

## Pollen traits of some American ashes investigated by a scanning electron microscope

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Over last 130 years the American ash was often used for afforestation of Croatian lowland areas where the native narrow leafed ash (*Fraxinus angustifolia* Vahl) did not grow well. Due to unequivocal species identification of the American ash *F. americana* L. or *F. pennsylvanica* Marshall, pollen samples of concerned specimens were collected. Based on species pollen samples of *F. americana* and *F. pennsylvanica*, collected from trees growing at the Royal Botanical Gardens Hamilton in Canada, and on their distinctive morphological features, pollen samples of American ash trees from a plantation Črnovšćak in Dugo Selo near Zagreb and from arboricultures in Zagreb were determined as *Fraxinus pennsylvanica*.

**Key words:** pollen, morphology, scanning electron microscopy, *Fraxinus americana* L., *Fraxinus pennsylvanica* Marshall

### Introduction

American ash species *Fraxinus americana* L. and *F. pennsylvanica* Marshall were introduced to Europe in 1723 and in 1824 respectively (HEGI 1908). In Croatia these two species are present for more than 130 years. One of the first papers mentioning those species growing in Croatian parks was ETTINGER (1889, 1892). *F. americana* was recommended as species for afforestations in Croatia by ČORDAŠIĆ (1894) and KOZARAC (1898). Some decades after that first data about successful afforestation with *F. americana* and *F. pennsylvanica* in lowland area were reported by PERUŠIĆ (1925), STREPAČKI (1931), ŠPANOVIĆ (1931), CIVIDINI and MIRTH (1941) and FUKAREK (1956). Most of the authors have mentioned *F. americana* as a species involved in afforestations, and only a few mentioned *F. pennsylvanica* as well (ŠPANOVIĆ 1954, ANIĆ 1954, DEKANIĆ 1974). More recent papers by RAUŠ (1993), RAUŠ et al. (1985), RAUŠ and MATIĆ (1990), and MAJER (1994) were dealing with the presence of *F. americana* in Croatian forests and arboricultures. KREMER (2001) and KREMER and BORZAN (2001) mentioned that *F. pennsylvanica* is primarily present in

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Croatian lowland forests in the Kupa river basin; the Sava river basin; and in part of the Danube river basin.

In Croatian forestry practice North American ash species are known today under one name – American ash. The primary reason for performing investigations of morphological traits of trees identified from the Royal Botanical Gardens, Hamilton in Canada was to compare them with samples taken from Croatian forests and arboricultures. Owing to micromorphological differences in the pollen of *F. americana* and *F. pennsylvanica* investigation by use of scanning electron microscopy was applied.

## Materials and methods

Pollen samples of *Fraxinus pennsylvanica* were collected in spring 2002 from the tree at the Botanical Garden of the Faculty of Sciences, University of Zagreb and in spring 2003 from a tree at Royal Botanical Garden in Hamilton, Canada. Pollen of *F. americana* was also collected in spring 2003 from a tree at RBG, Hamilton, Canada.

Samples of pollen of American ash were collected in spring 2002 from 12 trees in Management unit Črnovščak (subcompartment 4e, Forestry office Dugo Selo) and from 3 trees growing in arboricultures in Zagreb. Trees were randomly selected. The Management unit Črnovščak is situated about 20 km east from Zagreb. Borders of that unit are: railway line Sesevete – Božjakovina on the north, River Lonja on the east, villages Lipovec Lonjski, Trebovec and Rugvica on the southeast, the River Sava on the southwest and Ivanja Reka on the west. The altitude ranges from 99 to 107 m, according to the Management plan for Management unit Črnovščak (ANONYMOUS 1997).

At the beginning of bloom setting, flowering branches were cut and kept in the bottle with water until shedding of pollen. Pollen samples were stored in glass vials at  $-20^{\circ}\text{C}$ .

