differences in wood cell sizes can be largely attributed to the larger cambial initial length found in the polyploid trees. This agrees with most other studies of plant anatomy in relation to polyploidy, where the greatest difference was the increase in cell size found in the polyploids. This difference is the basis for using such characters as stomatal guard cell size to identify polyploids and ploidy levels. Unfortunately, even large sample sizes of wood cell measurements would not be adequate to identify specific ploidy levels, although, diploids and polyploids could be identified.

Since the intrusive growth of fibers in all the study trees remained in proportion to the cambial initial length, it is obvious that because they started as longer derivatives, the fibers in the polyploid trees had to undergo a larger absolute amount of ontogenetic elongation to achieve the proper proportionate length of about four times the length of the vessel elements. The increase in the amount of elongation resulted, particularly in the hexaploid trees, in a reduced angle of the cellulose microfibril helix. Since the helix angle is a function of both the elongation of the fiber (increased elongation reduces the helix angle) and the cell width (increased cell width increases the helix angle) (Panshin and deZeeuw 1970), and since both the fiber elongation and diameter increased with increased

ploidy level, the cell elongation appears to have the greatest influence. It is possible that the decreased cellulose microfibril helix angles of the hexaploid fibers might positively influence wood quality.

While the polyploid trees in this study had larger diameter and significantly longer fibers, there was no difference in the specific gravity. This is somewhat surprising since the specific gravity of wood is a measure of the relative amount of cell-wall material in a unit volume, and it would be logical to expect the increased cell size to reduce the specific gravity. However, the concomitant increase in cell-wall thickness may be responsible, in part, for keeping the amount of cell-wall material constant, relative to the cell volume. In addition, the fibers of the polyploid trees, while larger in diameter in the median portion, had longer, narrower, more gradually tapering cell ends. In these portions of the cell, the cell wall would account for a larger portion of the cell volume.

Specific gravity is considered one of the more important properties that influence wood strength, while differences in cell size and arrangement are usually associated with physical aspects of the grain. Wright (1944) suggested that differences between the wood of diploid and polyploid white ash might account for inexplicable differences observed in the strength of white ash wood. This suggestion was based on the fact that the observed differences in wood strength occurred in the same geographic region where differences in ploidy level were observed in white ash. If the differences in wood strength were related to differences in ploidy level, the parameter being measured would have had to be capable of being influenced by different cell sizes and not specific gravity. Wright (1944) did not specify the nature of the observed differences in wood strength, so it is impossible to speculate. These data suggest that great differences in wood quality between diploids and polyploids would not necessarily be expected; however, the increased fiber length, the increased fiber cell-wall thickness, and the reduced microfibril helix angle of the hexaploid trees may positively influence wood quality. One of the limitations of studying such small wood samples is that not all of the mechanical properties of the wood can be determined.

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