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MORPHOMETRIC VARIABILITY OF NARROW-LEAVED ASH SAMARAS AT THE TEST-TREE LEVEL

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Abstract: The aim of this research is to assess *F. angustifolia* test tree variability based on the morphometric traits and shape of samara from the locality Karapandža. Twenty adult trees were randomly selected. From each individual 100 fully developed samaras were analyzed. The following traits were analyzed: the length and width of samara, the length of seed, ratio between the length of seed and samara, the weight of 100 seeds by individual, samara shape and samara top shape. The obtained results indicated a considerable variability within the studied individuals at the level of samara morphometric traits. Considering that all sampled individuals were from the same populations we could suppose that this variability was not induced by different environmental factors (climate or soil), then with the existence of specific genotypes. Classification based on samara size says that our individuals could belong to large size samaras, i.e. the type known as f. *macrocarpa*. However, since there is no uniformity of samara shapes within the majority of studied individuals we cannot classify them in accordance with samara shape classification.

Key words: *Fraxinus angustifolia* Vahl, samara variability, natural population, the Danube River, the Karapandža locality

INTRODUCTION

Fraxinus angustifolia Vahl (the narrow-leaved ash) is a widely distributed wind-pollinated tree species naturally extending throughout Southern and Eastern Europe, from Portugal and Spain in the west to Slovakia and south Moravia in the north and Turkey, Syria, Caucasus and southern Russia in the east (*FRAXIGEN, 2005; Fukarek, 1983; Wallander, 2008*). Despite its wide distribution, this species is a local habitat specialist restricted to humid areas and waterways throughout the Mediterranean, where as it can be notably abundant and dominant in the temperate flood plain and riparian forests along large rivers and wet lands in Central and South-eastern Europe (the Danube and the Pannonian basin) (*Temunović et al., 2013*). The current distribution of this species is largely confined to areas of glacial refugia proposed to be located in the Balkan, Iberian and northern Apennine Peninsulas, as well as in the Dinaric Alps (*Heuertz et al., 2006*).

F. angustifolia is a widely distributed tree species of monodominant and mixed forests in the alluvial area of large rivers (*Tomić, 2004*). Monodominant forests grow on gley soils where F. angustifolia is a pioneer species and has the coecological optimum (*Bobinac et al., 2010*).

Mixed forests, with black and white poplar, common oak, common elm, European white elm, hornbeam and field maple, grow on developed alluvium and semigley soils. In mixed forests, it has ecological and productivity optimum and its stands are distinguished by high productivity (*Bobinac et al., 2010*). However, in the past period on alluvial soils, the growth of *F. angustifolia* was significantly neglected, predominantly because of introduced Euroamerican poplars and *F. americana* and *F. pennsylvanica* that have been more than a hundred years well adapted on the *F. angustifolia* natural stands (*Bobinac et al., 2010*).

In Serbia, *Fraxinus* genus is represented by 4 native species: *F. angustifolia* Vahl, the narrow-leaved ash, *F. excelsior* L., the common ash, and *F.ornus L.*, the manna ash and *F. pallisae* Willmott, the Balkan ash (*Jovanović, 1973*). The last two species are closely related and many authorities consider them to be conspecific (e.g. FRAXIGEN, 2005). *F. angustifolia* is a hermaphrodite with variable morphology that enabled the establishment of several taxa based on differences in the samara and leaf morphology (*Papi et al., 2011*). In addition, hybridization between *F. angustifolia* and *F. excelsior* is possible, which has been reported by various groups (*Papi et al., 2011*) based on the observation of intermediate morphologies (*Fernández-Manjarrés et al., 2006; Jarni et al., 2011*), the genotyping of natural populations (*Jeandroz et al., 1996; Papi et al., 2011; Temunović et al., 2012*) and the assessment of the morphological and genetic structure of putative hybrid populations (*Fernández-Manjarrés et al., 2006; Gerard et al., 2013*). Due to hybridization and description of many subspecies based on high variation in morphology, the taxonomic status of *F. angustifolia* is most complicated (*Wallander, 2008*).

