US00PP15283P3

(12) **United States Plant Patent**
Beineke

(10) **Patent No.: US PP15,283 P2**
(45) **Date of Patent: Nov. 2, 2004**

(54) **BLACK WALNUT TREE NAMED 'BEINEKE 2'**

(50) Latin Name: ***Juglans nigra*** L.
Varietal Denomination: **Beineke 2**

(75) Inventor: **Walter Beineke**, West Lafayette, IN (US)

(73) Assignee: **American Forestry Technologies, Inc.**, West Point, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/141,029**

(22) Filed: **May 8, 2002**

(65) **Prior Publication Data**

US 2003/0213030 P1 Nov. 13, 2003

(51) **Int. Cl.**[7] **A01H 5/00**
(52) **U.S. Cl.** **Plt./154**
(58) **Field of Search** Plt./154

(56) **References Cited**

U.S. PATENT DOCUMENTS

PP4,132	P	10/1977	Genn
PP4,388	P	2/1979	Forde
PP4,389	P	2/1979	Forde
PP4,405	P	4/1979	Forde
PP4,542	P	6/1980	Beineke
PP4,543	P	6/1980	Beineke
PP4,614	P	1/1981	Beineke
PP4,954	P	11/1982	Beineke
PP4,955	P	11/1982	Beineke
PP4,964	P	12/1982	Beineke
PP4,966	P	12/1982	Beineke
PP4,968	P	12/1982	Beineke
PP4,971	P	1/1983	Beineke
PP6,973	P	8/1989	Madison
PP9,906	P	6/1997	Jones
PP9,924	P	6/1997	Jones
PP9,925	P	6/1997	Jones

OTHER PUBLICATIONS

Appleton, Bonnie, et al. (2000) "Trees for problem Landscape Sites—The Walnut Tree: Allelopathic Effects and Tolerant Plants" *Virginia State University* Publication No. 430–021.
Beineke, Walter F. (1989) "Twenty Years of Black Walnut Genetic Improvement at Purdue University" *NJAF* 6:68–71.
Coladonato, Milo (1991) "*Juglans Nigra*" 1–11.
Esser, Lora. (1993) "*Juglans Californica*" 1–11.
Pavek, Diane S. (1993) "*Juglans Major*" 1–13.
Tirmenstein, D.S (1990) "*Juglans Microcarpa*" 1–11.
Website: http://www.treeguide.com/naspecies.asp?treeid=jungrl: printed Aug. 30, 2001: pp. 1–11.
Website: http://virual.clemson.edu/groups/FieldOps/Cgs/walnut.htm: printed Aug. 30, 2001: pp. 1–3.
Woeste, K. et al. (2002) "Thirty Polymorphic Nuclear Microsatellite Loci From Black Walnut" *The Journal of Heredity* 93(1):58–60.

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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 West Lafayette, 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 BW408, a seedling progeny of patented Fayette-1 (U.S. Plant Pat. No. 4,964) 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 2.'

3 Drawing Sheets

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 West Lafayette, Ind. in a black walnut planting of seedling progeny from previously selected trees for outstanding timber producing potential. This selection has been designated as BW408, a seedling progeny of patented Fayette-1 (U.S. Plant Pat. No. 4,964), 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 2.' 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.) which is distinctly characterized by extremely

rapid growth rate, very strong central stem tendency, and excellent straightness, thereby producing excellent timber qualities at age 17 years. The new variety has good nut bearing qualities meaning abundant nuts are produced biennially early in the life of the tree, about 3–4 years. Beineke 2 was 17 years old when described at a location near West Lafayette, Ind.

After the original clone was selected, and assigned an identity number of BW408 the aforesaid tree was reproduced by collecting scions from it and grafting these onto common black walnut rootstocks at American Forestry Technologies Company, West Point, Ind. These asexual reproductions ran true to the original tree and to each other in all respects.

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

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a photograph showing the timber form of 'Beineke 2.'

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

FIG. **3** is a photograph showing the nuts of 'Beineke 2.'

BOTANICAL DESCRIPTION OF THE PLANT

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

Tree:

Size.—Large — 46 ft. tall, at 17 years old, crown diameter of 16 ft.

Vigor.—Vigorous.

