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INTER AND INTRA-POPULATION VARIATION OF LEAF STOMATAL TRAITS OF *QUERCUS ROBUR* L. IN NORTHERN SERBIA

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Abstract – The research involved the examination of inter- and intra-population variation of stomatal traits: stomatal density, stomatal length and width, stomatal pore surface, potential conductance index and stomatal shape coefficient, in *Quercus robur* L. leaves. The research was conducted in northern Serbia and included five populations ("Ada Ciganlija", "Bojčinska šuma", "Subotica", "Sombor" and "Vršac"). The stomatal characteristics were examined in fully expanded leaves, from two leaf positions - the sun-exposed and shaded side of the tree. The leaf position in the tree crown, forming a part of the phenotypic variance, was relevant for the stomatal dimension traits. Within populations, the differences between the genotypes (i.e. trees), were relevant for all analyzed traits. On the basis of the analysis of the inter-populational differences, the "Bojčinska šuma" population had a statistically significantly lower stomatal density in comparison to the other populations.

Key words: Stomatal density, stomata pore surface, potential conductance index, *Quercus robur* L.

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INTRODUCTION

Plant anatomical traits are good indicators of habitat quality, since they manifest variability in relation to microclimatic conditions (Barber et al., 2004). Both stomata (the tiny plant structures on the epidermis which form the main system that controls photosynthesis and respiration) and stomatal traits (stomatal density, stomatal apparatus and guard cell architecture) respond to environmental and physiological cues (Nadeau and Sack, 2002; Gitz and Baker, 2009). According to numerous studies, the stomatal traits are very sensitive to alteration of light conditions; more precisely, the stomatal density decreases with decreasing light intensity (Cao and Booth, 2001; Baltzer and Thomas, 2005; Hovenden and Vander Schoor, 2006; Avramov et al., 2007).

The leaf stomatal traits of Pedunculate Oak in the area of Serbia have been studied by a small number of researchers (Mitrović et al., 1997; Nikolić et al., 2005; Batos et al., 2006). The results of the studies produced in this paper present the first research into Pedunculate Oak stomata from natural populations in Serbia. Most researches of the Serbian Pedunculate Oak are in the areas of systematics, forest silviculture and forest production, (Jovančević, 1966; Jovanović, 1971; Erdeši, 1985; Gajić and Tešić, 1992; Nikolić et al., 2006; Bobinac, 2007; Kovačević and Orlović, 2007).

In the present study we have assessed the stomatal differences between the sun-exposed and shaded leaves of *Quercus robur* from five populations in northern Serbia. We have attempted to evaluate the differences of stomatal characteristics between and within five populations in northern Serbia.

MATERIALS AND METHODS

Study area

While the Pedunculate Oak (*Quercus robur* L.) is one of the most esteemed woody species, the area it covers has considerably decreased in the last century (Yakovlev and Kleinschmidt, 2002; Thomas et al., 2003; Hinkov et al., 2005; Balboa-Murias et al., 2006; Kutnar, 2006; Kovačević and Orlović, 2007; Helama et al., 2009). It occurs throughout the European area, except for Spain, central Portugal and the northern parts of Scandinavia. Ecologically, the species is adapted to both continental-forest and forest-steppe climates and sub-Mediterranean and Mediterranean in the south. However, the Pedunculate Oak is not that flexible when it comes to soil, requiring deep and fertile soils which are influenced by underground waters and occasionally flooded. The largest areas covered by Pedunculate Oak forests in Serbia are in the valleys of the Sava (Srem being the area with the best quality Pedunculate Oak forests), Danube and Morava rivers.