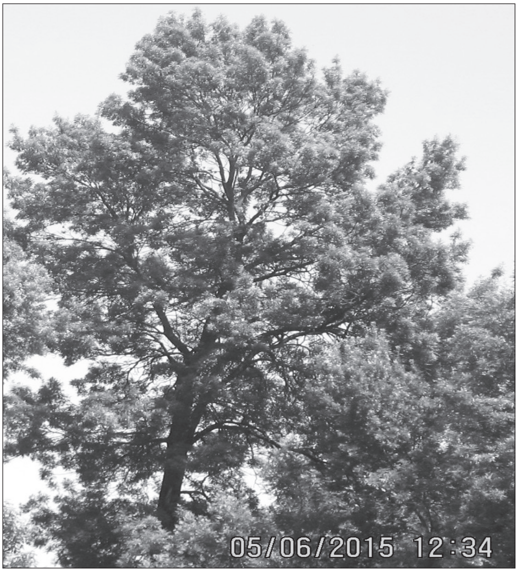

Figure 1. Selected *F. angustifolia* tree from its natural stand, the locality Karapandža

However, in Serbia *F. angustifolia* is characterized by great variability of samara (Flora SR Srbije, V), where a large number of species forms is distinguished by samara shapes and size: f. ***obtusa*** – long wing with a rounded top; f. ***emarginata*** – with extended top of the wing; f. ***acuta*** - elliptically narrowed with gradually tapered top; f. ***rostrata*** - tapered top like a beak; f. ***leptocarpa*** - characterized by smaller samaras (1-1.5 cm) and f. ***macrocarpa*** - characterized by larger samaras in general (5.5 cm length and 1 cm width) (Jovanović, 2000).

F. angustifolia is considered as threatened species, which in Serbia covers 25.200 ha (*Banković et al., 2009*) in riparian and floodplain forests (*Jovanović, 2000*). *F. angustifolia* is an important constituent of European mixed broadleaved woodlands, and in recent years, there is an increased interest in the planting of *F. angustifolia* as an autochthonous species, not only for timber production, but also for long-term sustainability, conservation of biodiversity and restoration of degraded riparian ecosystems and temporary flood-prone areas. Therefore, the selection of genetic material with adaptability and good growth indifferent ecological environments is of high importance (*FRAXIGEN, 2005; Spanos et al., 2004; Papi et al., 2011*).

Considering that this is an endangered species and that there is not enough information about *F. angustifolia* stands and variability in Serbia, the aim of this research is to make an assessment of *F. angustifolia* variability at the level of individuals selected based on morphometric traits and the shape of samara from the locality Karapandža, as the starting point in future conservation and breeding measures at the present area.

MATERIALS AND METHODS

In order to determine the morphometric variability of *F. angustifolia* samara, we studied *F. angustifolia* in its natural population from the locality Karapandža, a northern part of the Special Nature Reserve Gornje Podunavlje. The SNR Gornje Podunavlje covers an area of 19.648 ha in total; with *F. angustifolia* stands representing less than 2% of its total area (about 240 ha), while in studied locality there is about 16 ha of *F. angustifolia* in total. The selected population is located in the Valley of the Danube River, in the northern part of Serbia, on flat ground, without significant exposure and without regular flooding. The climate of the study area is semiarid, with a mean annual air temperature of 11.1°C and a mean annual precipitation of 569 mm (*Bobinac et al., 2010*). Forest vegetation is primarily conditioned by flood water or ground water influences related to the Danube water level.

Twenty adult test trees with fully developed crowns (at least at the one side) were randomly selected. From each individual 100 fully developed samaras were randomly selected and measured, in total 2000 seeds. The following traits were analyzed: the length and width of samara, the length of seed, ratio between the length of seed and samara, the weight of 100 seeds by individual, samara shape and samara top shape. Samara shapes were analyzed using the following classification – elliptic samara with narrow top and elliptic samara with extended top; and samara top shape – whole oval and tapered top, and oval and tapered top like a beak.

