Variation in White ash

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Summary

Seed of individual white ash parent trees from most of the species range were studied and used to establish a provenance-progeny test. Variation in seed length and length of wing on the seed appeared to be clinal while ploidy variation accounted for most of the difference in fruit length, seed width, seedling height, and date of leaf fall. In the nursery, tetraploid seedlings were taller and held their leaves longer than those of other ploidy levels. Among-stand variation was significant for all seed traits, nursery traits, and seedling survival and height after 3 years in the plantations. Little within-stand variation was found, suggesting that individual tree selection would not be an effective tree improvement method in white ash. Collection of seed up to 300 km south of the plantation location appears advantageous and safe.

Key words: Fraxinus americana, Provenances, Progenies, Clinal Variation, Polyploidy, Seed Zones, Tree Improvement.

Zusammenfassung

Samen individueller *Fraxinus americana* Mutterbäume aus dem größten Teil des natürlichen Verbreitungsgebietes wurde untersucht und für eine Nachkommenschaftsprüfung verwendet. Samenlänge und Länge des Flügels auf dem Nüßchen zeigten eine klinale Variation, während Unterschiede in den Ploidiestufen den größten Teil der Unterschiede in Fruchtlänge, Samenbreite, Sämlingshöhe und Laubfalltermin erklärten. In der Baumschule waren tetraploide Sämlinge höher und sie behielten ihre Blätter länger als die der anderen Ploidiestufen. Alle Samen- und Sämlingsmerkmale, Überlebensfähigkeit sowie Höhe der Bäume nach drei Jahren im Feld zeigten signifikante Herkunftsunterschiede. Nur geringe Variation wurde innerhalb eines Bestandes festgestellt. Deshalb scheint die Einzelbaumauslese in *F. americana* eine nicht effektive Züchtungsmethode zu sein. Es wird angenommen, daß eine Saatguternte bis zu 300 km südlich des Pflanzortes vorteilhaft und sicher ist.

Introduction

White ash (*Fraxinus americana* L.) is an important hardwood species that grows rapidly, produces large quantities of seed, and has higher-than-average market value. The natural range extends from Texas to Minnesota and Nova Scotia to Florida. It is found from near sea level in the coastal plain to 1,200 m in the Cumberland Mountains. Extreme climatic and soil differences exist within this broad natural range.

Wright (1944) studied the geographic variation in the species and on the basis of seedling morphology and ploidy level delineated three ecotypes. Chromosome numbers range from 46 for diploids to 138 for hexaploids (Wright 1944). White ash is dioecious and the sex ratio of male to female trees has been reported both as 2:1 (Anderson and Whelden 1936) and as 1:1 (Hunt 1964). Thus, the species appears to be variable and well suited for genetic improvement.

The high-value hardwood research unit of the North Central Forest Experiment Station in Carbondale, Illinois, began a provenance-progeny test of white ash in 1976. In this paper we examine seed and seedling characteristics, study the source and magnitude of variation components, and discuss their impact on practical tree improvement programs for white ash.

Materials and Methods

Seed Collection and Sowing

Seeds were collected from up to 10 native parent trees in each of 59 locations (*Figure 1*) throughout the natural range during 1973 and 1974. The seed lots were sown in the Illinois Division of Forestry Nursery at Jonesboro, Illinois, in the fall of 1974 and the spring of 1975. The design used was randomized complete blocks with three blocks, each containing 339 families from 63 stands. The seeds were sown in 30 cm strips across the nursery bed at the rate of 800 seeds/m^2. The beds were then covered with 1 cm of soil and 5 to 8 cm of sawdust mulch. After germination in 1975, seedlings were thinned to about 5×5 cm spacing. Data were collected in the fall. Seedlings were then lifted, stored, and packaged for outplanting.

Plantation Design

Plantations were established at 22 locations throughout the eastern United States and Canada. Because we did not have enough seedlings in each family, southern plantations contained more southern sources and northern plantations had more northern seedlings. However, in this paper we will only discuss plantations established in Louisiana, Illinois, Ohio, and Wisconsin as randomized complete blocks with five replications. These four plantations have 45 families in common and are suitable for study of genotype-environment interactions.

