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Characteristics of shagbark hickory in the Niepołomice Primeval Forest of Southern Poland

Eigenschaften von Schuppenrinden-Hickory im Niepołomice Urwald in Südpolen

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Stichworte: Carya ovata, Polen, genetische Variation, Verwandtschaft des Bäumes, Isoenzyme, Höhe, Brusthöhendurchmesser, Mariabrunn

Abstract

The paper presents an analysis of growth features and genetic variation of 21 shagbark hickory (Carya ovata) trees growing on one site in Niepołomice Primeval Forest (southern Poland), which are reminiscent of the experiment founded in 1898 by the Department of Forestry in Mariabrunn. Height and breast height diameter of particular individuals were measured, and in order to verify their relationship the genetic variability was estimated by analysis of 5 enzymes encoded in 5 loci. The distribution of hickories and small distances between them suggested that they may be vegetative progeny created from the root sprouts of an unknown number of trees. This hypothesis was aexcluded both based on phenotypic analysis and isoenzymatic

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examinations. However most of the enzymes analyzed were characterized by monomorphism, the analysis of phosphogluconate isomerase demonstrated that 3 various genotypes are present in hickories growing in Niepołomice Primeval Forest. Thus, a tree growing in the study area did not arise in a vegetative way, but by sexual reproduction as natural regeneration.

Zusammenfassung

Der Artikel enthält eine Analyse von Wachstumsmerkmalen und genetischer Variation von 21 Schuppenrinden-Hickorys-Bäumen, die im Niepołomice Urwald (Südpolen) wachsen. Diese Bäume sind von einem Experiment geblieben, das von der Abteilung für Forstwirtschaft in Mariabrunn im Jahr 1898 durchgeführt wurde. Die Höhen und die Brusthöhendurchmesser jedes Baumes wurden gemessen. Um die Verwandtschaft zu bestimmen, wird die genetische Variation der fünf Enzyme untersucht, die bei fünf Loci kodiert wurde. Der geringe Abstand zwischen einzelnen Schuppenrinden-Hickorybäumen deutet auf eine vegetative Vermehrung aus Wurzelausschlägen hin. Auf der Grundlage der Isoenzymuntersuchungen konnte diese Hypothese teilweise bestätigt werden. Die Analyse des Phosphoglucose-Isomerase hat gezeigt, dass die untersuchten Schuppenrinden-Hickorybäume des Niepołomice Urwaldes drei verschiedene Genotypen aufweisen und somit nicht aus einer vegetativer sondern aus generativer Vermehrung stammen.

1. Introduction

Introduction of alien species from another geographical region, is carried out due to better growth and more valuable wood than native trees and possibility to use for restoration of degraded area [Szwagrzyk 2000]. In Poland, introduced species are rarely encountered, and their participation in the forests is estimated at 0.5% [Kozioł and Matras 2004]. The shagbark hickory (Carya ovata (Mill.) K. Koch) was introduced to Europe from North America in the 17th century, mainly due to profitable mechanical properties of the wood which is characterized by high resistance on bending, compression and high elasticity [Sargent 1947; Graney 1990; Seeling 1998]. The analysis of technical parameters of wood of various hickory species growing in Germany [Sachsse 1980] and in Poland [Hubert 1970] demonstrated their high similarity to hickories growing in North America. In European conditions C. ovata is characterized by high photophilicity and relatively high resistance on frosts, which are close to requirements for Quercus robur. The hickory is a long-lived species and it grows slowly, slower than an oak. During the first years the trees grow about 20–30 cm annually on a height, reaching growth accumulation between 10th and 30th year of life, and then the growth decreases. In natural conditions it tolerates a shade well grows well inside the tree stand being concurrently a species of a long production cycle (over 200 years) [Graney 1990]. The shagbark hickory has a strong root system, covered with numerous lateral roots with resting buds on them. This creates the possibility of root sprouts forming, what may be a main manner of its reproduction, especially on burned areas [Kruger and Reich 1997]. The analysis conducted by Chylarecki [1963] also demonstrate a significant role of such reproduction in a formation of new generation developing under the tree stand canopy. The reproduction of hickory via sprouts from the trunk in turn, has according to Schenck [1939] small forest forming value, since the trees are susceptible to fungal infections at young age (about 20 years). Also natural generative reproduction is of little effectiveness since the nuts are a food for numerous animal species and are often collected by people, therefore only small proportion of the seeds has the chance to germinate [Chylarecki 1963].

