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Morphometric research of samaras of North American ash species from Croatian plantations

DARIO KREMER[1]
RENATA JURIŠIĆ GRUBEŠIĆ[2]
TOMISLAV DUBRAVAC[3]

[1]Pharmaceutical Botanical Garden »Fran Kušan«, Faculty of Pharmacy and Biochemistry, University of Zagreb
Schrootova 37
HR-10000 Zagreb, Croatia

[2]Department of Analytics and Control Medicines, Faculty of Pharmacy and Biochemistry, University of Zagreb
A. Kovačića 1, 10000 Zagreb, Croatia

[3]Division of Silviculture
Croatian Forest Research Institute
Cvijetno naselje 41
10450 Jastrebarsko, Croatia

Correspondence:
Dario Kremer
Pharmaceutical Botanical Garden »Fran Kušan«
Faculty of Pharmacy and Biochemistry University of Zagreb
Schrootova 37, 10000 Zagreb, Croatia
E-mail: dkremer@pharma.hr

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Abstract

Background and Purpose: Fraxinus americana *L. and* F. pennsylvanica *were introduced 100 years ago in forest management in the lowland regions of Croatia to afforestation sites where the native narrow-leaved ash could not survive. These species are not distinguished in the practice in Croatia. The aim of the article was to determine distinguishing traits between these introduced North American ash species based on morphological traits of samaras. Reliable determination is the necessary precondition for successful forest management of plantations of these two species.*

Materials and Methods: *Samaras were collected from 50 trees in Croatian plantations on 5 localities (Karlovac, Dugo Selo, Đurđevac, Batina, Gunja), and from 3 voucher trees of* F. americana *and from 1 voucher tree of* F. pennsylvanica. *The following traits were measured: length of samara, length and width of samara wing, length and width of seed cavity, and the length of wing along the cavity. The results were evaluated using UPGMA method of cluster analysis with Euclidean distance (D_E).*

Results: *The most variable trait was absolute length of wing along seed cavity with coefficient variability (CV) from 8.38% to 23.41%, while the least variable trait was the ratio between the length of seed cavity and the length of samara (CV = 3.64–7.84%). Samaras from Croatian plantations showed similarity with* F. pennsylvanica. *Cluster analysis separated voucher trees of* F. americana *from trees from Croatian plantations of North American ash species, while the tree of* F. pennsylvanica *fitted among the trees from Croatian plantations.*

Conclusions: *Only the presence of* F. pennsylvanica *in Croatian plantations was confirmed.*

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INTRODUCTION

White ash (*Fraxinus americana* L.) and green ash (*F. pennsylvanica*) were introduced 100 years ago in forest management in the lowland regions of Croatia to afforestation sites where the native narrow-leaved ash could not survive *(1)*. These two species are not distinguished in practice in Croatia, and they are known usually under one name – »American ash«. Generally, only the distribution of North American ash species in Croatia was researched *(2, 3, 4, 5, 6, 7)*. Only few authors, trying to reliably determine these species in plantations in Croatia, researched their morphological traits. Kremer *(8)* analyzed macromorphological traits of leaves while Borzan *et al*. *(9)* researched micromorphological traits of leaves of the introduced North American ash species.

The determination of *F. americana* and *F. pennsylvanica* based on macromorphological traits of leaves is not always reliable *(10)*. On the other hand, determination based on micromorphological traits of leaves is reliable but requires light microscope which is not practical for field work. The introduced North American ash species have abundant fructification almost each year. Accordingly, the possibility of determination of the introduced North American ash species based on morphological traits of samaras was researched.

Reliable determination is the necessary precondition for successful forest management of plantations of these two species. Actually, *F. americana* and *F. pennsylvanica* grow in different ecological conditions and have different quality of trunk. *F. pennsylvanica* grows at or below the high water line and on poorly drained backwater sites, often with its roots in water. It is a small to medium-size tree with a broad irregular crown, and a short, usually poorly formed trunk. *F. pennsylvanica* is a typical pioneer species which is adapted well to periodical flooding and swamp conditions, and exceedingly hardy to climatic extremes. Unlike *F. pennsylvanica*, *F. americana* grows at or above the level of high flood water, on higher and drier slopes, on deep, moist, fertile upland soils. It is a medium-size tree with long, straight, clear, and cylindrical bole. *F. americana* is the most important ash (*Fraxinus* L.) species in the North Eastern part of USA *(10)*. Collecting samaras in Croatian plantations of North American ash species for nursery practice without knowing the distinguishing traits between *F. americana* and *F. pennsylvanica* leads to establishing plantations of these two species in inadequate ecological conditions. Also, the poor growth (low increment, bent trunk) of *F. pennsylvanica* is ascribed to *F. americana*. It was very often the case in forest management of lowland forest in Croatia in the middle of the 20th century.