Data Collection and Analysis

Total fruit length (*i. e.*, seed plus wing), seed length, seed width, and length of the wing on the seed were measured on 10 seeds each of 451 parent trees. The percentage of filled seed in each seedlot was determined by a cutting test (Daniels 1979). Because the variation in the data was gradual, we could not recognize any distinct ecotypes. Instead, we divided the natural range of the species into five arbitrary regions—Northeast, Lake States, Central, Southeast, and Southwest (*Figure 1*). A two-level nested ANOVA (Model II) was performed on the seed traits to test for differences among regions and stands within regions. One

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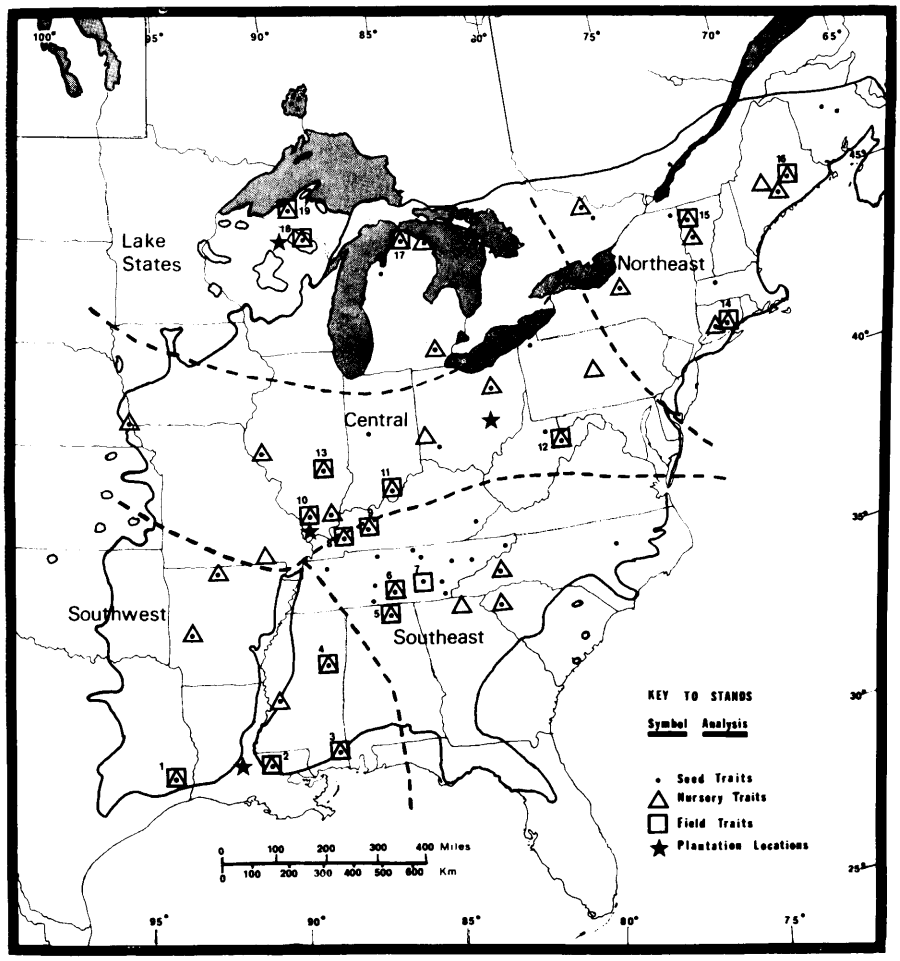

Figure 1. — Natural range of white ash showing location of regions, stands, and plantations. Stands included in plantation analysis are numbered as in *Table 5*.

way ANOVA was used to test for differences among ploidy levels in these traits. Correlations were run between seed traits and seed source data.

In the nursery, leaf color was scaled from 1 for yellow-green to 5 for dark green. A rank of 1 for leaf angle denoted acute leaf angles while 5 signified a horizontal position. Date of leaf fall was recorded as the number of days after October 1, 1975, when all the leaves in a plot had fallen. Height was measured on 10 randomly chosen trees in each plot. Only data from 228 fall-sown families were used in the analyses. Randomized blocks with three-level nested ANOVA (Modell II) was used to test for differences in nursery performance among regions, stands within regions, and mother trees within stands. For families of known ploidy, the effects of ploidy level were tested by one-way ANOVA.