The analysis of shagbark hickory so far has been focused so far mainly on its adaptation abilities. Cultivation of the trees beyond the natural extent of its occurrence and obtaining of possibly the highest economic profits requires the cognition of relationships between the plant of foreign origin and the environment. The study presented includes an analysis of shagbark hickory growing in the Niepołomice Primeval Forest. The experiment was initiated in 1898 by Dr. Adolf Cieslar, the employee of the Department of Forest Research in Mariabrunn near Vienna (Austria), who established nu-

merous experimental sites with different introduced species on an area of southern Poland.

The only mention of studies of C. ovata was found in the work of Cieslar [1901], which stated that the seven experimental plots including 3908 hickories were planted. However, there are no exact data on the number of hickories planted of the experiment in the Niepołomice Primeval Forest and their origins.

The arrangement of the examined individuals in the Niepołomice Primeval Forest (Fig. 1) and small distances between them suggested that they may be a vegetative progeny (root sprouts) of an unknown number of trees. To establish the parentage of hickories verification the technique of isoenzymatic markers was used.

Figure 1: Location of the research area with C. ovata (Central Europe, southern Poland, Niepołomice Primeval Forest)

Abbildung 1: Die Lage der Versuchsfläche mit C. ovata (Central Europe, Südpolen, Niepołomice Urwald)

2. Material and methods

2.1 The analysis of growth and phenotypic features

The analysis included 21 trees of shagbark hickory growing in Niepołomice Forest District, Chobot forest range, sub-compartment 423j. The trees grow on fecund river fen soils which create profitable development conditions for C. ovata. Phenotypic features and growth parameters of particular trees were determined: their height using Vertex height meter (with an accuracy of 1 m) and breast height diameter (with an accuracy of 0.5 cm) were measured. Mean, standard deviation and coefficient of examined feature variability were calculated, and also histograms of percentage share of trees in particular height and thickness classes were drawn, accepting five-meters and five-centimeters range of each class, respectively. Also an azimuth and distance between particular trees and the highest and the thickest hickory marked with number 1, were determined. It was the only blossoming individual, and probably the only rising from the foundation of the experiment. The best method of checking the age of individual trees would perform drilling and identify the number of annual growth, but in the context of these studies not performed such analyzes. Most of the trees were characterized by good quality, only two were diagnosed with the curvature of the trunk (Tab. 1).

2.2 Isoenzymatic analysis

Six isoenzymatic systems encoded in 6 loci were analyzed (Tab. 2). Isolation of the enzymes was performed using 20 ml 0.5M Tris-HCl buffer (pH=7.5), 37 mg EDTA, 75 mg KCl, 200mg $MgCl_2 \times 6H_2O$, 3g PVP K25 and 100 µl Triton. The solution obtained was completed with distilled water up to the volume of 100 ml. 20 µl of mercaptoethanol was added for each 5 ml of the extraction buffer [Odrzykoski and Gottlieb 1984]. Due to enzymes degradation observed during plant material storage (–20°C), the buffer composition was modified by three-fold increase in an amount of mercaptoethanol and two-fold increase in PVP amount. That is commonly used method to remove mentioned secondary compounds during DNA and proteins isolation, especially from biological material which is stored for a longer time at a temperature of –20°C [Fan and Xueqin 2009].

Separation of proteins on particular fractions was performed in 12% starch gel [Conkle et al., 1992], and particular buffers for visualization and electrophoresis were prepared according to the methodology described for spruce [Konnert and Maurer 2004, modified] (Tab. 3 and 4).

Table. 1: Growth characteristics and localization of shagbark hickory trees growing in the Niepołomice Primeval Forest.

Tabelle 1: Wachstumsmerkmale und der Lage des Schuppenrinden-Hickorys, die in dem Niepołomice Urwald wachsen.