The analyzed populations are situated in the localities in Serbia with the largest representation of Pedunculate Oak, and differ between themselves in relation to habitat and environmental conditions. Two populations are located in the surrounding area of Belgrade (Central Serbia: Ada Ciganlija - AC (N 44˚48', E 20˚24') and Bojčinska šuma - BS (N 44˚43', E 20˚10')) and three are located in the area of Vojvodina (western Serbia Sombor - SO (N 45˚46', E 18˚56'), the north - Subotica - SU (N 45˚46', E 18˚56'), and the east - Vršac - VR (N 45˚07', E 21˚25') (Fig.1). These are areas of continental/moderate continental climate, with no large differences in altitude, and with autochthonous Pedunculate Oak forests (except for the artificially planted culture at the Subotica locality) of an average tree age of 80 years, a different pedological substratum (silty loam) and different geological stratum (silty sediments, lessoid, lessoid clay, crystal shale).

Study species

The Pedunculate Oak belongs to the species-rich and significant genus *Quercus* within the family *Fagaceae* (Nixon, 1993). It is a long-lived, tall deciduous tree, with a branchy crown, deeply cracked bark and a large, morphologically, highly irregular, leaf. A considerable intra-population variation is a result of the wide area this species covers as well as the occurrence of spontaneous hybridization with the Sessile Oak (*Quercus petraea* L.), which greatly hinders its determination. *Quercus robur* L. is important in forestry, ecology, medicine, pharmacy etc. (Orlović et al., 1999; Kovačević and Orlović., 2007; Rakić et al., 2006; Rakić et al., 2007).

Foliar analyses

The leaf sampling was conducted in September of the same year in the afternoon, on a day without wind and precipitation. From the south/south-west side of the crown, at a height of between 3 -5m; several twigs in the first phase of growth were taken from the outer (exposed position) and inner (shade position) part of the crown (Brewer, 1992; Beerling et al., 1993; Bruschi et al., 2000; Chen et al., 2001; Lin et al., 2001). Leaf conservation was carried out in a laboratory under identical conditions; a sudden or extended dark or a high temperature and strong wind can close the stomata or influence their degree of openness (Brewer, 1992). Fresh and dried leaf imprints were taken by employing a thin collodion film fast fixation method from the abaxial leaf side, central part, along the main leaf nerve (5 cm/1 cm), in the direction from the petiole to the leaf top (Sun et al., 2003). In this manner prepared permanent preparations were utilized for the analysis of stomata variation parameters.

The size and number of stomata from the leaf imprints were determined by a light microscope with the Leica Galen III screen at magnification of 400X and 100X. Stomata counting and measuring was conducted on randomized visual areas at one epidermis imprint per plate. For each individual tree, five sun-exposed and five shade leaves were analyzed, totaling ten leaves per tree. Ten trees per population, i.e. locality, were examined.

Of the primary parameters, the ones that were analyzed were: stomatal density, the number of stomata per mm^{-2} leaf area (SD); the size of stomata guard cell, length (L_A) and width (W_B); the size of stomatal aperture length (L_a) and width (W_b) (Phelps et al., 1976; Sha Valli Khan and Hausman, 1999).

Fig. 1. The map of sampling populations of *Quercus robur* L. in northern Serbia *(www.melnica.com/slike/Srbija_karta.gif;) (*mod. Batos, 2008).

The following obtained parameters were estimated:

- stomatal pore surface of stomata guard cell (Balasooriya et al., 2009) :

$$SPS_{LAWB} = (L_A\ W_B\ \pi)/4],$$

- stomatal pore surface, the surface area of a widely opened stomatal pore, μm^2 (Balasooriya et al., 2009):

- $SPS_{LaWb} = (L_a\ W_b\ \pi)/4$,

- stomatal shape coefficient (SSC)

$$SSC = 100\ W_b / L_a,$$

- potential conductance index, [=(guard cell length)^2 x stomatal density x 10^{-4}] (Holland and Richardson, 2009)

$$PCI = (L_A)^2 \text{ x SD x } 10^{-4}.$$

Data analysis

The statistical data processing was conducted by means of application of relevant programs - SAS 9.1 (SAS Institute, 2002) and STATISTIKA (Version 8.0) for Windows '95.