Height and survival were measured in each plantation after 3 years. A mixed factorial, nested ANOVA was used to test plantation, stand, and family within stand effects. Differences among ploidy levels were tested by-one-way ANOVA.

Ploidy levels of 98 parent trees were determined cytophotometrically on the basis of DNA content by Schaefer and Miksche (1977) and Leser (1978). Their findings were used in this study because accurate chromosome counts are difficult to make in white ash due to the large number and small size of the chromosomes.

Results

Geographic Variation

Trees from the different regions varied significantly in all seed traits. Those from the Southwest and Southeast had shorter fruits, seed, and wing on the seed than those from the other regions (*Table 1*). On the other hand, trees from the Lake States and the Northeast had higher percentages of filled seed. Trees from the Southwest held their leaves longer in the nursery and were taller than those from the other regions. After 3 years in the field, these trees had the poorest survival in all but the Louisiana plantation but were the tallest in the Louisiana and the Illinois plantations (*Table 2*). In contrast, they were the shortest trees in the Ohio and Wisconsin plantations.

Several traits were significantly correlated with source origin. In the nursery, trees from lower latitudes were larger and dropped their leaves later than northern trees. All seed traits except seed width were also positively correlated with latitude. Trees from lower elevation sources tended to be greener and to have a wider leaf angle than trees from higher elevation sources (Bey *et al.* 1977), Both survival and height were negatively correlated with latitude in the Louisiana plantation; just the reverse was true in the Wisconsin plantation. The trends for these two traits were in opposite directions in the Illinois plantation, while correlations were poor in the Ohio plantation.

Table 1. — Seed and seedling characteristics of white ash from five geographic regions.

Trait	Mean of region1/ North-east	Lake States	Central	South-east	South-west	F Statistics	Variance component
			Seed traits2/				Percent
Total fruit length (mm)	35.99	34.98	35.21	32.24	29.55	20.38***	18
Seed length (mm)	15.17	14.83	13.16	10.78	10.10	100.27***	54
Seed width (mm)	2.54	2.54	2.79	2.64	2.51	7.66***	7
Wing on seed (mm)	4.90	4.74	4.02	3.20	2.82	86.54***	50
Filled seed (percent)	67.44	72.00	53.50	57.23	55.09	6.02***	5
			Nursery traits3/				
Leaf color (score)	2.96	3.15	3.05	2.91	2.98	1.05	0
Leaf angle (score)	2.81	2.81	2.72	2.59	2.73	3.12*	2
Leaf fall (days4/)	44.33	41.33	44.73	47.03	50.72	61.98***	31
Height (ft)	1.00	1.01	1.08	0.99	1.40	21.98***	14

1/ Underlined means are not significantly different from each other.
2/ Based on 438 trees.
3/ Based on 228 families
4/ Days after October 1, 1975.

* = Significant at the .05 level.
*** = Significant at the .001 level.

Genotypic Variation

Diploid trees occurred throughout the natural range of the species but tetraploids were only found south of latitude 35° N in Texas, Louisiana, and Mississippi. All pentaploids and hexaploids originated between 35° and 40° N latitude except for one pentaploid from Louisiana.

The analysis of seed traits in parent trees of different ploidy levels included 65 diploids, 8 tetraploids, 7 putative pentaploids, and 18 hexaploids. Pentaploids and hexaploids had significantly longer fruits and wider seeds than diploids and tetraploids *(Table 3)*. There were no differences among ploidy levels in seed length, length of wing on seed, and percent of filled seed.

The ANOVA's for nursery performance of 54 diploid, 8 tetraploid, 7 pentaploid, and 16 hexaploid families showed that the tretraploids retained their leaves longer and were significantly taller than trees of any other ploidy level *(Table 3)*. Pentaploids and hexaploids had the slowest growth in the nursery. Ploidy level had no effect on leaf color or leaf angle.

The magnitude of the variance components was different in the ANOVA's of nursery data for ploidy levels and for regions. They were, respectively, 52 and 14 percent for height growth and 54 and 31 percent for date of leaf fall. This suggests that ploidy level of the trees probably has a stronger effect on these traits than their geographic origin.

Table 2. — Three-year survival and height of 45 white ash families from five regions in four plantations.