Descriptive statistics was calculated for all morphometric traits: mean value, min-max, and the coefficient of variation. The significance of differences between the mean values of selected individuals was tested using the One-Way ANOVA test. The differences between individuals were examined by the post hoc multiple comparison test (Tukey HSD test). To measure the strength of the linear relationship between the variables, Pearson correlations between each pair of morphometric traits have been examined. Furthermore, the results were evaluated using the unweighted pair-group method with an arithmetic mean (UPGMA) with Euclidian distances. Prior to this cluster analysis, each morphometric variable was standardized. The frequency of certain samara shapes was shown by percentage in each individual. Statistical analyses and tests were performed in the STATISTICA software package (StatSof. Inc., USA).

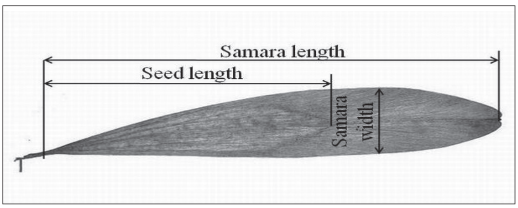

Figure 2. Morphometric traits measured on samara

RESULTS

The results showed that all studied traits were statistically significant between 20 selected individuals ($p < 0.05$), which were grouped in 8-10 homogenous groups according to the Tukey's HSD test. The average coefficient of variation per tree for all traits was about 7%, and in total about 18%. The mean value of samara length was 34.29 mm, ranging from 26.94 mm (tree 4) to 49.01 mm (tree 16), while the mean value of samara width was 7.74 mm, ranging from 5.79 mm (tree 4) to 9.84 mm (tree 17). The mean value of seed length was 18.59 mm and it ranged from 14.53 mm (tree 13) to 26.17 mm (tree 16). Seed and samara length ratio showed a mean value of 0.55, which means that the seed represents about 50% of the whole samara length, and other 50% represented wing. Furthermore, the mean value of 100 seeds weight was 6.99 g and it ranged from 4.5 g (tree 4) to 12.95 g (tree 16). According to these results (table 1) it could be seen that tree 16 is distinguished as the tree with the largest samara and seed dimensions. Cluster analysis also confirmed that this tree was the most separated from the others, while other individuals were separated within several groups as shown in Figure 3. This grouping was performed by dimensions and weight analyses and did not show any kind of connection with the samara shape.

Table 1. Descriptive Statistics of F. angustifolia samara morphological traits.