By utilization of the PROC MEANS procedure, descriptive statistic for the primary parameters were obtained – mean value ($\bar{x}$), standard error (SE) and the coefficient of variation (CV %). The analysis was carried out separately for each locality.

The estimation of statistical importance of the different level sources of phenotypic variance was conducted by analysis of variance (ANOVA). The localities were regarded fixed factors (being clearly defined), while a tree was regarded to be a random factor (since the selection of trees within the locality was randomized). The testing of the main variance effects was carried out by the F-test, by using RANDOM option in PROC GLM procedure. The Scheffe's test of arithmetic mean values comparison between the different localities was done in the same option.

For the analysis of factors in the total anatomical traits variation, more precisely, of stomatal density, a model with three phenotypic variance sources was utilized: locality, tree, position and interaction between the locality and the position. The results of this analysis describe the statistical importance of the following sources of phenotypic variance:

- locality - phenotypic variance conditioned by the environmental differences of localities
- tree (nested in a locality) – genetic differences within a locality,
- position – differences in branch positions (sun and shade),
- locality x position – differences in trait phenotypic values at certain position, depending on environment, that is locality

The trait phenotypic value in this model included:

$$X_{ijkl} = \mu + S_i + T_{ij} + P_{ijk} + L_{ijkl} +$$

where X_{ijkl} represents the leaf trait phenotypic value and μ the arithmetic mean value, the main effect. Irregularities that occurred were the result of the following effects: S_i the locality effect and ,T_{ij} the effect of the tree j within the locality i, the effect P of the branch k position on the tree j in the locality i, and finally, L_{ij} the effect of the leaf l at the position k within the tree j of the locality i and being the error.

The comparison of differences between the tree leaf positions (sun and shaded), within every population was carried out by utilization of the PROC TTEST option in SAS, while the comparison of average mean values between localities was done by Scheffe's test in PROC GLM option.

RESULTS

The differences between the leaf tree positions (sun-exposed and shaded) were specific for both individual populations and for each Pedunculate

Table 1. Descriptive statistics (mean, standard error (SE) and coefficients of variation (CV %)) for each measured stomatal trait in five *Quercus robur* L. populations in northern Serbia. The trait acronyms are defined in Material and Methods.

