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# (12) United States Plant Patent Beineke

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## (54) BLACK WALNUT TREE NAMED 'BEINEKE 4'

Latin Name: Juglans nigra L.

Varietal Denomination: Beineke 4

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(US)

(73) Assignee: American Forestry Technologies, Inc.,

Westpoint, IN (US)

(\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 6 days.

(21) Appl. No.: 10/141,021

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(65) Prior Publication Data

US 2004/0025210 P1 Feb. 5, 2004

(51)	Int. Cl. <sup>7</sup> A0	01H 5/00
(52)	U.S. Cl	Plt./154
(58)	Field of Search	. Plt./154

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## (57) ABSTRACT

A new and distinct cultivar of black walnut tree (Juglans nigra L.) which is distinctly characterized by extremely rapid growth rate, very strong central stem tendency, and excellent straightness, thereby producing excellent timber qualities. This new variety of black walnut tree (Juglans nigra L.) was discovered by the applicant near South Raub, Tippecanoe County, Ind. in a black walnut planting of seedling progeny from a previously selected tree for outstanding timber producing potential. This selection has been designated as BW500, a seedling progeny of BW 249 in records maintained by the applicant on the performance of the selection and grafts made from the selection and will be known henceforth as 'Beineke 4.'

## 3 Drawing Sheets

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Latin name of the genus and species: Juglans nigra L.

## BACKGROUND OF THE INVENTION

This new variety of black walnut tree (*Juglans nigra* L.) was discovered by the applicant near South Raub, Tippeca- 5 noe County, Ind. in a black walnut planting of seedling progeny from previously selected trees for outstanding timber producing potential. This selection has been designated as BW500, a seedling progeny of BW 249 (unpatented) in records maintained by the applicant on the performance of 10 the selection and grafts made from the selection and will be known henceforth as 'Beineke 4.' The male parent is unknown, as is generally the case with black walnut trees (Beineke, 1989).

#### SUMMARY OF THE INVENTION

A new and distinct cultivar of black walnut tree (Juglans nigra L.) is distinctly characterized by extremely rapid

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growth rate, very strong central stem tendency, and excellent straightness, thereby producing excellent timber qualities at 7 years. Beineke 4 was 7 years old when described at South Raub, Ind.

After the original clone was selected, and assigned an identity number of BW500 the aforesaid tree was reproduced by collecting scions from it and grafting these onto common black walnut rootstocks at American Forestry Technology Company, West Point, Ind. These asexual reproductions ran true to the original tree and to each other in all respects. Comparisons of BW 249 with Beineke 4 at this location were not made because no BW 249 trees were planted at this site, and the parent tree was cut down.

Color values used were from the Munsell Color Chart for Plant Tissues. However, color is too dependent on weather conditions and fertilization to be consistent or distinctive. For example, leaves can be made a deeper green by applying

nitrogen. Walnut tree leaves turn yellow as the season progresses, especially if there is a lack of rainfall. As black walnut meats dry, they become darker. Simply being on the ground for a week causes the outer shell to darken. Bark color involves many shades of gray through brown and black.

Beineke 4 is hardy in zones 4, 5, 6, 7, and 8.

#### DESCRIPTION OF THE DRAWINGS

FIG. 1 is a photograph showing the timber form of 'Beineke 4.'

FIG. 2 is a photograph showing the leaves of 'Beineke 4.'

FIG. 3 is a photograph showing the nuts of 'Beineke 4.'

#### BOTANICAL DESCRIPTION OF THE PLANT

The botanical details of this new and distinct variety of walnut tree are as follows:

Tree:

Size.—Large, 26 ft. at 7 years; crown diameter is 13 ft. Vigor.—Vigorous.

Growth rate.—Very rapid, 37% larger in diameter than the average of Purdue 1 (U.S. Plant Pat. No. 4,543) grafts, planted the same year on the same land. Diameter growth rate (at 4½ feet above the ground) averages 0.743 inches per year over 7 years, to 5.2 inches.