MATERIALS AND METHODS

The selection of locality for collecting samaras was based on area distance. In this way, the possibility that the seedlings used for establishing plantations were grown up in the same nursery or that the plants in plantations were not grown up from seeds which came by wind or water from neighboring plantations was avoided. In that way the following forestry offices were selected: Batina (Management unit »Zmajevačke podunavske šume«; trees 1 – 10), Gunja (M. u. »Trizlovi – Rastovo« and M. u. »Desićevo«; trees 11 – 20), Đurđevac (M. u. »Đurđevačke nizinske šume«; trees 21 – 30), Dugo Selo (M. u. »Črnovščak«; trees 31 – 40), Karlovac (M. u. »Rečički lugovi«; trees 41 – 50). Ten adult trees at the edge of plantations with fully developed crown (at least at the one side) were selected randomly on each locality (Figure 1).

Fully developed and fully illuminated crown enables complete development of leaves and fruits *(11)*. Fully developed samaras were removed from short shoots and put into paper bag. From each bag, 50 samaras were pulled out by chance and measured. The following traits were analyzed: the length of samara, the length and width of samara wing, the length and width of seed cavity and the length of wing along seed cavity (Figure 2). The relative length of wing along seed cavity, the ratio between the length of seed cavity and samaras, and the ratio between the width and length of seed cavity were obtained by mathematical calculation.

Figure 1. *Plantation of North American ash species in Management unit »Desićevo«, Forestry office Gunja. Photo: Slavko Tomašević.*

Samaras of voucher trees of *F. americana* were collected in Fort Worth in Texas (tree no. 51), State College Forest in Pennsylvania (tree no. 52) and in Royal Botanical Gardens Hamilton in Canada (tree no. 53), while samaras of voucher tree of *F. pennsylvanica* were collected in RBG Hamilton in Canada (tree no. 54). Voucher specimens are deposited in the Collection of the Pharmaceutical Botanical Garden »Fran Kušan«, Zagreb, Croatia.

Descriptive statistics was done for all traits. The results were evaluated using unweighted pair-group method with arithmetic mean (UPGMA) with Euclidean distance (D_E). UPGMA generally yields results which are the most accurate for classification purposes. Prior to cluster analysis, each variable was standardized *(12, 13)*. The statistical analysis was performed using software Statistica 7 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Descriptive statistics

The average length of samaras collected from trees in plantations in Croatia ranged from 25.32 mm (tree no.

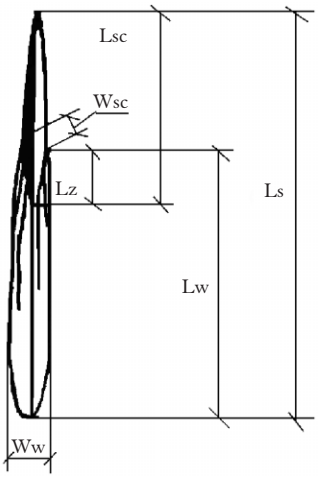

Figure 2. *Morphological traits measured on samara. Ls = samara length, Lw = wing length, Lsc = seed cavity length, Lz = wing length along seed cavity, Ww = wing width, Wsc = seed cavity width.*

50, Karlovac) to 50.24 mm (tree no. 45, Karlovac). Samaras from plantations in Croatia generally were longer than samaras of *F. americana* (x = 30.40–36.83 mm) and *F. pennsylvanica* (x = 27.88 mm).

The average length of samara wing from plantations in Croatia ranged from 20.41 mm (tree no. 50, Karlovac) to 42.05 mm (tree no. 21, Đurđevac), in trees of *F. americana* from 23.32 mm (tree no. 53) to 26.43 mm (tree no. 52), and in *F. pennsylvanica* (tree no. 54) 24.58 mm.