Population	position		L_A	W_B	L_a	W_b	SSC	SPS_{LAWB}	SPS_{LaWb}	SD	PCI
Ada ciganlija	sun	Mean	25.51	20.53	14.84	4.23	81.50	413.18	49.80	352.45	23.27
		SE	0.12	0.08	0.09	0.04	0.42	2.88	0.57	6.81	0.59
		CV%	12.94	10.25	17.46	22.80	14.16	19.10	31.08	23.66	31.17
	shaded	Mean	25.60	20.42	14.74	3.88	80.94	411.32	45.16	352.69	23.78
		SE	0.11	0.07	0.09	0.04	0.46	2.52	0.55	5.96	0.53
		CV%	12.18	9.69	17.20	26.51	15.58	16.76	33.33	20.68	27.40
Bojcinska suma	sun	Mean	26.58	20.15	15.01	3.77	76.57	422.24	44.45	255.71	18.79
		SE	0.11	0.07	0.09	0.04	0.37	2.74	0.54	4.18	0.42
		CV%	11.59	10.07	16.06	27.57	13.16	17.76	33.06	20.04	27.63
	shaded	Mean	26.31	20.08	14.75	3.42	76.99	416.59	39.71	256.93	17.74
		SE	0.11	0.07	0.09	0.04	0.35	2.69	0.52	4.76	0.41
		CV%	11.33	10.18	16.54	31.14	12.60	17.66	35.65	22.67	28.22
Sombor	sun	Mean	24.91	19.90	14.07	4.14	80.56	391.10	46.38	337.52	21.63
		SE	0.10	0.07	0.08	0.03	0.36	2.53	0.54	6.45	0.61
		CV%	11.29	9.99	16.44	21.58	12.11	17.71	31.90	23.39	34.29
	shaded	Mean	24.17	19.14	13.48	3.64	80.06	367.15	39.88	339.04	20.49
		SE	0.13	0.09	0.11	0.04	0.38	3.11	0.57	7.36	0.61
		CV%	14.32	12.37	22.51	27.80	12.97	23.21	39.17	26.58	36.73
Subotica	sun	Mean	26.12	20.17	15.00	4.12	77.93	415.58	48.86	325.12	22.36
		SE	0.11	0.06	0.09	0.03	0.33	2.60	0.49	5.60	0.52
		CV%	11.52	8.77	16.09	19.07	11.50	17.14	27.31	21.08	28.30
	shaded	Mean	26.41	19.85	15.19	3.56	75.90	413.32	42.89	343.81	24.17
		SE	0.11	0.07	0.09	0.03	0.35	2.62	0.52	6.62	0.58
		CV%	11.59	9.40	17.00	25.20	12.78	17.37	33.06	23.56	29.22
Vrsac	sun	Mean	26.11	20.47	15.12	4.16	79.12	421.34	49.89	343.95	22.92
		SE	0.11	0.07	0.09	0.04	0.34	2.64	0.56	5.48	0.50
		CV%	11.29	9.04	16.52	23.05	11.90	17.19	30.76	19.50	26.46
	shaded	Mean	25.88	19.75	14.93	3.70	77.15	403.29	44.16	321.09	20.97
		SE	0.11	0.08	0.09	0.03	0.39	2.80	0.57	4.77	0.49
		CV%	12.10	10.46	16.84	25.48	13.84	19.04	35.57	18.18	28.71

Oak *(Quercus robur* L.) leaf stomatal apparatus trait (Table 1). In fact, most of the analyzed statistically important stomatal apparatus traits possessed higher mean values in the sun-exposed leaves in comparison to shade leaves.

According to the t-test results (Satterthwaite method), the arithmetic mean values of stomata width trait (W_b) (see Fig. 2) were significantly higher in the sun-exposed leaves in comparison to shade leaves in all populations (t-value = 6.89 for