tree	weight of 100 seeds (g)	Samara length (mm)				Samara width (mm)				Seed length (mm)				Seed and samara length ratio			
		Mean	Min	Max	CV (%)	Mean	Min	Max	CV (%)	Mean	Min	Max	CV (%)	Mean	Min	Max	CV (%)
1	6.37	31.10d	25.5	41.5	7.99%	8.90g	8.0	11.5	7.19%	15.53bc	12.0	20.0	8.30%	0.50b	0.42	0.54	4.11%
2	6.18	30.86d	25.5	36.0	6.31%	7.14d	6.0	8.5	6.97%	15.12abc	11.5	17.5	7.36%	0.49b	0.40	0.53	4.43%
3	6.84	36.16f	27.5	40.0	6.79%	6.20b	5.0	7.0	7.07%	18.89ef	15.0	21.5	6.32%	0.52c	0.47	0.58	4.51%
4	4.5	26.94a	23.5	35.0	5.74%	5.79a	5.0	7.0	7.16%	14.85ab	13.0	19.0	6.13%	0.55ef	0.51	0.61	4.15%
5	8.01	35.14ef	25.5	43.0	10.07%	8.74g	6.0	11.0	10.24%	19.38f	14.5	24.0	9.68%	0.60ef	0.44	0.67	5.03%
6	6.58	33.72e	28.5	40.0	6.89%	6.75c	5.0	8.0	7.63%	19.19ef	12.5	22.5	9.27%	0.57g	0.41	0.67	7.28%
7	6.19	38.42g	31.0	44.5	7.80%	7.89e	6.0	19.0	16.08%	18.63e	15.0	22.5	7.40%	0.49b	0.43	0.55	5.25%
8	6.05	28.69bc	24.0	37.5	7.90%	5.88a	5.0	7.0	7.94%	15.57bc	13.5	18.5	5.59%	0.54de	0.41	0.60	4.82%
9	6.1	29.16c	26.0	34.5	6.44%	6.48bc	5.5	8.0	7.23%	15.42bc	13.0	19.0	6.60%	0.53cd	0.47	0.59	4.72%
10	6.26	30.80d	24.0	37.0	7.55%	7.72e	6.0	9.0	7.30%	17.00d	14.0	28.5	10.17%	0.55ef	0.49	0.97	9.28%
11	6.13	29.34c	20.5	35.0	7.67%	7.86e	6.5	9.0	6.16%	17.16d	14.0	20.0	6.70%	0.59h	0.51	0.71	5.63%
12	8.19	40.28hi	27.5	48.0	6.94%	7.93e	7.0	9.0	5.82%	18.48e	15.5	21.5	7.35%	0.46a	0.39	0.60	6.79%
13	5.9	27.72ab	23.0	43.5	10.72%	7.87e	6.5	9.0	6.23%	14.53a	12.5	23.0	11.66%	0.53c	0.48	0.59	4.36%
14	6.33	28.52bc	23.0	33.5	8.13%	7.98e	6.5	9.0	6.94%	15.65c	12.0	23.0	13.59%	0.55e	0.47	0.74	7.56%
15	7.86	41.42i	37.0	48.0	6.02%	8.35f	7.5	9.5	5.42%	22.74h	20.5	26.0	5.78%	0.55e	0.51	0.59	3.30%
16	12.95	49.31j	39.5	57.0	6.72%	8.32f	7.0	9.5	5.44%	26.17i	21.5	30.0	6.19%	0.53cd	0.48	0.58	3.53%
17	6.17	34.40ef	30.0	38.5	5.54%	9.84h	8.5	11.0	5.36%	21.24g	18.5	24.5	5.24%	0.62i	0.56	0.68	3.95%
18	7.97	38.21g	31.5	47.5	7.69%	8.98g	7.5	10.0	6.97%	22.64h	20.0	25.5	6.04%	0.59h	0.52	0.66	4.84%
19	7.17	35.89f	29.0	41.5	7.22%	7.86e	7.0	9.0	6.14%	21.17g	18.0	24.5	7.41%	0.59h	0.46	0.67	5.78%
20	8.13	39.81h	32.5	47.5	7.86%	8.42f	7.0	9.5	6.84%	22.54h	17.0	28.0	9.35%	0.57fg	0.51	0.61	4.01%
tot	139.88	34.28	20.5	57.0	18.09%	7.74	5.0	19.0	15.62%	18.59	11.5	30.0	18.97%	0.55	0.39	0.97	8.92%
avg	6.99	34.29	27.7	41.5	7.40%	7.74	6.4	9.5	7.31%	18.59	15.2	23.0	7.81%	0.55	0.47	0.64	5.17%

*Means followed by the same letter in the same row are not significantly different at $p > 0.05$ according to Tukey's test.

Table 2. The analysis of variance (one-way ANOVA) for the analyzed morphometric traits.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
wing length	63027.1	19	3317.21	504.04	0.0000
wing width	2221.9	19	116.9	330.79	0.0000
seed length	20580.4	19	1083.2	498.97	0.0000
seed and samara ratio	3.00543	19	0.15818	184.5	0.0000