The SAM investigations were done at the Institute of Plant Physiology, Karl-Franzens-University, in Graz, Austria. Pollen samples were mounted on carbon-coated double-sided tape, sputter coated with gold and investigated with a Phillips XL30 scanning electron microscope (BORZAN and STABENTHEINER 2002, MÜLLER et al. 2003). A length of 35 pollen grains from each tree, along the longer pollen grain axis, was measured. Data were tested by Kolmogorov-Smirnov test and by ANOVA analysis. Differences among trees were established by means of Scheffé's post-hoc test. The statistical analysis was performed using Statistica 6.0 software.

Pollen morphology was described using common terminology as in papers by ERDTMAN (1952), MOORE and WEBB (1978), STRASBURGER et al. (1978), PUNT et al. (1994), RICCIARDELLI (1997), VON DER OHE and VON DER OHE (2000).

## Results

Statistical results of pollen length measurements are presented in table 1 and Scheffé's post-hoc test in table 2. Largest pollen grains were measured in samples of *Fraxinus pennsylvanica* from the RBG Hamilton (mean =  $27.71\ \mu\text{m}$ ). They were statistically longer only from pollen grains belonging to 5 trees (trees no. 1, 6, 7, 14, and 18), i.e. from trees marked and identified as *F. pennsylvanica* (no. 1) in Botanical Garden Zagreb, and as American ash from the forest plantation Črnovščak (no. 6, 7, 14) and arboriculture (no. 18). The

**Tab. 1.** Statistical analysis of pollen grain length. Thirty five pollen grains were measured. Minimum and maximum values are in boldface. C. V. = coefficient of variability. 1 = *Fraxinus pennsylvanica* (Zagreb), 2 = *F. pennsylvanica* (Hamilton), 3 = *F. americana*, (Hamilton), 4 to 18 = American ash = introduced ash species (not identified)

Tree no:	Mean ( $\mu\text{m}$ )	Std. dev. ( $\mu\text{m}$ )	C. V. (%)	Minimum ( $\mu\text{m}$ )	Median ( $\mu\text{m}$ )	Maximum ( $\mu\text{m}$ )
1	25.25	1.06	4.20	23.00	25.10	28.30
2	<b>27.71</b>	1.21	4.37	<b>24.90</b>	<b>27.90</b>	29.50
3	26.17	1.17	4.47	24.00	25.90	28.70
4	26.21	1.46	5.57	23.60	26.40	29.70
5	26.20	1.15	4.39	24.00	26.10	28.40
6	25.36	<b>1.62</b>	<b>6.39</b>	22.40	25.20	28.70
7	24.50	1.43	5.84	22.30	24.50	28.40
8	27.66	1.40	5.06	24.00	27.70	30.00
9	26.78	1.38	5.15	24.00	26.80	29.00
10	26.05	1.21	4.64	24.00	25.90	28.60
11	26.32	1.23	4.67	23.30	26.20	28.90
12	26.50	1.27	4.79	22.30	26.40	28.80
13	26.38	1.18	4.47	24.20	26.20	28.70
14	25.31	<b>0.98</b>	<b>3.87</b>	23.00	25.10	28.00
15	26.15	<b>1.62</b>	6.20	23.90	25.80	<b>30.90</b>
16	27.06	1.25	4.62	24.70	26.90	29.70
17	27.13	1.21	4.46	24.20	26.90	29.80
18	<b>23.09</b>	1.41	6.11	<b>20.00</b>	<b>22.90</b>	<b>25.90</b>

smallest pollen grain (mean = 23.09  $\mu\text{m}$ ) was from tree no. 18 (= American ash from arboriculture in Zagreb), and was statistically smaller from pollen grains of all other trees except tree no. 1 (*F. pennsylvanica*, Botanical garden Zagreb) and no. 7 (forest plantation Črnovščak). Pollen grains from identified *F. americana* at the RBG Hamilton (tree no. 3) were statistically significantly different from the smallest pollen grains of the American ash tree from the arboriculture in Zagreb (tree no. 18) only.

Pollen grains of *F. pennsylvanica* (Fig. 1a) have irregular elliptic shape (more or less angular) in equatorial view with clearly visible apertures. In *F. americana* (Fig. 1d) pollen grains have irregular elliptic shape as well, somewhat less angular than the pollen of *F. pennsylvanica*. In both species the polar view is usually quadrangular with poorly visible apertures. *F. pennsylvanica* and *F. americana* have commonly 4 (*stephanocolpate*) apertures. Apertures in both species are long and narrow (known as *colpi*), sunken with irregular and diffuse margins (Fig. 1b, e). *Mesocolpium* is large in both species.