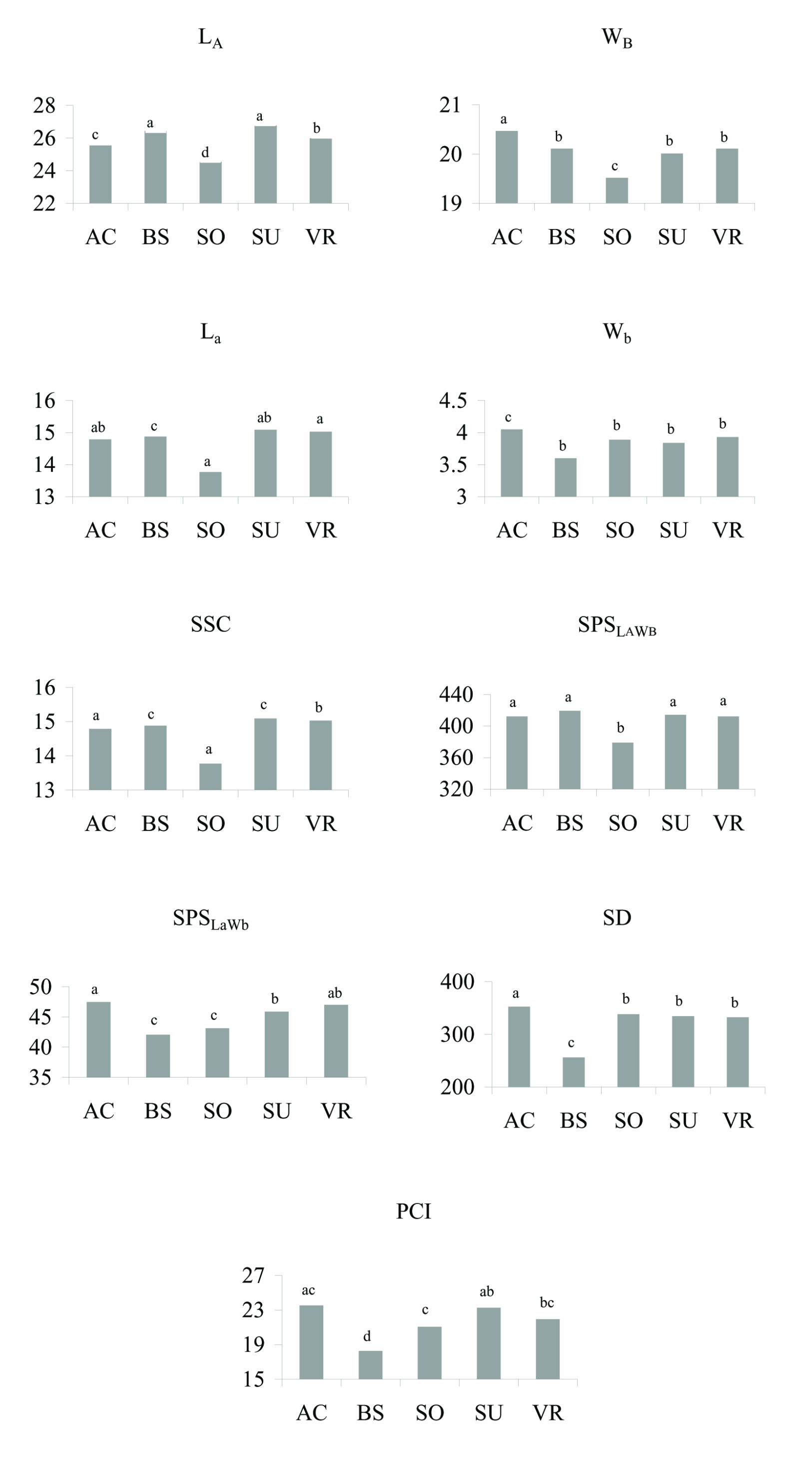

Fig. 2. The histogram presents the mean values of nine stomatal apparatus traits of a *Quercus robur* L. leaf in five populations in northern Serbia ("Ada Ciganlija" (AC), "Bojcinska šuma" (BS), "Sombor" (SO), "Subotica" (SU) and "Vršac" (VR)), with the data pooled for individual trees. The values of the different letters are significantly different at the 0.05 probability level according to the results of the Scheffe's test. The trait acronyms are defined in Material and Methods.

Table 2. *F*-statistics for eight stomatal apparatus traits in the *Quercus robur* L leaf from a nested-crossed analysis of variance. The trait acronyms are defined in Materials and Methods.

Source of variation	df	SD	PCI	SSC	L_A	W_B	L_a	W_b	SPS_{LAWB}	SPS_{LaWb}
Population	4	6.07***	4.25**	5.16**	4.05**	1.47	2.49	1.61	2.36	1.49
Position	1	0.05	1.4	0.01	6.76**	87.69**	12.28**	479.48**	43.90****	304.55****
Tree (Population)	45	27.94****	9.47****	30.72****	25.65****	35.60****	32.24****	34.43****	36.07****	30.79****
Population x position	4	5.13***	5.05****	6.01****	7.02****	12.14****	5.57****	4.29****	8.26****	1.3

* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

AC, t-value = 6.31 for BS, t-value = 10.23 for SO, t-value = 12.85 for SU and t-value = 9.33 for VR; all df = 1498 and all p = 0.0001). Accordingly, the SPS_{LaWb} also statistically significantly differed within each of the analyzed populations (t-value = 5.89 for AC, t-value = 6.36 for BS, t-value = 8.28 for SO, t-value = 8.40 for SU and t-value = 7.13 for VR; all df = 1498 and all p = 0.0001). In the Sombor population, along with the above-mentioned traits, the traits that significantly differed in relation to the leaf position were: the length of the stomata guard cell (L_A) and width (W_B) (t-value = 4.59, and t-value = 6.72, respectively), and SPS_{LAWB} (t-value = 5.97).