Tree no. 29 (Đurđevac) had the closest (3.47 mm), and tree no. 19 (Gunja) the widest (6.83 mm) samara wing. The average width of samara wing in *F. americana* ranged from 4.62 mm (tree no. 52) to 5.86 mm (tree no. 53), and in *F. pennsylvanica* (tree no. 54) 6.19 mm.

The average length of seed cavity in samaras from Croatian plantations ranged from 11.54 mm (tree no. 50, Karlovac) to 24.44 mm (tree no. 21, Đurđevac), in *F. americana* from 12.64 mm (tree no. 51) to 16.25 mm (tree no. 52), and in *F. pennsylvanica* (tree no. 54) it was 11.74 mm. Seed cavity in samaras collected in Croatian plantations was generally longer than in *F. americana*. Only a few trees from Croatian plantations had the seed cavity as long as *F. pennsylvanica*.

The average width of seed cavity ranged from 1.37 mm (tree no. 22, Đurđevac) to 2.14 mm (tree no. 22, Gunja). Only samaras of trees no. 18 (Gunja) and 39 (Dugo Selo) had the seed cavity wider than 2 mm. In *F. americana,* seed cavity width was 2.35 mm (tree no. 52), 2.41 mm (tree no. 51), and 2.95 mm (tree no. 53), while in *F. pennsylvanica* (tree no. 54) seed cavity width was 1.84 mm (Table 1).

The absolute length of wing along seed cavity in samaras collected in Croatian plantations ranged from 6.62 mm (tree no. 50, Karlovac) to 16.71 mm (tree no. 21, Đurđevac), in *F. americana* it ranged from 5.68 mm (tree no. 51) to 7.42 mm (tree no. 53), and in *F. pennsylvanica*

TABLE 1

Statistics for average width of seed cavity in samaras from trees in Croatian plantations (1–50), and from voucher trees of *Fraxinus americana* (Fort Worth – F. a. F. W; State College Forest – F. a. S. C.; Botanical Gardens Hamilton – F. a. H.) and *F. pennsylvanica* (Botanical Gardens Hamilton – F. p. H.). Minimum and maximum values are bolded.

Locality:	Tree number:	Mean (mm)	S. D. (mm)	CV (%)	Min (mm)	Max (mm)
	1	*1.75*	0.11	6.29	1.43	2.06
	2	1.59	**0.22**	**13.84**	1.16	2.01
B	3	1.78	0.10	5.62	1.58	2.00
A	4	1.80	0.13	7.22	1.52	2.07
T	5	1.80	0.10	5.56	1.59	2.00
I	6	1.83	0.11	6.01	1.58	2.08
N	7	1.80	0.11	6.11	1.48	2.06
A	8	1.48	0.12	8.11	1.21	1.80
	9	1.93	0.14	7.25	1.49	2.25
	10	1.71	0.12	7.02	1.50	2.11
	11	1.48	0.09	6.08	1.30	1.65
	12	1.72	0.11	6.40	1.38	1.88
G	13	1.86	0.10	5.38	1.60	2.05
U	14	1.89	0.11	5.82	1.60	2.06
N	15	1.73	0.17	9.83	1.41	2.12
J	16	1.71	0.09	5.26	1.52	1.86
A	17	1.93	0.19	9.84	1.38	2.29
	18	**2.14**	0.16	7.48	1.66	**2.43**
	19	1.96	0.19	9.69	1.51	2.30
	20	1.98	0.17	8.59	1.63	2.23
	21	1.56	0.16	10.26	**1.03**	1.93
Đ	22	**1.37**	**0.08**	5.84	1.16	**1.56**
U	23	1.62	**0.08**	4.94	1.40	1.84
R	24	1.59	0.11	6.92	1.31	1.89
Đ	25	1.90	0.10	5.26	1.73	2.13
E	26	1.87	0.10	5.35	1.60	2.01
V	27	1.53	0.09	5.88	1.34	1.76
A	28	1.88	0.12	6.38	1.48	2.09
C	29	1.45	0.11	7.59	1.24	1.74
	30	1.46	0.11	7.53	1.26	1.70
D	*31*	1.90	0.11	5.79	1.69	2.20
U	32	1.74	0.15	8.62	1.45	1.95
G	33	1.60	0.13	8.13	1.24	1.87
O	34	1.97	0.19	9.64	1.30	2.29
	35	1.70	0.10	5.88	1.39	1.91
S	36	1.61	0.09	5.59	1.40	1.95
E	37	1.63	0.10	6.13	1.43	1.83
L	38	1.79	0.16	8.94	1.37	2.22