Growth rate.—Very rapid, faster than Purdue 1 (U.S. Plant Pat. No. 4,543), Tippecanoe 1 (U.S. Plant Pat. No. 4,954), and Fayette 1 (U.S. Plant Pat. No. 4,964). 26%, 8% and 39% larger in diameter than the average of the patented trees respectively planted on the same site. Diameter growth rate (at 4½ feet above the ground) averages 0.588 inches per year, over 17 years about 10 inches.

Form.—Excellent timber form, as good as Purdue 1 (U.S. Plant Pat. No. 4,543) and Tippecanoe 1 (U.S. Plant Pat. No. 4,954) — 36% straighter than average of the entire planting. 67% straighter than the patented parent tree Fayette 1 (U.S. Plant Pat. No. 4,964). Beineke 2 averages 1. 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 (no crooks); 3, about average straightness; 4, several severe crooks or a single fork; and 5, a very crooked, forked and/or leaning central stem. 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 — Much longer than average; average length — 19.0", average width — 3.6".

Leaflets.—Size — Very large; average length — 4.6"; average width — 1.7"; average number of leaflets — 17.6 — lanceolate; acutely pointed, rounded. Thickness — thin; Texture — smooth; Margin — serrated; Color — Topside — dark green (2.5G4/4 by the Munsell Color Chart for Plant Tissues); Underside — lightgreen; (5GY5/4 on the Munsell Color Chart for Plant Tissues) base — rounded; Petioles — short.

Anthracnose resistance.—Excellent.

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.

Size of catkins.—Short.

Time of pollen shed.—Mid-season, between May–July.

Age at which tree starts producing pistillate flowers.—Early, 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.—None.

Number of pistillate flowers per inflorescence.—2 to 4, but flower numbers vary with the age of the trees.

Nut crop:

Bearing.—Biennial.

Productivity.—Heavy.

Ripening period.—Early.

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

Distribution of nuts on tree.—Throughout.

Nut:

Size.—Small; average length — 1.4"; average diameter in suture plane — 1.1"; average diameter cheek to cheek — 1.3".

Uniformity of size.—Not much variation.

Form.—Somewhat elongated; oblong; flattened in suture plane.

Blossom end.—Pointed.

Basal end.—Slightly pointed to rounded.

Thickness of shell.—Very thick.

Ridges.—Rounded off; not sharp.

Winter hardiness: Hardy in east and Midwest, no dieback due to cold.

GENETIC METHOD OF IDENTIFICATION

DNA fingerprint for identification of 'Beineke 2:'

DNA was isolated from the leaves of 'Beineke 2.' 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 2.'

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/μL. 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 μL reactions in 96-well plates. The PCR reaction mix contained 2 μL of the aforementioned black walnut DNA, 5 μL Sigma Taq ReadyMix (Sigma Aldrich, St. Louis, Mo.), 0.4 μL of a 20 pmol mixture of forward and reverse fluorescence labeled primer, and 3 μL 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 μL PCR product was added to 0.75 μL blue dextran and 0.25 μL of CXR 350bp 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)
WGA33	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)
	Reverse	
WGA2	GTACGGCTCTCCTTGCAGTC	(SEQ ID NO: 10)
WGA6	CATCCCAAGCGAAGGTTG	(SEQ ID NO: 11)
WGA24	TTCTCGTGGTGCTTGTTGAG	(SEQ ID NO: 12)
WGA32	ACGGGCAGTGTATGCATGTA	(SEQ ID NO: 13)
WGA33	GGTTCGTCGTTTGTTGACCT	(SEQ ID NO: 14)
WGA86	TGAGTGGCAATCACAAGGAA	(SEQ ID NO: 15)
WGA89	TGCCTAATTAGCAATTTCCA	(SEQ ID NO: 16)
WGA90	TACCTGCAACCCGTTACACA	(SEQ ID NO: 17)
WGA97	TTGAACAAAAGGCCGTTTTC	(SEQ ID NO: 18)

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 below is less that 1 in 10^{-17}.

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

WGA2		WGA6		WGA24		WGA32		WGA90	
116	152	162	162	224	236	179	189	152	168
WGA86		**WGA97**		**WGA33**		**WGA89**			
214	236	153	153	208	208	215	215		

DOCUMENTS CITED

Beineke, Walter F. (1989) Twenty years of black walnut genetic improvement at Purdue University *North. J. Appl. For.* 6:68–71.