Plantation	Mean of region1/ North-east	Lake States	Central	South-east	South-west	F Statistics	Variance component
	- - - - - - -Survival (percent)- - - - - -						Percent
Louisiana	72	64	80	82	81	1.81	8
Illinois	95	95	96	95	80	5.21**	32
Ohio	70	71	86	73	51	4.04**	26
Wisconsin	76	74	74	65	21	27.20***	75
	- - - - - - - - Height (cm)- - - - - - - -						
Louisiana	45.7	44.0	42.6	43.2	70.8	18.91***	67
Illinois	77.4	71.0	122.3	153.8	176.7	24.15***	72
Ohio	151.4	148.0	175.4	157.7	135.9	4.12**	26
Wisconsin	37.0	38.4	36.6	30.6	25.6	5.20**	32

1/ Underlined means are not significantly different from each other.
** = Significant at the .01 level.
*** = Significant at the .001 level.

Twenty four families of known ploidy levels were common to the four plantations. After 3 years the tetraploids were significantly taller than trees of other ploidy levels in the Louisiana plantation but significantly shorter in the Ohio and Wisconsin plantations *(Table 4)*. Although they were tallest in the Illinois plantation, the difference was not significant. Height growth of the pentaploids and hexaploids was not significantly different from that of the diploids in any of the four plantations.

Table 3. — Seed and nursery traits of white ash of different ploidy levels

Trait	Mean of ploidy level1/ 2X	4X	5X	6X	F Statistics	Variance component
		Seed traits2/				Percent
Total fruit length (mm)	31.83	32.35	38.71	39.50	11.92***	39
Seed length (mm)	11.90	11.80	12.43	12.97	0.80	0
Seed width (mm)	2.50	2.54	3.14	3.17	29.49***	64
Wing on seed (mm)	3.55	2.90	3.21	3.43	1.07	0
Filled seed (percent)	62.89	80.50	62.86	63.06	1.45	3
		Nursery traits3/				
Leaf color (score)	2.83	3.14	2.50	2.82	1.99	7
Leaf angle (score)	2.73	2.66	2.46	2.51	1.28	2
Leaf fall (days4/)	45.71	54.00	48.11	47.49	19.28***	54
Height (ft)	1.13	1.64	0.94	0.91	18.31***	52

1/ Underlined means are not significantly different from each other.
2/ Based on 98 trees.
3/ Based on 85 families.
4/ Days after October 1, 1975.

*** = Significant at the .001 level.

Table 4. — Three-year survival and height of 24 white ash families of different ploidy levels in 4 plantations

Plantation	Mean of ploidy level1/ 2X	4X	5X	6X	F Statistics	Variance component
	- - - - Survival (percent)- - -					Percent
Louisiana	76	86	80	91	1.56	10
Illinois	95	81	96	99	2.30	20
Ohio	71	51	92	91	3.27*	30
Wisconsin	65	14	80	79	20.09***	79
		Height (cm)				
Louisiana	46.1	80.4	32.6	41.6	44.07**	89
Illinois	133.4	182.0	146.0	142.1	2.51	22
Ohio	156.4	131.3	181.6	182.6	6.61**	52
Wisconsin	34.5	21.1	38.3	35.0	7.19**	54

1/ Underlined means are not significantly different from each other.

* = Significant at the .05 level.
** = Significant at the .01 level.
*** = Significant at the .001 level.

The superior early growth of the tetraploids was also apparent in several other plantations. When eight tetraploid and nine diploid families originating between 30° and 35° N were compared, the tetraploids were 11 to 62 percent taller than the diploids in six southern plantations, including the Illinois plantation (CLAUSEN *et al.* 1980). In six northern phantations their growth was poorer than that of the diploids.

Because all tetraploids identified so far originated south of latitude 33.5° N, they had poorer survival than trees of other ploidy levels when planted farther north. Percent survival of the tetraploids decreased with increasing northerly displacement but was significantly different only in the northern-most plantation (Wisconsin) (*Table 4*). However, the origin effect was not limited to tetraploid families. Diploid families from south of 35° N also had poor survival in northern plantations (CLAUSEN *et al.* 1980).