O	39	2.08	0.10	**4.81**	**1.81**	2.23
	40	1.55	0.09	5.81	1.41	1.80
	41	1.68	0.09	5.36	1.50	1.94
K	42	1.62	0.10	6.17	1.42	1.83
A	43	1.75	0.14	8.00	1.46	2.00
R	44	1.83	0.14	7.65	1.53	2.11
L	45	1.87	0.16	8.56	1.60	2.32
O	46	1.82	0.11	6.04	1.56	2.00
V	47	1.75	0.10	5.71	1.52	1.98
A	48	1.99	0.10	5.03	1.73	2.14
C	49	1.70	0.10	5.88	1.50	1.95
	50	1.86	0.10	5.38	1.56	2.04
F. a. F. W.	51	2.41	0.13	5.39	2.08	2.69
F. a. S. C.	52	2.35	0.13	5.53	2.07	2.67
F. a. H.	53	2.95	0.22	7.46	2.40	3.59
F. p. H.	54	1.84	0.13	7.07	1.52	2.09

(tree no. 54) it was 8.43 mm. The relative length of wing along seed cavity (Table 2) in samaras from plantations in Croatia ranged from 41% (tree no. 13, Gunja) to 80% (tree no. 18, Gunja), in *F. americana* it ranged from 36% (tree no. 52) to 51% (tree no. 53), and in *F. pennsylvanica* it was 72% (tree no. 54).

The ratio between the length of seed cavity and the length of samara collected from trees in Croatian plantations ranged from 0.41 (tree no. 10, Batina) to 0.63 (tree no. 39, Dugo Selo), in *F. americana* it ranged from 0.41 (tree no. 51) to 0.48 (tree no. 53), and in *F. pennsylvanica* (tree no. 54) it was 0.42. The ratio the between width and length of seed cavity ranged from 0.06 (tree no. 21, Đurđevac) to 0.16 (tree no. 50, Karlovac), in *F. americana* it ranged from 0.14 (tree no. 52) to 0.20 (tree no. 53), and in *F. pennsylvanica* (tree no. 54) it was 0.16.

The most variable trait was the absolute length of wing along the seed cavity with coefficient variability (CV) ranging from 8.38% to 23.41%, while the least variable trait was the ratio between the length of seed cavity and the length of samara (CV = 3.64–7.84%).

Cluster analysis

As regards analyzed traits of samaras, UPGMA separated investigated trees of North American ash species as shown in Fig. 3. Cluster analysis connected two trees (trees no. 51 and 52) of *F. americana* at the Euclidean distance (D_E) of 2.797 in one cluster. The third tree of *F. americana* (tree no. 53) was connected to this cluster at the Euclidean distance of 8.729. A cluster with trees no. 51 (*F. americana*, Fort Worth) and 52 (*F. americana*, State College) was connected at the distance of 5.449 to the group of trees no. 5, 42, 21 and 45 from Croatian plantations. *F. pennsylvanica* (tree no. 54) was fitted among the trees from Croatian plantations and it was connected

TABLE 2

Statistics for the relative length of wing along seed cavity in samaras from trees in Croatian plantations (1–50), and from voucher trees of *Fraxinus americana* (Fort Worth – F. a. F. W; State College Forest – F. a. S. C.; Botanical Gardens Hamilton – F. a. H.) and *F. pennsylvanica* (Botanical Gardens Hamilton – F. p. H.). Minimum and maximum values are bolded.