Woeste, K., Burns, R., Rhodes, O., and Michler, C. (2002) Thirty polymorphic nuclear microsatellite loci from black walnut. *Journal of Heredity,* 93(1): 58–60.

SEQUENCE LISTING

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ttgaacaaaa ggccgttttc 20

I claim:

1. A new and distinct variety of black walnut tree named 'Beineke 2' substantially as illustrated and described, which has excellent timber quality, is fast growing, has strong central stem tendency, no sweep, and no crooks.

* * * * *

FIG. 1

FIG. 2

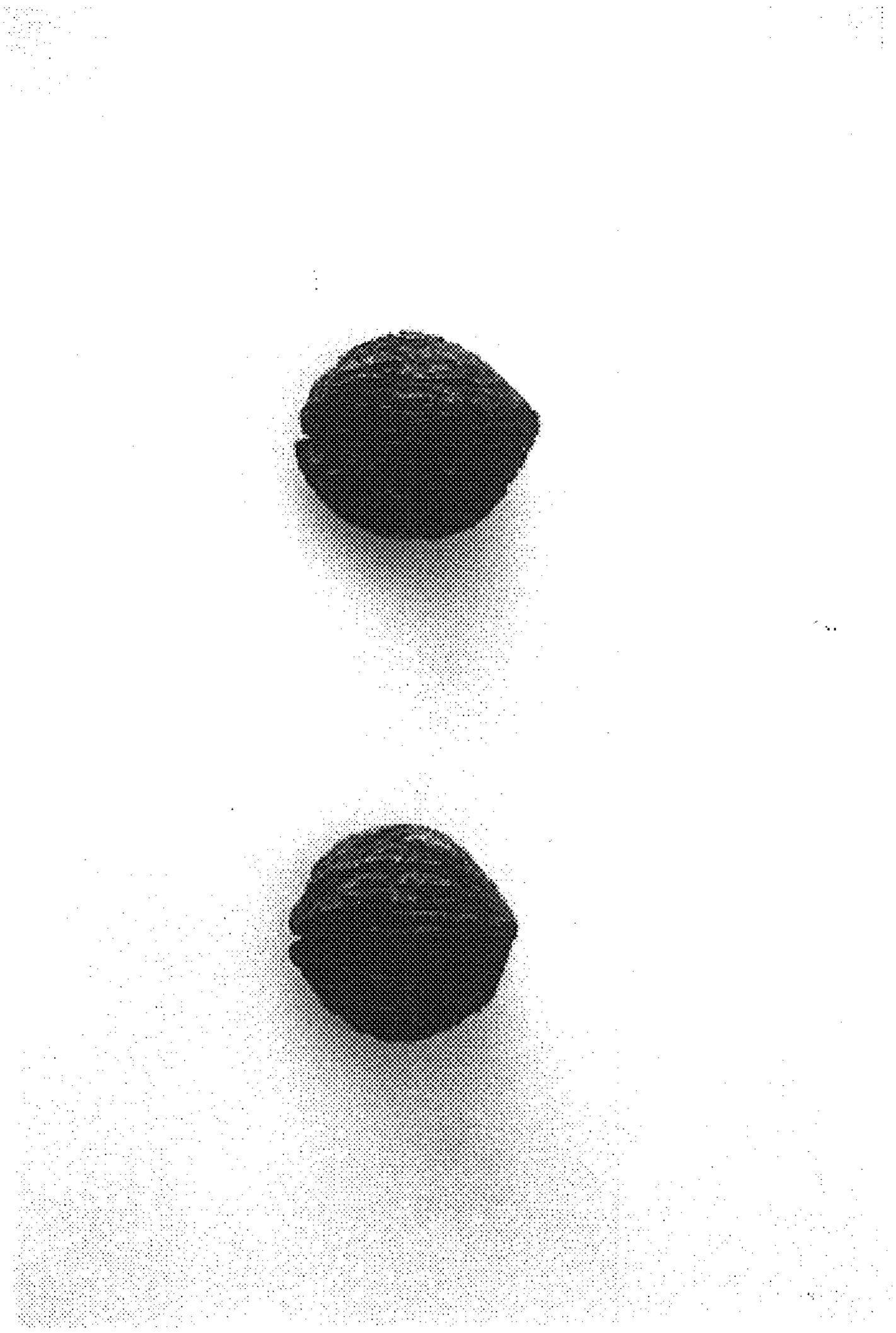

FIG. 3