Stand Variation

Stand within-region differences were significant for all seed traits measured in parent trees from 59 stands. The fall-sown seedlings from 39 stands also showed significant differences in the four nursery traits with the stand/region component accounting for 13 percent of the variation in leaf color and in date of leaf fall. The spring-sown seedlings from 24 other stands only differed in leaf angle and height growth. The 19 stands represented in the four plantations showed significant differences within regions in both survival and height after 3 years in the field. The stand/region component accounted for 19 percent of the genetic variation in seedling height in the nursery, but only contributed 3 percent in the plantations where environmental variation was greater than in the relatively uniform nursery environment. About 9 percent of the total variance among seedlings in 3-year survival was due to stand/region variation.

Tree/Stand Variation

Except for leaf angle, trees within stands varied little in the nursery traits. Noteworthy is stand No. 3 (Mississippi) in which the family means for leaf angle ranged from acute to nearly horizontal. Within-stand variation accounted for 19 and 12 percent of the total variance among fall- and spring-sown seedlings, respectively.

The tree/stand variation was also small in the four plantations. Family differences in 3-year height were not significant in the Ohio and Wisconsin plantations and there were no significant differences in survival in the Illinois plantation. This component contributed from less than 1 to 13 percent of the total variance in survival and between 6 and 9 percent of the genetic variation in height in each plantation.

Several stands contained trees of more than one ploidy level. When their progenies were compared after three growing seasons in the field, diploid, pentaploid, or hexaploid families from the same stand differed little in height growth. However, tetraploid families were 14 to 53 percent taller than diploid families from the same stand, except in the Wisconsin plantation in which the diploids had slighly better growth than the tetraploids.

Plantation Variation

The plantation effect on growth and survival of the white ash seedlings was substantial. The probability of this effect was beyond 0.9995 and it contributed 72 and 17 percent of the total variance for 3-year height and survival, respectively.

Table 5. — **Height (in cm) of 45 white ash families in 4 plantations after 3 growing seasons[1])**

Family		Mean height in plantation				
No.	State	Louisiana	Illinois	Ohio	Wisconsin	Average
1-04	TX	87.6	170.2	132.0	24.0	103.4
1-05		67.6	164.0	171.0	15.0	104.4
1-06		90.2	216.4	150.8	27.0	121.1
2-01	LA	89.6	192.2	109.4	14.0	101.3
2-04		68.4	121.8	183.0	12.0	96.3
2-08		70.8	145.0	103.0	10.0	82.2
3-01	MS	59.0	164.8	141.8	24.6	97.5
3-02		55.6	181.0	120.8	43.5	100.2
4-04	MS	76.6	204.0	121.8	36.4	109.7
4-07		50.0	176.6	107.0	39.0	93.2
4-08		63.8	207.6	154.2	36.2	115.4
5-06	AL	54.6	139.4	142.2	33.8	92.5
5-08		47.2	131.4	127.8	32.6	84.8
6-02	TN	47.2	162.4	138.0	28.2	94.0
6-04		45.8	128.2	176.8	28.8	94.9
6-05		49.6	173.2	155.2	34.0	103.0
7-06	TN	49.6	167.8	143.0	29.0	97.4
8-01	KY	38.4	170.6	150.3	26.6	96.5
8-02		39.6	161.2	164.0	33.0	99.4
8-03		35.4	168.0	168.2	27.0	99.6
9-03	KY	37.2	127.0	168.4	36.2	92.2
9-09		30.2	162.8	201.2	27.8	105.5
10-09	IL	49.0	165.0	195.4	35.0	111.1
10-12		41.0	136.8	189.0	32.8	99.9
10-13		52.4	163.6	163.2	34.2	103.4
11-04	IN	34.2	176.0	197.8	34.8	110.7
11-06		39.0	139.6	177.6	35.8	98.0
11-10		31.6	116.0	165.4	41.8	88.7
12-04	WV	37.2	56.0	139.8	28.0	65.2
12-05		45.4	70.6	161.4	35.4	78.2
12-10		44.8	73.0	169.5	38.8	81.5
13-04	IL	52.4	126.4	214.6	49.0	110.5
14-02	CT	45.8	89.2	158.0	35.2	82.0
14-04		47.4	93.0	145.8	34.4	80.1
14-05		45.6	82.6	158.8	39.0	81.5
15-01	VT	56.0	62.2	185.8	34.8	84.7
15-02		41.8	83.2	188.2	40.2	88.4
16-02	ME	45.2	86.8	128.0	46.2	76.6
16-07		37.4	57.8	128.6	33.0	64.2
16-09		46.2	64.2	118.4	32.8	65.4
17-01	MI	39.6	70.4	147.0	36.6	73.4
18-03	WI	51.2	75.4	163.8	44.6	83.7
19-04	MI	47.0	71.4	128.2	35.0	70.4
19-06		42.2	74.0	149.0	40.2	76.4
19-07		39.8	64.0	152.0	35.4	72.8
	Average	50.4	129.6	154.6	32.7	91.8

1/ Families and plantations are listed from south to north.