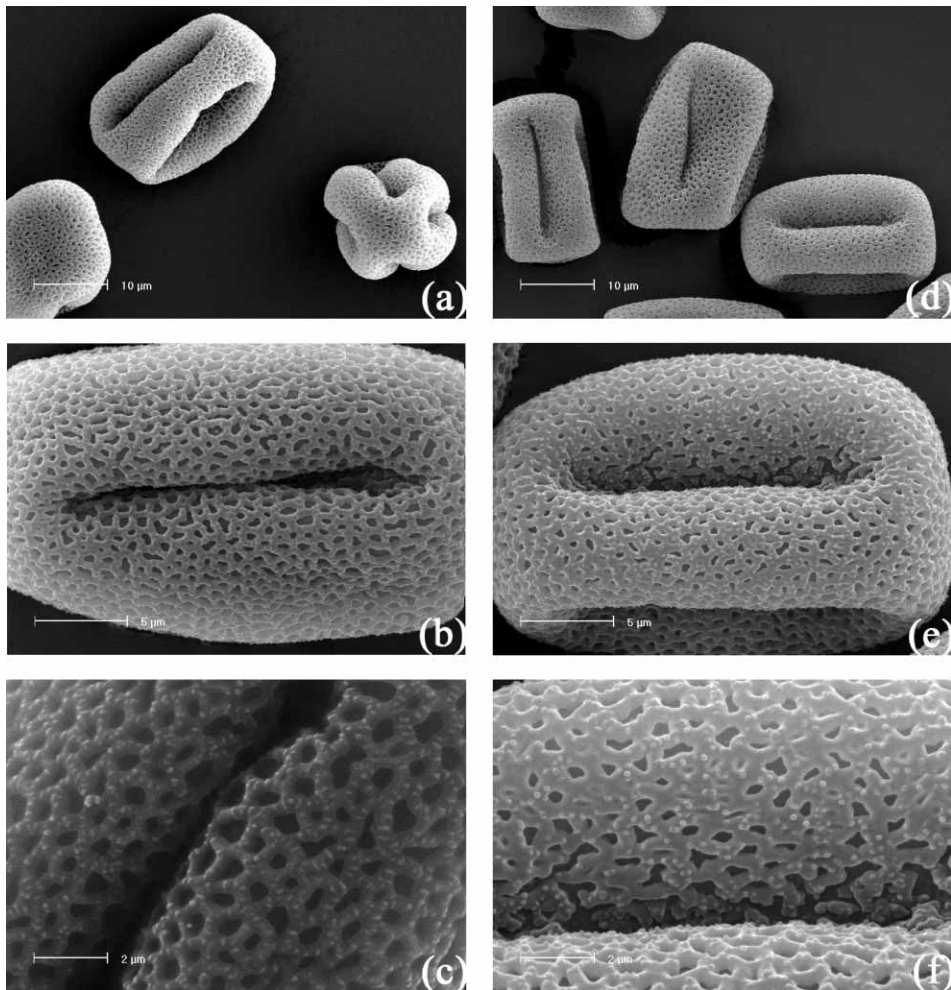
In *F. pennsylvanica* and *F. americana* the outer layer (*exina*) of the *sporodermis* are reticulate (have reticulum-shaped sculptural elements). The inner areas of a reticulum mesh (*lumina*) are of similar size in both species. Ribs in the reticulum (*muri*), which limits the lumina, are firmly fused in both species and have the same width or are wider (seldom nar-

**Tab. 2.** Results of Scheffé's post-hoc test for length of pollen grains. \* = statistically significant difference ( $p < 0.05$ ). 1 = *Fraxinus pennsylvanica* (Zagreb), 2 = *F. pennsylvanica* (Hamilton), 3 = *F. americana* (Hamilton), 4 to 18 = American ash – introduced ash species (not identified).