Form.—Excellent timber form, as good as Purdue 1 (U.S. Plant Pat. No. 4,543), 57% straighter than average of the entire planting. Stem form was obtained by subjectively rating the straightness of the main stem on a scale of 1 to 5 with 1 representing a perfectly straight stem; 2, slight crook or deviation of the central stem; 3, about average straightness; 4, several severe crooks or a single fork; and 5, a very crooked, forked and/or leaning central stem. Beineke 4 averages 1. The trees of the present invention are grown in plantations, not open fields (not natural stands). In plantations, trees are upright and have no distinctive or characteristic crown shape because all branches are seeking to grow upwards.

Branches: Diameter depends on age and size of tree, varies from ½" to 12", bark color varies from grays to browns. Leaves:

Compound leaves.—Size — Shorter than average; average length —13.60".

Leaflets.—Size — Smaller than average; average length — 3.48"; average width — 2.9"; average number of leaflets — 18.0 — lanceolate; acutely pointed, rounded base; Petiole— short.

Thickness—Thin; Texture — smooth; Margin — serrated; Color — Topside — dark green, (2.5 G 4/4 on the Munsell Color Chart); Underside — light green, (5GY5/4 on the Munsell Color Chart for Plant Tissues).

Anthracnose resistance.—Good.

Nut:

Size.—Small; average length — 1.33"; average diameter in suture plane — 1.10"; average diameter cheek to cheek — 1.37".

Uniformity of size.—Not much variation.

Form.—Rounded; flattened in suture plane. See FIG. 3. Blossom end.—Pointed.

Basal end.—Slightly pointed to rounded.

Thickness of shell.—Thick.

Ridges.—Sharp.

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Color.—Mottled, 5 YR 3/2 and 2.5 YR 3/4 on the Munsell Color Chart.

Flowering habit:

Age at which trees start producing catkins.—Early, it takes about 4–5 years to flower, but the flower number varies with the age of the tree.

Number of catkins produced.—Abundant.

Age at which tree starts producing pistillate flowers.— Early, about 4–5 years.

Number of pistillate flowers produced by young trees.— Abundant.

Number of pistillate flowers produced by mature trees.—Abundant.

Lateral shoots producing pistillate flowers.—Few.

Number of pistillate flowers per inflorescence.—2 to 4. Flowering season: Flowers typically in May in Indiana. There are probably 1- million pollen per catkin. Female flowers are about ½16" long and grow to two "pollen pick up point" which subsequently break apart. Pollen exits as "dust" which is not feasible to quantitate.

Nut crop:

Bearing.—Annual.

Productivity.—Heavy.

Ripening period.—Late, September-October.

Evenness of maturity (period between first and last nuts are ready for harvest). —Even.

Quality.—Good.

Distribution of nuts on tree.—Throughout.

## GENETIC METHOD OF IDENTIFICATION

DNA "fingerprint" for identification of Beineke 4:

DNA was isolated from the leaves of Beineke 4. For purposes of DNA fingerprinting, nine highly polymorphic loci from a suite of microsatellites developed by Woeste et al. (2002) were chosen. Microsatellites sizes were checked against previously published standards and verified by a second independent analysis. The "fingerprint" is the collection of microsatellite allele sizes at each locus for Beineke 4.

DNA was isolated from the leaves of 10 black walnut trees obtained from Walter Beineke using CTAB extraction buffer (50 mM TRIS-HCL, pH 8.0, 20 mM EDTA, pH 8.0, 0.7 M NaCl, 0.4 M LiCl, 2% SDS, 2% TAB, nd 1% PVP). After isolation the DNA from each tree was quantified and diluted with nanopure distilled water to a final concentration of 5 ng/ $\mu$ L. The samples were stored in 96-well plates at 20° C.