Locality:	Tree number:	Mean	S. D.	CV (%)	Min	Max
	1	*0.57*	0.09	15.79	0.34	0.76
	2	0.54	0.07	12.96	0.40	0.73
B	3	0.57	0.07	12.28	0.43	0.74
A	4	0.51	0.07	13.73	0.33	0.66
T	5	0.68	0.07	10.24	0.50	0.82
I	6	0.59	0.10	16.95	0.31	0.82
N	7	0.50	0.07	14.00	0.37	0.72
A	8	0.61	0.10	16.39	0.42	0.87
	9	0.72	0.08	11.11	0.50	0.88
	10	0.67	0.09	13.43	0.48	0.83
	11	0.74	0.07	9.46	**0.63**	0.89
	12	0.56	0.09	16.07	0.29	0.75
G	13	**0.41**	0.08	19.51	0.28	0.67
U	14	0.50	0.07	14.00	0.38	0.69
N	15	0.64	0.11	17.19	0.45	0.95
J	16	0.63	0.06	9.52	0.51	0.78
A	17	0.53	0.10	18.87	0.33	0.89
	18	**0.80**	0.06	7.50	**0.63**	0.90
	19	0.77	0.07	9.09	**0.63**	0.92
	20	0.64	0.09	14.06	0.46	0.86
	21	0.68	0.10	14.71	0.48	0.87
Đ	22	0.62	0.06	9.68	0.50	0.79
U	23	0.57	0.07	12.28	0.36	0.77
R	24	0.55	0.08	14.55	0.38	0.80
Đ	25	0.55	0.07	12.73	0.42	0.72
E	26	0.64	0.06	9.38	0.50	0.83
V	27	0.56	0.05	8.93	0.43	0.64
A	28	0.60	0.09	15.00	0.40	0.81
C	29	0.44	0.07	15.91	**0.24**	**0.61**
	30	0.55	0.07	12.73	0.38	0.74
D	*31*	0.64	**0.04**	**6.25**	0.55	0.75
U	32	0.73	0.08	10.96	0.53	0.90
G	33	0.70	0.06	8.57	0.53	0.81
O	34	0.74	0.09	12.16	0.50	0.90
	35	0.74	0.07	9.46	0.56	0.88
S	36	0.65	0.09	13.85	0.40	0.88
E	37	0.69	0.09	13.04	0.53	0.91
L	38	0.72	0.07	9.72	0.58	0.94

O	39	0.63	0.11	17.46	0.41	0.99
	40	0.61	0.07	11.48	0.47	0.78
	41	0.66	0.09	13.64	0.48	0.87
K	42	0.63	0.07	11.11	0.50	0.79
A	43	0.60	0.07	11.67	0.48	0.78
R	44	0.66	0.06	9.09	0.53	0.82
L	45	0.57	0.07	12.28	0.43	0.79
O	46	0.73	0.06	8.22	0.54	0.84
V	47	0.72	0.06	8.33	0.57	0.94
A	48	0.65	0.08	12.31	0.52	0.86
C	49	0.57	0.07	12.28	0.42	0.73
	50	0.57	**0.13**	**22.81**	0.40	**1.00**
F. a. F. W.	51	0.45	0.06	13.33	0.34	0.61
F. a. S. C.	52	0.36	0.06	16.67	0.23	0.48
F. a. H.	53	0.51	0.05	9.80	0.42	0.63
F. p. H.	54	0.72	0.10	13.89	0.52	0.93

(D_E = 3.438) with the cluster formed by trees from all researched localities (trees no. 9, 41, 38, 25, 39, 30 and 50). This group and *F. pennsylvanica* (tree no. 54) were connected (D_E = 4.096) to the large group formed by 38 trees from Croatian plantations.

DISCUSSION

According to results of research of samaras of *F. americana* and *F. pennsylvanica* by American authors *(14, 15, 16),* the average length of samaras in *F. americana* does not surpass 4 cm. Based on that fact, we can conclude that at least trees from Croatian plantations with long samaras belong to *F. pennsylvanica*. Miller (Table 3) found that *F. americana* has somewhat wider (0.66 cm) samaras than *F. pennsylvanica* (0.60 cm). In Croatian plantations, only samaras of trees no. 19 and 28 had the wing wider than 0.66 cm. Also, the length of seed cavity in *F. americana* does not surpass 1.6 cm *(14, 15, 16)*. In most samaras collected in Croatian plantations, the length of seed cavity surpassed 1.6 cm.

The length of wing along seed cavity is a very important characteristic for determination of samaras of *F. americana* and *F. pennsylvanica*. Wing extends to the upper third part (or 33%) of the seed cavity in *F. americana*, and at least to the upper half (often through to the base) in *F. pennsylvanica (14, 17, 18, 19, 20)*. In samaras collected in Croatian populations wing extends along seed cavity from 41% to 80% (in 40 trees more than 55% and in 11 trees more than 70%).