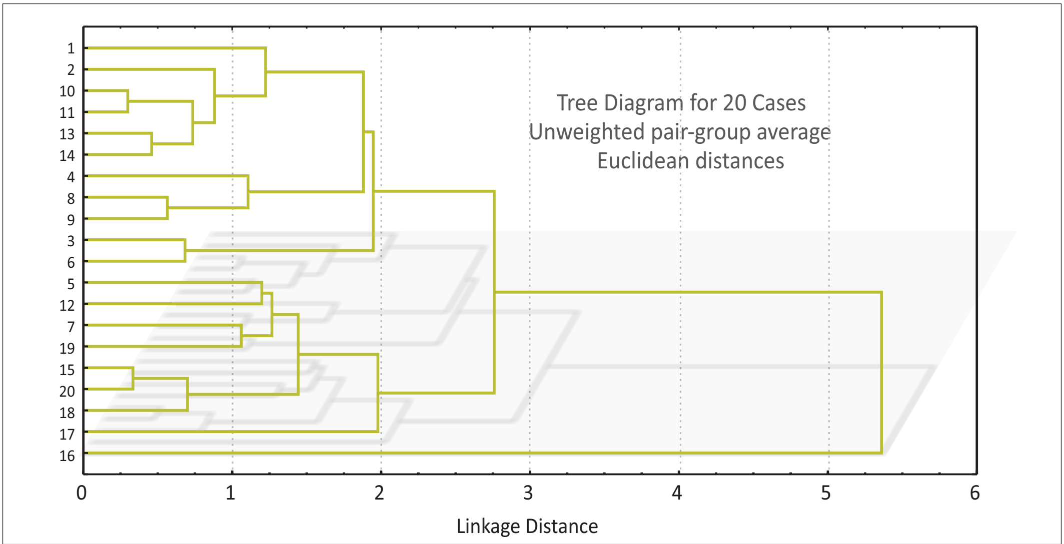

Figure 3. UPGMA dendrogram obtained by analysis of *F. angustifolia* samaras

The Pearson coefficient of correlation shows positive correlation between all morphometric traits at the samara level, which are statistically significant at the 95.0% confidence level (p<0.05). Correlation was not found between the dimensions and shape traits.

Based on variability results of samara shape, we can see that the highest number of samara (61%) has elliptic shape with a narrowed top, while 37.8% of samaras have an elliptic shape with an extended top. Regarding the samara top shape it has been recorded that most of samaras had a whole top (54.9%), while 31.6% of analyzed samaras had a tapered top. The smallest percentage of samara (8.95%) had a top like a beak. In total, 12 individuals had elliptic samaras with a narrow top, while 8 individuals had samaras with an extended top. If we look at the top shape of samara, dominant were samaras with the oval top (54.9%), then with the tapered top (36.1%), while only three samaras had an oval top like a beak.

Table 3. Pearson correlations between samara morphometric traits (***p<0,05).

	samara length	samara width	seed length
samara length	-	0.4158***	0.8697***
samara width	0.4158***	-	0.4815***
seed length	0.8697***	0.4815***	-

Table 4. Samara shape analysis

tree	Samara shape (%)		Samara top shape (%)			
type	1	2	1	2	3	4
1	1	99	74	8	18	0
2	41	59	69	25	6	0
3	100	0	0	100	0	0
4	99	1	95	1	4	0
5	78	22	81	11	6	2
6	92	8	25	75	0	0
7	98	2	5	92	2	1
8	17	83	80	17	3	0
9	42	58	68	29	3	0
10	0	100	0	0	100	0
11	56	44	97	2	1	0
12	100	0	0	100	0	0
13	15	85	83	1	16	0
14	4	96	83	0	17	0
15	95	5	73	27	0	0
16	100	0	1	99	0	0
17	5	95	100	0	0	0
18	100	0	42	58	0	0
19	100	0	64	36	0	0
20	100	0	59	41	0	0
∑	1234	757	1099	722	176	3
	61.70%	37.80%	54.90%	36.10%	8.80%	0.15%

*samara shape – (1)elliptic with narrow top and (2)elliptic with extended top; samara top shape – (1) whole oval and (2) tapered top, and (3) oval and (4) tapered top like a beak

DISCUSSION

High variability of *F. angustifolia* samara was previously recorded, such as in studies of *Stilinović (1985), Ivetić (2012), Jarni (2012), Jovanović (2000). Stilinović (1985) and Ivetić (2012)*, who recorded that *F. angustifolia* samara length could be in the range from 25 mm to 40 mm, while in Slovenia at several localities *Jarni (2009)* a samara length of 38.8 mm was recorded. On average, our results (26.94 - 49.01 mm) match up with these results. The highest deviation was noticed within test tree 16, with an average samara length of 49.01 mm and with a maximum samara length of 57.0 mm, which is out of the mentioned ranges from other studies. This test tree was also distinguished within the highest length of seed measure (26.17 mm). However, the average values were in the range of 14.53-26.17 mm, 18.59 mm on average, which represent about half of the whole samara length (ratio value about 0.5). The same test tree also had the highest weight of 100 samaras, valued at 12.95 g.