The Subotica and Vršac populations possessed the highest number of stomatal traits which statistically showed significant differences between the different leaf tree positions. Consequently, apart from the important differences mentioned above, both populations showed a significant difference in the trait width of the stomata guard cell (W_B) (t-value = 3.47 for SU, and t-value = 7.13 for VR), SSC (t-value = 4.21 for SU, and t-value = 3.78 for VR), and SPS_{LAWB} (t-value = 4.68 only for VR).

With respect to the SD and PCI traits, only the following populations showed significant difference among the leaf tree positions: SD t-value = 2.16 for SU and t-value = 3.15 for VR; PCI t-value = 2.34 for SU and t-value = 2.78 for VR. They indicated a reverse trend; in the Sombor population the SD was higher for the shade leaves, while in the Vršac population the opposite trend was recorded – the mean value was higher in the sun-exposed leaves. Other populations did not show significant differences in SD mean values for the different leaf positions.

The least individual variation quantified by the variance coefficient (CV%) possessed the W_B trait (ranging from 8.77% to 12.37%) in all populations. With respect to the variance coefficient range, the highest variation was obtained for the SPS_{LaWb} trait (ranging from 27.31% to 39.17%) (Table 2).

Three-way ANOVA results concerning the nine traits of the *Quercus robur* L. leaf stomatal apparatus are presented in Table 2. The share of phenotypic stomatal trait variation conditioned by the environmental differences among populations was relevant for the L_A, SD and PCI traits. The leaf position in a tree crown as a part of the assessed phenotypic variation was relevant for stomata dimension traits (L_A,W_B, L_a, L_b, SPS_{LAWB} i SPS_{LaWb}). Within populations, the differences among the genotypes (i.e. trees) were relevant for all traits. Differences among trees with respect to all measured traits were statistically highly significant and presented by one-way ANOVA tests (all $P <$ 0.0001), which confirmed the intra-population variation of the analyzed stomatal traits. Also, a statistically significant interaction between a position and locality was confirmed for all analyzed stomatal traits, (except for SPS_{LaWb}).

The mean values of the analyzed stomatal traits in the five populations are presented in Fig. 2. The difference between the populations depended on the traits. Consequently, on the basis of the results of Scheff's test, the Sombor population had the lowest values of L_A,W_B, L_a, and SSC traits. Bojčinska šuma had the lowest values for SPS_{LaWb}, SD and PCI in comparison to the other populations. A statistically significant interaction between position and locality of all the analyzed stomatal traits (except for SPS_{LaWb}) was confirmed.

DISCUSSION

The heterogeneity of external factors (light, humidity, temperature and other) presents an external factor which is a generator of intra-individual variation and directly influences leaf traits (Balasooriya et al. 2009). Stomata are one of the key regulation factors of the relation between plant and external environment. Stomatal traits are characteristics of the species, although certain variation can present a response to micro-environmental conditions and global ecosystem changes (Brewer, 1992; Malone et al., 1993; Bruschi et al., 2000; Van Hoof et al., 2006). The differences between sun-exposed and shade positioned leaves present a plant adaptation aiming at the best possible exploitation of available light intensity. By observing a tree crown, one can define two micro-climatic sites. More precisely, the outer crown circle presents a more stressful environment for leaves in comparison to the inner part, being more exposed to greater temperature variations, sun rays, pests (Bruchi et al., 2003).