Miller *(14)* found that the average width of seed cavity in *F. pennsylvanica* was 1.9 mm, and in *F. americana* 3.0 mm (Table 3). According to Daniels *(15),* the width of seed cavity in *F. americana* ranged from 2.52 mm to 2.65 mm. Clausen *et al. (16)* found that the width of seed cavity in *F. americana* ranged from 2.50 mm do 3.17 mm (Table 4). The average width of seed cavity in samaras from Croatian plantations surpassed 2 mm only in two trees (2.14 mm in tree no. 18, and 2.08 mm in tree no. 39). Based on that fact, samaras from Croatian populations belong to *F. pennsylvanica*.

The results of descriptive statistics were confirmed by cluster analysis which separated voucher trees of *F. americana* from trees of Croatian plantations. On the other hand, tree of *F. pennsylvanica* fitted among the trees from Croatian plantations.

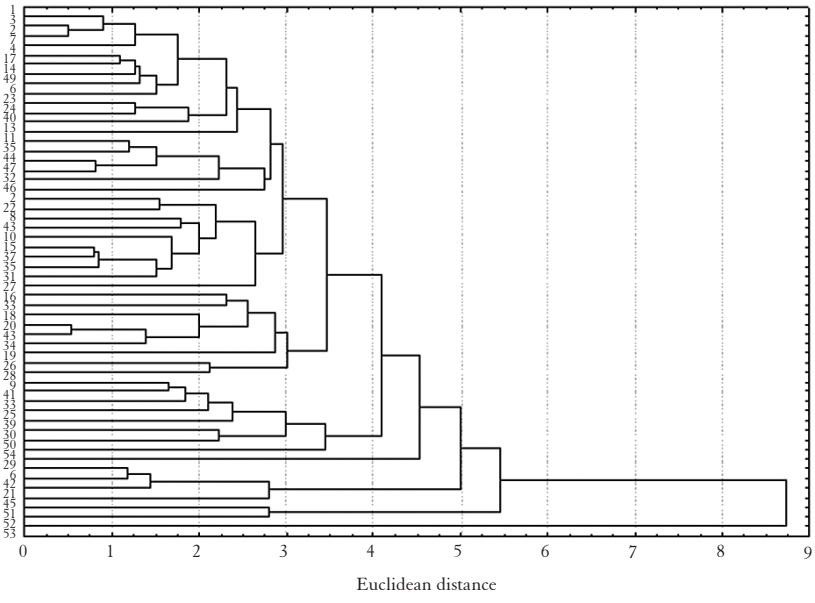

Figure 3. *UPGMA dendrogram obtained by analysis of samaras of North American ash species: nondetermined trees from Croatian plantations (1–50), determined voucher trees of* Fraxinus americana *(51–53), and* F. pennsylvanica *(54).*

TABLE 3

Differences between samaras of *Fraxinus americana* and *F. pennsylvanica* (Miller 1955).

Samara measurement	Species	No. of fruits	Mean (cm)	S. D. (cm)
Samara	*F. pennsylvanica*	803	4.2	± 0.721
Length	*F. americana*	704	3.8	± 0.431
Samara	*F. pennsylvanica*	803	0.60	± 0.1112
Width	*F. americana*	704	0.66	± 0.074
Length of	*F. pennsylvanica*	803	2.0	± 0.352
Body of Samara	*F. americana*	704	1.6	± 0.044
Diameter of	*F. pennsylvanica*	803	0.19	± 0.033
Body of Samara	*F. americana*	704	0.30	± 0.042

TABLE 4

Characteristics of *Fraxinus americana* samaras regarding ploidy level (Clausen *et al*. 1981).

Traits	Chromosome number			
	2×	4×	5×	6×
Samara Length (mm)	31.83	32.35	38.71	39.50
Seed Length (mm)	11.90	11.80	12.43	12.97
Length of Wing on Seed (mm)	3.55	2.90	3.21	3.43
Length of Wing on Seed (%)	29.83	24.58	25.82	26.45
Seed Width (mm)	2.50	2.54	3.14	3.17

Based on morphometric researches of samaras of North American ash species collected in Croatian plantations and on the available results of American authors, it could be concluded that samaras from Croatian plantations belong to *F. pennsylvanica*. Similarity is noticeable especially on the basis of the length of wing along seed cavity, width of seed cavity, as well as the length of samaras. Using traits of samaras instead of (or together with) macromorphological traits of leaves it could be possible to reliably differentiate *F. americana* and *F. pennsylvanica* which were introduced in the lowland regions of Croatia. This is the necessary precondition for successful forest management of plantations of these two species.

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