For purposes of DNA fingerprinting, nine highly polymorphic loci from a suite of microsatellites developed by Woeste et al. (2002) were chosen. Amplification of each locus was performed with an MJ Research Tetrad Thermocycler (Waltham, Mass.) using  $10 \mu L$  reactions in 96-well plates. The PCR reaction mix contained 2  $\mu$ L of the aforementioned black walnut DNA, 5  $\mu$ L Sigma Taq ReadyMix (Sigma Aldrich, St. Louis, Mo.),  $0.4 \mu L$  of a 20 pmol mixture of forward and reverse fluorescence labeled primer, and 3  $\mu$ L PCR grade water supplied with the Sigma ReadyMix. PCR amplification was for 30 cycles of 94° C. for 20 sec, 55° C. for 30 sec, and 72° C. for 1 min. All primers were annealed at 55° C. The products were then held at 4° C. until aliquots could be loaded into 6% Long Ranger (polyacrylamide) denaturing gels (BMA, Rockland, Me.). For each individual  $0.5 \mu L$  PCR product was added to  $0.75 \mu L$  blue dextran and  $0.25 \mu L$  of CXR 350 bp Ladder Standard (Promega, Fitchburg Center, Wis.) in a new 96-well 1 late. The samples were denatured for 2 min at 95° C. and loaded onto a CAL96 96-well laminated membrane comb (The Gel Company, San

Francisco, Calif.). Electrophoresis was at 3,000 V, 60 mA, 200 Watts, 50° C. for 2 hours using an ABI 377 (Perkin Elmer) with 36 cm plates and 0.2 mm spacers. The resulting data was analyzed using ABI's GeneScan 3.1.2 and Genotyper 2.5 (Perkin Elmer). Microsatellite sizes were checked against previously published standards and verified by a second independent analysis. The "fingerprint" is the collection of microsatellite allele sizes at each locus for each tree.

Locus	Forward				
WGA2	GACGACGAAGGTGTACGGAT	(SEQ	ID	NO:	1)
WGA6	CCATGAAACTTCATGCGTTG	(SEQ	ID	NO:	2)
WGA24	TCCCCCTGAAATCTTCTCCT	(SEQ	ID	NO:	3)
WGA32	CTCGGTAAGCCACACCAATT	(SEQ	ID	NO:	4)
WG33	TGGTCTGCGAAGACACTGTC	(SEQ	ID	NO:	5)
WGA86	ATGCCTCATCTCCATTCTGG	(SEQ	ID	NO:	6)
WGA89	ACCCATCTTTCACGTGTGTG	(SEQ	ID	NO:	7)
WGA90	CTTGTAATCGCCCTCTGCTC	(SEQ	ID	NO:	8)
WGA97	GGAGAGGAAAGGAATCCAAA	(SEQ	ID	NO:	9)
		-			
Locus	Reverse				
Locus WGA2	Reverse GTACGGCTCTCCTTGCAGTC	(SEQ	ID	NO:	10)
		,		NO:	10) 11)
WGA2	GTACGGCTCTCCTTGCAGTC	,	ID	NO:	,
WGA2 WGA6	GTACGGCTCTCCTTGCAGTC CATCCCAAGCGAAGGTTG	(SEQ	ID ID	NO:	11)
WGA2 WGA6 WGA24	GTACGGCTCTCCTTGCAGTC CATCCCAAGCGAAGGTTG TTCTCGTGGTGCTTGTTGAG	(SEQ	ID ID ID	NO: NO:	11) 12)
WGA2 WGA6 WGA24 WGA32	GTACGGCTCTCCTTGCAGTC CATCCCAAGCGAAGGTTG TTCTCGTGGTGCTTGTTGAG ACGGGCAGTGTATGCATGTA	(SEQ (SEQ (SEQ	ID ID ID ID	NO: NO: NO:	11) 12) 13)
WGA2 WGA6 WGA24 WGA32 WG33	GTACGGCTCTCCTTGCAGTC CATCCCAAGCGAAGGTTG TTCTCGTGGTGCTTGTTGAG ACGGGCAGTGTATGCATGTA GGTTCGTCGTCGTTTTGACCT	(SEQ (SEQ (SEQ (SEQ	ID ID ID ID	NO: NO: NO:	11) 12) 13) 14)
WGA2 WGA6 WGA24 WGA32 WG33 WGA86	GTACGGCTCTCCTTGCAGTC CATCCCAAGCGAAGGTTG TTCTCGTGGTGCTTGTTGAG ACGGGCAGTGTATGCATGTA GGTTCGTCGTTTGTTGACCT TGAGTGGCAATCACAAGGAA	(SEQ (SEQ (SEQ (SEQ	ID ID ID ID ID	NO: NO: NO: NO: NO:	11) 12) 13) 14) 15)

The best interpretation of the current data indicates that the probability that any other black walnut tree would have the collection of microsatellite allele sizes listed is less that 1 in  $10^{-17}$ .