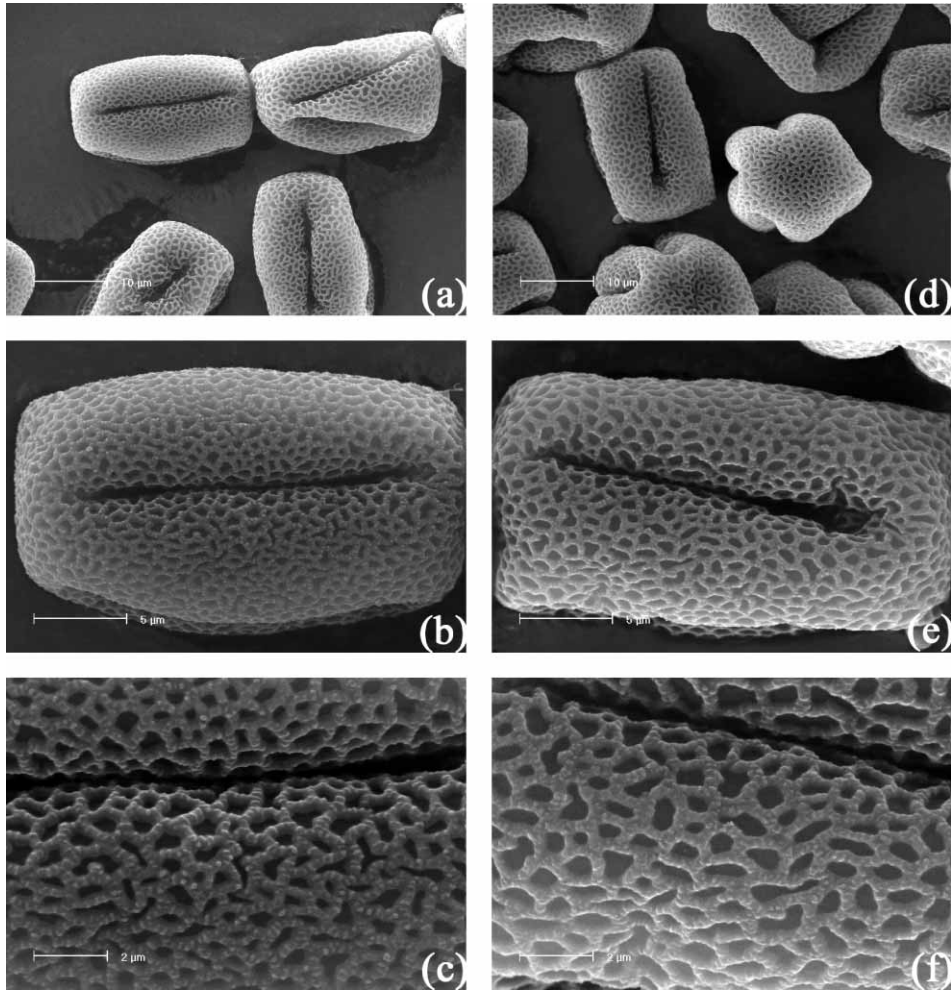
Tree no:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1																		
2	0.000*																	
3	0.586	0.463																
4	0.147	0.896	1.000															
5	0.539	0.509	1.000	1.000														
6	1.000	0.002*	0.999	0.921	0.998													
7	1.000	0.000*	0.297	0.040*	0.259	0.997												
8	0.000*	1.000	0.539	0.930	0.586	0.003*	0.000*											
9	0.025*	0.992	1.000	1.000	1.000	0.640	0.004*	0.996										
10	0.479	0.569	1.000	1.000	1.000	0.996	0.215	0.644	1.000									
11	0.365	0.684	1.000	1.000	1.000	0.988	0.142	0.751	1.000	1.000								
12	0.159	0.885	1.000	1.000	1.000	0.930	0.045*	0.921	1.000	1.000	1.000							
13	0.280	0.769	1.000	1.000	1.000	0.976	0.097	0.826	1.000	1.000	1.000	1.000						
14	1.000	0.000*	0.997	0.888	0.995	1.000	0.999	0.002*	0.569	0.992	0.980	0.898	0.961					
15	0.615	0.433	1.000	1.000	1.000	0.999	0.323	0.509	1.000	1.000	1.000	1.000	0.998	0.998				
16	0.002*	1.000	0.995	1.000	0.997	0.246	0.000*	1.000	1.000	0.998	0.999	1.000	1.000	0.195	0.993			
17	0.001*	1.000	0.987	1.000	0.991	0.174	0.000*	1.000	1.000	0.995	0.998	1.000	0.999	0.133	0.984	1.000		
18	0.353	0.000*	0.000*	0.000*	0.000*	0.005*	0.648	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.007*	0.000*	0.000*	0.000*	0.000*