Regarding the samara width *Stilinović (1985)* and *Ivetić (2012)* mentioned a samara width of 6-7 mm, while *Jarni (2009)* recorded a width of 7.8 mm. Compared these values, we could say that our results, which show average values in range of 5.79-9.84 mm have higher values. But, if we look at the average value of samara width at the level of all studied individuals (7.74 mm), our results are close to results that *Jarni (2009)* recorded in Slovenian populations.

Samara size classification distinguishes individuals with a small samara about 1-1.5 cm (f. *leptocarpa*) and individuals with a large size samara that could be 5.5 cm in length and about 1 cm in width (f. *macrocarpa*) (*Jovanović, 2000*). According to this classification we could say that our samples could belong to these large size samaras type known as f. *macrocarpa*.

Samara shape analysis showed that the highest percentage of the analyzed individuals (61.70%) have an elliptic samara with a narrowed top. Only six individuals (tree 3, 12, 16, 18, 19, 20) had all sampled samaras with an elliptic shape and a narrowed top. However, just one individual (tree 10) had a completely elliptic samara shape with an extended top, while other individuals had mixed samara shapes. Regarding the samara top shape results, we could notice great variability of samaras shapes within each individual. There were only four individuals with uniform samara shapes (tree 17 – oval samara top, tree 3 and 12 –tapered top and tree 10 – tapered top like a beak). In total, the most represented was the shape with oval top (54.90%), then with tapered top (36.10%), while only three samaras, from a total 2000 analyzed samaras, had a tapered shape top like a beak. *Ivetić (2012)* noticed that *F. angustifolia* samara top shape could be oval, tapered and sometimes like a beak. Samara shape classification (*Jovanović, 2000*) recognized four species forms: f. *obtusa* – long wing with a rounded top; f. *emarginata* – with an extended top of the wing; f. *acuta* - elliptically narrowed with a gradually tapered top; f. *rostrata* - tapered top like a beak. According to this classification and our results, most of our studied individuals belong to the f. *obtuse*, but considering that there is no uniformity within most of the studied individuals, we could conclude that samara variability is at a high level, but we cannot classify individuals for sure.

Anyway, the obtained results confirmed considerable variability within the studied individuals at the level of samara morphometric traits. Considering that all sampled individuals were from the same populations, we could suppose that this variability was not induced by different environmental factors (climate or soil), but by the existence of specific genotypes. Therefore, it is crucial, for the conservation and restoration programmes, to maintain the existing variability of the given species in its natural stands and especially to maintain the adaptive variation that improves the fitting of the species and defines their adaptation/survival under various environmental conditions (*Papi et al., 2011; Maksimović et al., 2014*). By prescribing appropriate conservation measures, we will neutralize losses and preserve the specific gene pool (*Čortan et al., 2015*) of this species.

CONCLUSION

Based on the results of the morphometric variability research of *Fraxinus angustifolia* Vahl samaras, from Karapandža locality, we could see that:

- The results indicate the existence of considerable variability between the selected individuals, which was statistically significant for all morphometric traits ($p < 0.05$);
- Considering that these are individuals from a single population, we suppose that this variability was not influenced by different environmental factors, but by specific genotypes;
- The Pearson coefficient of correlation shows positive correlation between all morphometric traits at the samara level, which are statistically significant ($p<0.05$), while correlation was not shown between the dimensions and shape traits;
- In total: Samara length ranged from 22.7 to 41.5 mm; Samara width from 6.4 to 9.5 mm; Seed length from 15.2 – 23.0 mm; The weight of 100 seeds ranged from 4.5 to 12.95 grams;
- Individual number 16 was the one that stood out by its dimensions, which showed the highest values;
- According to seed dimensions traits, it can be concluded that these individuals belong to f. *macrocarpa*;
- According to seed shape, it could be classified as f. *obtuse*, but considering high samara variability within one individual we cannot classify individuals by their shape for sure;
- These results should be further combined with broader genetic analyses, so that we can get a complete picture of the studied population and the specific genotype structure of the species. Having all this in mind, we could go further with directed conservation and sustainable use of the available gene pool and further breeding of this endangered species.

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