Tree No	Height (m)	Breast height diameter (cm)	Azimuth in relation to tree 1 (°)	Distance in relation to tree 1 (m)	Remarks
1	30	44,0	0,0	0,00	Fruiting tree
2	12	12,0	120,0	3,35	Trunk with strong curvature
3	20	21,0	64,0	4,75	–
4	16	13,5	86,0	12,00	–
5	12	10,5	49,0	12,95	Umbrella-shape tree
6	17	12,5	15,0	16,70	–
7	15	13,0	38,0	20,40	–
8	22	18,0	23,0	20,30	–
9	13	9,0	19,0	20,00	–
10	21	19,5	18,0	26,15	–
11	16	11,0	33,0	24,85	–
12	5	7,5	41,0	24,00	Broken tree top
13	15	14,5	49,5	25,40	–
14	6	6,5	57,0	18,40	Umbrella-shape tree
15	5	6,5	74,0	11,00	–
16	11	9,5	76,5	19,85	Broken tree top but regenerated
17	10	8,0	65,0	24,00	–
18	14	11,0	69,0	25,50	–
19	6	6,0	84,5	29,30	Umbrella-shape tree
20	7	8,5	42,0	14,50	Umbrella-shape tree
21	11	9,0	96,5	15,10	Trunk with strong curvature
Mean	13,5	12,9	–	–	–
Std.	6,3	8,3	–	–	–
V%	46,7	64,3	–	–	–

Table 2: The analyzed isoenzymatic systems

Tabelle 2. Untersuchte Enzymsysteme

Name of enzyme	Abbreviation	Number E.C.	Gene
Isocitrate dehydrogenase	IDH	1.1.1.42	*Idh-A*
Malate dehydrogenase	MDH	1.1.1.37	*Mdh-A*
Shikimic dehydrogenase	SHDH	1.1.1.25	*Shdh-A*
Malic enzyme	ME	E.C.1.1.1.40	***Me-A***
Phosphogluconic isomerase	PGI	5.3.1.9	***Pgi-A***

Based on the results of analyses of enzymes exhibiting polymorphism (PGI), the average number of alleles and the observed heterozygosity (heterozygotes share of the total number of analyzed trees) was calculated [Bergman and Gregorius 1979, Nei and Roychoundry 1974]. The distribution of allelic variants in populations of spruce was based on the effective number of alleles at the locus. Based on the ratio of genetic distance by Nei'a [1972] the genetic similarity between the analyzed hickories was estimated and dendrogram based on unweighted pair group method with arithmetic mean (UPGMA) was elaborated [Sneath and Sokal 1973]. The calculations were performed using PopGene ver. 1.3 software [Yeh et al., 1999].

Table 3: Composition of buffers for enzymes electrophoresis [Konnert, Maurer 2004, modified]

Tabelle 3: Puffer für die Elektrophorese von Enzymen (Konnert, Maurer [2004], modifiziert)

Buffer system	Composition of the electrode buffer	The composition of the gel buffer	Voltage	Enzymes
Poulik ph = 8,2/8,7	0,3M H_3BO_3 0,06M NaOH	0,07M TRIS 0,035M $C_3H_8O_7$	210 V	PGI
Tris-Citrat ph = 7,5	0,14M TRIS 0,041M $C_3H_8O_7$	Electrode buffer mixed with a gel buffer in the ratio 1:6.5	250V	IDH, MDH, ME, SHDH

Table 4: Composition of buffers for enzymes visualization (according to Konnert and Maurer [2004], modified]

Tabelle 4: Puffer für die Visualisierung der Enzyme (von Konnert und Maurer [2004], modifiziert)

Enzymes	Coloring buffer composition
IDH	6 ml 0,2 M Tris-HCl buffer (pH = 7,5), 5 mg acid trisodium salt izocitrate, 2,5 mg NADP, 150 µl 10% $MgCl_2$, 80 mg phenazine methosulfate (PMS), 140 mg Thiazolyl blue tetrazolium bromide (MTT)
MDH	6 ml 0,2 M Tris-HCl buffer (pH = 7,5), 257 mg DL – malic acid, 30 mg NAD, 1,5 g NaOH, 150 µl PMS, 200 µl a MTT
SHDH	6 ml 0,2 M Tris-HCl buffer (pH = 7,5) 40 mg shikimic acid, 2,5 mg NADP, 150 µl 10% MgCl2, 150 µl PMS, 200 µl MTT
ME	6 ml 0,2 M Tris-HCl buffer (pH = 7,5), 10 mg DL-malic acid, 1,2 mg NADP, 1,5 g NaOH, 150 µl 10% $MgCl_2$, 150 µl PMS, 200 µl MTT
PGI	6 ml 0,2 M buffer Tris-HCl o pH = 7,5 2,4 mg fructose-6-phosphate, 1,8 mg NADP, 1,7 mg EDTA, 20 µl G-6P-dehydrogenase, 150 µl 10% $MgCl_2$, 150 µl PMS, 200 µl MTT