rower) than the lumina. On the muri of *F. pennsylvanica* there are suprategal transversal ridges and sometimes granules, which are poorly visible because of the present ridges (Fig. 1c). Muri of *F. americana* have small number of ridges so that the granules are more noticeable (Fig. 1f). The lumina in both species are different in size and irregularly shaped with obtuse angles and are not smaller toward apertures.

The pollen morphology of trees under the name American ash was also investigated in details. The muri of their pollen grains have suprategal transversal ridges and granules (Fig. 2). Because of that they were identified as pollen grains of *F. pennsylvanica*.



**Fig. 1.** SEM micrographs of pollen grains of *Fraxinus pennsylvanica* (a–c) and *F. americana* (d–f). Apertures in pollen grains of *F. pennsylvanica* (b) and *F. americana* (e). Reticulate pollen grain ornamentation in *F. pennsylvanica* (c) and *F. americana* (f). Ridges on muri of *F. pennsylvanica* pollen grains are abundantly present (c). Muri (ribs in reticulum which limits the lumina) are more smooth in *F. americana* with lesser ridges and granules (f).



**Fig. 2.** SEM micrographs of pollen grains of two American ash trees: Tree no. 4 from forest plantation Črnovščak (a–c), and tree no. 17 from arboricultures in Zagreb (d–f). Similar ornamentation on those and other American ash trees led to the conclusion that all those belong to *F. pennsylvanica*.

## Discussion

Pollen grains of *Fraxinus pennsylvanica* and *F. americana*, based on their average length, might be classified according to KREMP's (1965) classification as middle large. The length of middle large pollen according to his classification is 25–50 µm.

Based on the pollen length it is not possible to distinguish the pollen of *F. americana* and *F. pennsylvanica*. The variation of pollen length in *F. pennsylvanica* is broad so that the range of length includes ranges of the investigated pollen grains belonging to one identified *F. americana* tree and 13 (out of 15 investigated) American ash trees. Two smallest pollen grains are from two American ash trees, no. 7, and no. 18. This means that length of pollen



is not a trait which could help in determining to which species the »American ashes« are belonging.

The number of colpi (apertures) on the pollen grains in *F. americana* and *F. pennsylvanica* is still not certain. JONES et al. (1995) have described the pollen of both species as *stephanocolpate* (with more than 3 apertures). On the contrary MARTIN and DREW (1970) described the pollen of *F. pennsylvanica* as *tricolpate* (with 3 apertures). Some of our photos show that the pollen grains of *F. pennsylvanica* seen from a polar view have four apertures (Fig. 1a). Depending on the length of an aperture, if short, the aperture could be hidden when viewed from the polar side. When seen from an equatorial side the aperture could be completely hidden under the pollen grain laying on a carbon-coated tape when being prepared for microscopic research. On the other hand, if a pollen grain contains very sunken *mesocolpi* such a part can be misidentified as the aperture.

The best possibility in differentiating pollen grains of *F. americana* from *F. pennsylvanica* is in their differences in ornamentation of surface. The surface of pollen grains in both species is reticulate. Granules and ridges on the muri in *F. pennsylvanica* are more expressed (Fig. 1c) than in *F. americana* pollen grains. Ridges on the muri are rare in *F. americana* (Fig. 1f) and surface of the muri seems more smooth, with rarely present ridges and with fewer present, but with good visible granules. In all the samples of American ash ridges on the muri on the pollen grain surfaces are readily visible (Fig. 2c, f) and abundantly present. Thus they have been identified as *F. pennsylvanica*.

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