The average height of the 45 families ranged from 155 cm in the Ohio plantation to 33 cm in the Wisconsin plantation (*Table 5*). Thus, these two extremes differed by almost five-fold. In general, the Illinois plantation was comparable to the Ohio plantation except for trees from West Virginia, Connecticut, Vermont, Maine, Wisconsin, and Michigan. Seedlings from these stands were only half as tall in Illinois as they were in Ohio. The poor growth in the Louisiana plantation was partly due to insufficient weed control (KUNG and CLAUSEN 1980).

The four plantations were also significantly different in seedling survival. The average survival percentage was 92 in Illinois, 78 in Louisiana, 70 in Ohio, and 60 in Wisconsin and, thus, was not directly related to latitude of the plantations.

Although Texas family 1-06 was the tallest family in the Illinois and Louisiana plantations and had the best overall growth, its performance was below average in the Ohio and Wisconsin plantations (*Table 5*). Similarly, the tallest family in the Ohio and Wisconsin plantations (Illinois family 13-04) only had average growth in the other two plantations. Such family × plantation interactions were common for both height and survival.

Discussion

By comparing the F-statistics, the variance components, and the square of the correlation coefficients obtained in the various analyses, the variation pattern in white ash can be interpreted. The variation in seed length and length of wing on the seed appears to be clinal. Although date of leaf fall shows a latitudinal trend, it is also strongly affected by ploidy level. Survival in the plantations could be expected to show clinal variation but this was only true in the Wisconsin plantation. The strong effect of ploidy level in this plantation was mostly due to the poor survival of the tetraploids, which all were of extreme southern origin. The superior growth of trees from the Southeast and Southwest regions in the Illinois plantation could be interpreted as an ecotypic effect but the close correlation with latitude of seed origins suggests that clinal variation is more important. Due to the superior growth of the tetraploids in the Louisiana plantation and their poor performance in the Ohio and Wisconsin plantations, ploidy level accounted for most of the variation in these plantations. The variation in percent of filled seed, leaf color, and leaf angle appears to be random.

The close correlations with latitude and strong effects of ploidy level indicates that none of the traits studied in this test differ ecotypically. However, until the geographic variation in white ash has been studied in more detail, the five regions delineated in this paper can be used as interim seed collection zones. Our test results show that among-stand variation is ample and hence it is possible to make general seed collection recommendations.

This test indicates that no one specific area, stand, or family is suitable for all locations. In general, more or less local provenances performed well, but the best provenance in all four plantations originated south of the plantation location. As an example, the best provenance in the Illinois plantation (Mississippi provenance No. 4) was 26 percent taller than the local one (provenance No. 10), and the best family was 31 percent taller than the best local family. Thus, it appears advantageous and safe to use provenances from 100—200 miles (150—300 km) south of the intended location. Movement of provenances southward is not recommended because only in the Ohio plantation did a more northern provenance (No. 15 from Vermont) perform as well or better than the local one.

Due to the apparent superiority of tetraploids over trees of other ploidy levels, considerable gains could be made by using known tetraploid families in plantations in the southern half of the species range. However, this improvement method is not practical at present because we do not yet have a quick and simple method of determining number of chromosomes or ploidy level. Triploid trees have not been reported so far but, if they can be found, their growth should be compared with that of the diploids and other polyploids.

Except for tetraploid trees, individual tree selection would not be effective in a white ash tree improvement program due to the small within-stand variation observed. The small variation may be due to the seed production and natural seeding characteristics of the species. When there is a seed crop, a large white ash tree is capable of producing many thousands of seeds. White ash generally becomes established after some disturbance. It is conceivable that a stand of white ash could be derived from a few parents and thus be relatively uniform.

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