3. Results

3.1 Growth and phenotypic features

Twenty-one trees of the shagbark hickory were characterized by relatively high variability of growth features in the conditions of Niepołomice Primeval Forest. Mean height of the hickories on the research area was 13.5 m, while breast height diameter was 12.9 cm. The highest was the tree marked with number 1 (30 m), while the lowest with number 12 and 15 (5 m). The breast height diameter ranged from 6 (tree no 19) to 44 cm (tree no 1).

The class of the thinnest trees (5.5–10 cm) dominated among the hickories growing in the Niepołomice Primeval Forest, and the contribution of the trees in higher thickness classes decreased gradually (Fig. 2B). It turn, the frequency of trees in particular height classes was characterized by the course of values close to the normal contribution. The highest number of individuals was observed in class 11–15 m (Fig. 2A).

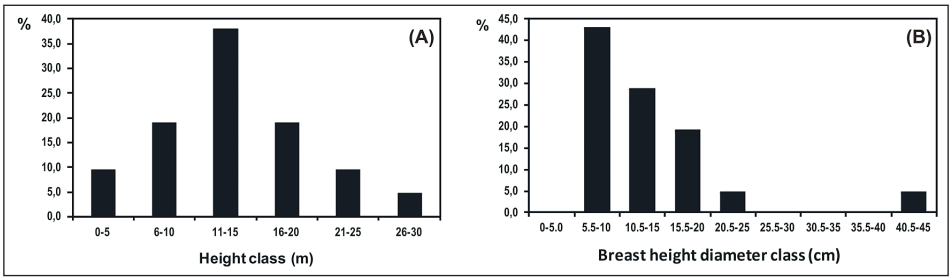

Figure 2: The histogram of frequency of height (A) and breast height diameter (B) of the shagbark hickory growing in Niepołomice Primeval Forest

Abbildung 2: Histogramm der Häufigkeit der Höhe (A) und Brusthöhendurchmesser (B) des Schuppenrinden-Hickory die in dem Niepołomice Urwald wachsen

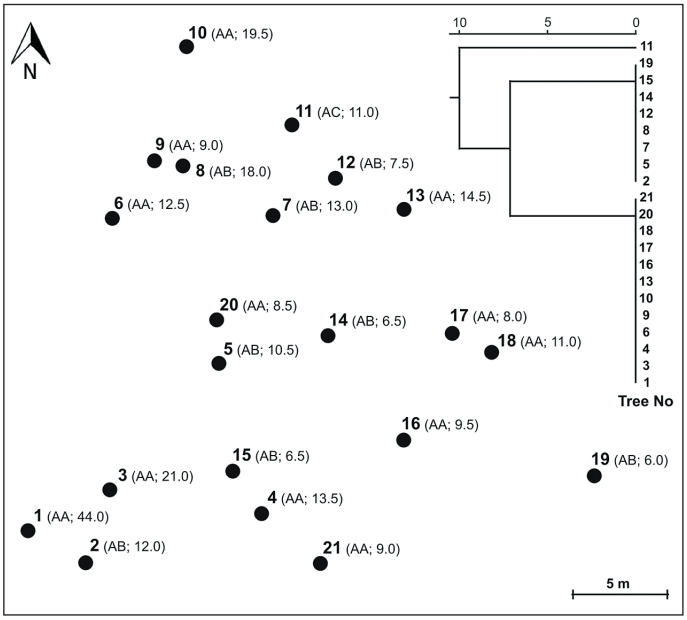

Figure 3: Localization of the trees and a dendrogram of genetic distance according to Nei [1978] of the shagbark hickory growing in Niepołomice Primeval Forest. Trees numeration according to Table 1; genotype (AA–AC) and b.h.d. (in cm) of the tree were put in the parenthesis

Abbildung 3: Die Lage der Bäumen und genetische Distanz durch Nei'a [1978] des Schuppenrinden-Hickory die in dem Niepołomice Urwald wachsen. Die Nummerierung der Bäumen enthält Tabelle 1;. Genotypen (AA–AC) und Brusthöhendurchmesser (in cm) des Bäumen sind in Klammern

Maternal / paternal tree	A	B
A	AA	AB
C	AC	~~BC~~

Figure 4: Probable genotypes of parental trees of the shagbark hickory on the research area in Niepołomice Primeval Forest.