Sizes (bp) of microsatellites at 9 loci used to fingerprint 'Beineke 4' (2 alleles at each locus)

We	òA2	WC	<b>∂A</b> 6	WG	A24	WG	A32	WC	<b>3A</b> 90
168	168	142	142	234	240	171	207	152	160
		WGA86		WGA86 WGA97 WGA33		A33	<b>WGA</b> 89		
		220	234	153	155	208	208	187	197

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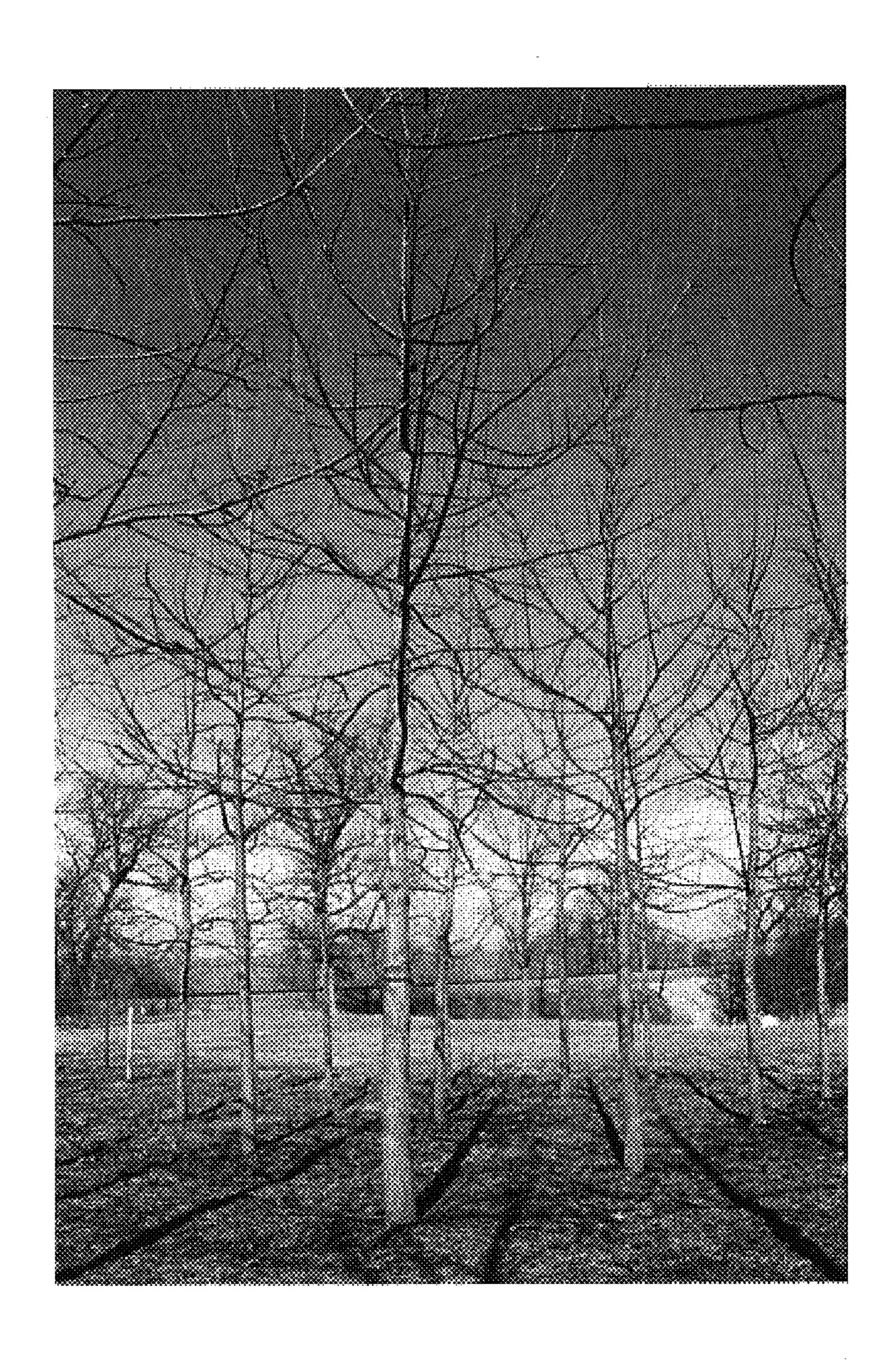
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I claim:

1. A new and distinct variety of black walnut tree named 'Beineke 4' substantially as illustrated and described, which

has excellent timber quality, is fast growing, has strong central stem tendency, no sweep, no crooks.

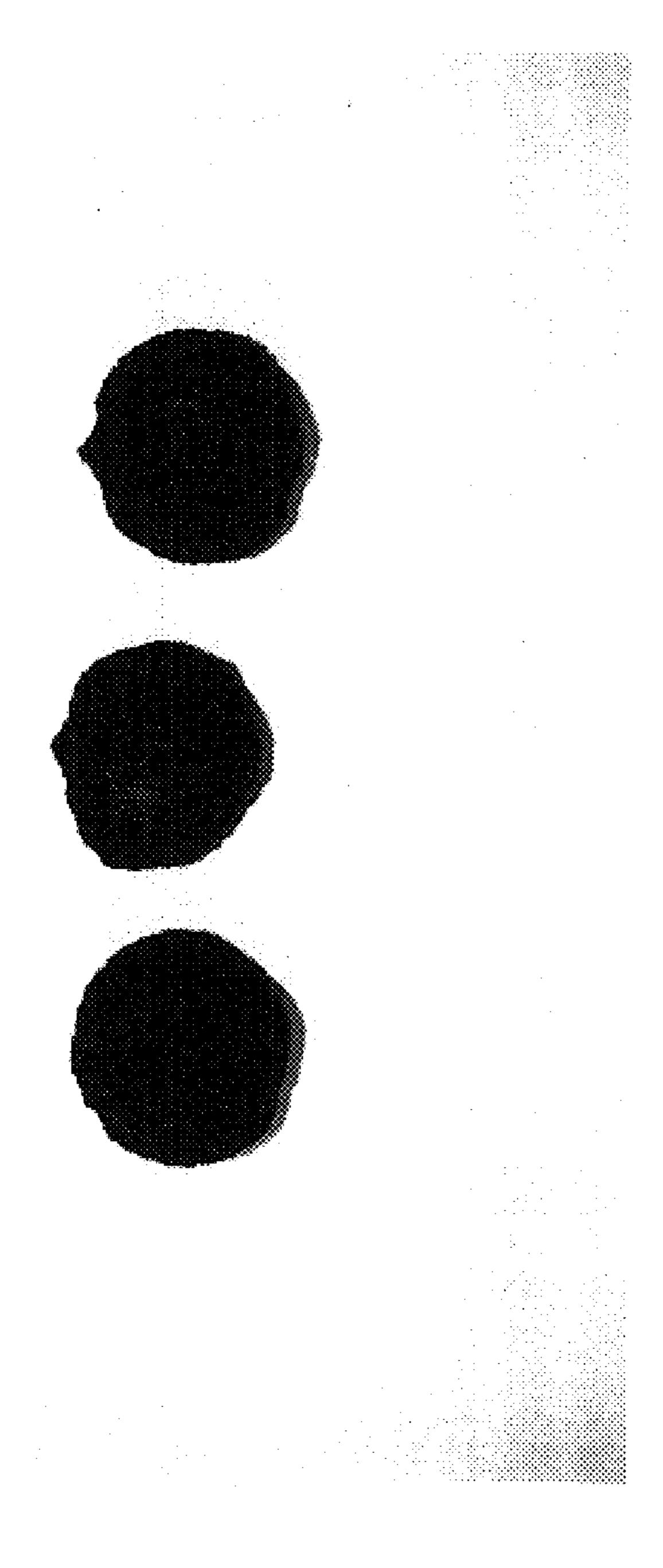
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