Abbildung 4: Mögliche Genotypen den Eltern des Schuppenrinden-Hickory auf der Versuchsfläche im Niepołomice Urwald

Based on growth features no affinity of growing hickories was demonstrated. The analysis of trees arrangement suggested that an individual no 3 would have been created from root sprouts of the highest and the thickest hickory, marked with number 1. Although the tree no. 2 grows closer to tree no. 1, but the other genotype excludes this possibility of vegetative origin.

With a lack of differences in an external appearance and small distance, the possibility of formation from root sprouts after cut off trees of a few other individuals was taken into consideration (e.g. 5 and 21, 8 and 9, 17 and 18), especially that such reproduction manner was observed on the research area.

3.2 Isoenzymatic polymorphism

The genetic loci of the examined isoenzymatic systems in the group of twenty-one trees were mainly homozygous. Among the examined enzymes, only phosphogluconate isomerase (PGI) was characterized by polymorphism. The analysis of the mentioned enzyme revealed the presence of three different alleles. Average and effective number of alleles at a locus were respectively 3.00 and 1.55. Observed heterozygosity was 0.43, and the expected 0.35. Analysis of phosphogluconate isomerase and an arrangement of particular trees suggest that the hickories growing in the Niepołomice Primeval Forest are not a vegetative progeny. Only the trees number 4 and 21, 6 and 9 or 17 and 18 would have been created via the sprouts, assuming that these individuals were created from trees that had been previously removed from the surface (Fig. 3).

Three various genotypes were obtained analyzing the genetic structure of 21 hickories using phosphogluconate isomerase (PGI), therefore it may be concluded that the examined individuals are not a vegetative progeny. In a minimum range they may be a descendants of one mother and one father. They were presumably formed from crossing of heterozygous individuals of AB and AC genotypes, assuming that no formation of BC genotype trees occurred in an offspring generation (Fig. 4).

4. Discussion

Currently in Poland the number of tree species of foreign origin observed in tree stands managed by the State Forests National Forest Holding does not exceed 0.5% and is maintained on a constant surface level reaching about 46 thousands ha [Kozioł and Matras 2011]. According to Tumiłowicz [1999] the introduced species may be successfully used in forestry when they are better than native species, however there is a need of a special care since they may be a menace as an invasive element.

The research areas in Poland on which the works concerning genetic variability of C. ovata have been conducted, are very sparse so far. Chylarecki [1963] localized them mainly on the basis of information left by Schwappach who established most of the experimental areas of in contemporary Prussia. After the Second World War, part of them (several tree stands) were located in Poland, with over one hundred years old trees characterized by high variability [Chylarecki 1963; Hubert 1970; Ratyska et al., 2011]. Kuzniar [2011], who was leading the research on one of these sites localized on an area of Oława Forest District, found a hickory of an impressive height of 38 meters and a breast height diameter of about 1 meter. Due to the changes in forest ranges and districts and in the economy conducted, the data contained in the study of Chylarecki [1963] are currently out of date and require some inventory works. It is, however, the richest description of Polish shagbark hickory sites, including an influence of climatic-soil arrangements on growth reactions and health status of hickories in 26 regions of Poland, covering in total an area of 9.08 ha in a form of 15–30 are sites.

An introduction of single C. ovata individuals to the tree stands is not recommended, since they are suppressed by the tress of native species. An introduction of trees to cultivation in a form of a larger group, such as hickories growing in Niepołomice Primeval Forest, enables to create large assimilation area and to achieve suitable technological properties of the wood. However, in order to obtain profitable results of an introduction, it is necessary to recognize genetic variability, adaptation ability, resistance and the course of growth and reproduction of various provenances of the

introduced species, what will allow to perform a proper assessment of its usefulness [Bellon 2006].

The simplest method to determinate the way of hickories reproduction on a given area is an analysis of phenotype and arrangement of the trees. Large distance between the individuals exclude their formation from root sprouts, since the probability of formation of resting buds decreases with a distance from the trunk [Janson 1967]. The hickories growing in the Niepołomice Primeval Forest would have been a vegetative progeny of an unknown number of trees. This was pointed by the cluster arrangement and small distances between them. However, it was not possible to confirm this assumption unequivocally based on an analysis of an external appearance and growth features.

Technique of isoenzymatic markers was used for verification of the hypothesis of vegetative formation of hickories growing in the Niepołomice Primeval Forest, what seemed to be more useful for that purpose than more complicated molecular techniques. Biochemical markers (isoenzymes) and simple molecular markers (RAPD) are used for determination of genetic variability of C. ilionensis population [Marquard 1987, 1991; Marquard and Skorpenske 1989; Marquard et al., 1995; Rüter et al., 2000, 2001], identification of varieties [Conner and Wood 2001; Marquard 1988] and in the research concerning the level of self-pollination on hickory plantations [Wood and Marquard 1992]. In turn, more complex molecular techniques, based on PCR reaction mainly for construction of genetic maps and qualitative and quantitative features mapping [Crespel et al., 2002].

The difficulties connected with an isolation and storage of protein extract were encountered during the analysis. This probably resulted from an activity of free radicals which released during an isolation and caused acidification of the cells leading thus to enzymes inactivation, and also from large amount of secondary compounds polluting an extract. According to methodology provided by Kephart [1990], an activity of free radicals was reduced by increasing an amount of beta-mercaptoethanol added to an extraction buffer. The content of polyphenols and polysaccharides in an extract was in turn lowered by addition of PVP Fan and Xueqin 2009].

The research work consisted of these enzymes, which exhibit at hickory high polymorphism (PGI, PGM, MDH) and the omission of other systems with high monomorphism [Marquard 1987, 1991, Marquard and Skorpenske 1989]. The genetic individuality of most of the trees growing in Niepołomice Primeval Forest was determined for sure using isoenzymatic analysis. Various genotypes were obtained based on an analysis of phosphogluconate

isomerase (PGI) which as the only of the examined enzymes was characterized by polymorphism. It was observed that the examined hickories may be progeny of one mother and one father, or one mother and numerous fathers. Probably only the one largest hickory remains from the beginning of the experiment, and the rest individuals are the offspring of trees that were removed in recent years. Unfortunately, there is no source materials characterizing the origin of the planting, which was used to establish the experimental cultivation. It is also possible that the hickories growing in research area were grown from the seeds originated from a few different trees, alternatively, a portion of them may be the vegetative origin (root sprouting). This may be verified when analyzing other tissues in terms of PGI expression, since phosphogluconate isomerase is an enzyme of a diversified spectral pattern dependent on a type of the examined hickory tissue [Marquard and Skorpenske 1989]. Probably higher genetic variability of the trees will be determined when analyzing e.g. cambium or pollen [Marquard 1987]. The precise verification of an affinity of hickories from Niepołomice Primeval Forest may be also established using more sensitive molecular methods. Quickly evolving markers (e.g. microsatellites) exhibit higher genetic variability when compared to slowly mutating markers which include isoenzymes [Freeland 2008]. The example is the examinations on the red pine (Pinus resinosa), for which the lack of isoenzymatic variability and small variability of RAPD markers was demonstrated in 159 individuals, while 25 alleles and 23 different haplotypes were separated based on 9 microsatellite loci [Echt et al., 1998]. Similar results were also obtained in the study on lodgepole pine (P. contorta) [Thomas et al., 1999].

The analysis of isoenzymatic polymorphism of the hickory from Niepołomice Primeval Forest is an example of a considerable role of genetic research in the "Program of conserving forest genetic resources and breeding of trees in Poland for the years 2011–2035" which one of the aims is determination and protection of forests genetic variability, taking into account also selected species of foreign origin. In terms of an isoenzymatic structure, the hickories from Niepołomice Primeval Forest form 3 groups of genotypes: AA, AB and AC. Cutting out of a few trees of AA or AB genotype will not cause genetic variability lowering, however it will happen in case of removing of an individual of AC genotype (only one tree). Thus, the breeding practices without previous recognition of trees genetic variability may result in decay of some alleles, valuable for preservation of gene resources of the shagbark hickory growing in Poland.

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