We found a high degree of inter- and intra-population variation among the populations of *Quercus robur* L in Serbia with respect to the examined stomatal apparatus traits. A significant population effect and a confirmation of an inter-population variation was obtained for the SD, PCI, i SSC i L_A traits (Table 2). Additionally, a significant tree nested in a population effect was obtained for all stomatal apparatus traits, which presented a confirmation of genetic variation and intra-population variation (Table 2). The obtained results concerning the stomatal traits variation in relation to the leaf position (sun-exposed and shaded) in a tree crown, proved that the position has no statistically significant influence on SD, PCI, and SSC traits, while a statistically relevant position influence was obtained for the traits concerning stomatal apparatus dimension - L_A,W_B L_a,W_b, SPS_{LAWB} and SPS_{LaWb}. The populations differed in the number of traits which indicated significant statistical differences among the leaf positions. The Subotica and Vršac populations possessed the largest number of stomatal traits showing statistically significant differences in relation to a leaf tree position. A statistically relevant interaction between the position and locality for all analyzed stomatal traits was confirmed (except for SPS_{LaWb}). The research by Bruchi et al., 2003 confirmed an inter-population variation with regard to stomatal density, stomatal apparatus length and width, and the surface area of a stomatal aperture on a *Quercus petraea* leaf in natural populations, as well as intra-population variation for all traits, apart from the surface area of the stomatal aperture, for which a significant leaf position effect was confirmed. By comparison of the species of *Quercus.sp* genus (*Quercus coccinea*, *Quercus rubra* i *Quercus velutina*), a significant difference among positions in relation to stomatal density was obtained, with the higher stomatal density mean value recorded in the sun-exposed leaves. The stomatal length of a *Quercus coccinea* leaf was significantly smaller in comparison to the other two species, but this trait did not significantly differ for any of the species in relation to a leaf position, that is, in relation to the light intensity (Ashton and Berlyn, 1994).

The majority of studies confirm that a sun-exposed leaf, which develops under thenstronger influence of heat and light intensity, has a larger number of stomata (Abrams, 1987; Abrams, 1990; Beerling et al., 1993; Onwueme and Johnston, 2000; Sun et al., 2003), although there are some studies according to which a sun-exposed leaf has a lower stomatal density and a greater guard cell length in comparison to a shade leaf (Bruschi et al., 2003). Results of three-way ANOVA confirm that there is no statistically significant difference for SD, PCI

Table 3. Some examples of Pedunculate Oak (*Quercus sp*.) leaf stomatal density and dimensions from recent literature, and the results of the present study analysis of Pedunculate Oak (*Quercus robur* L.) leaf stomatal traits from five populations in northern Serbia.

		SD (number of stomata per mm^{-2})		stomatal dimension (μm)			
				length		width	
	Species	sun	sheded	sun	sheded	sun	sheded
Abrams, 1987. Kansas USA	Quercus macrocarpa	818		20.1			
Abrams, 1987. Kansas USA	Quercus muehlenbergii	986		20.7			
Abrams, 1990. Wisconsin USA	Quercus ellipsoidalis	498	443	15.3	13.3		
Abrams, 1990. Wisconsin USA	Quercus macrocarpa	543	506	19.5	17.7		
Abrams, 1990. Wisconsin USA	Quercus velutina	540	429	13.2	12.4		
Abrams, 1990. Wisconsin USA	Quercus alba	547	402	16.5	16.1		
Abrams, 1990. Wisconsin USA	Quercus rubra	571	338	14.1	13.4		
Bruschi i sar., 2000. Italija	Quercus petraea Matt.	394.3	355.1	24.3	24.6	19.5	19.3
Mitrović i sar., 1997. Srbija	Quercus robur L.	133.7-148.5					
Naujoks i sar., 1995. Nemačka	Quercus robur L.			18.2-20.1			
Nikolić i sar., 2005. Srbija	Quercus robur L.	530-791		24.7-27.6		17.8-20.7	
Nobrega and Pereira 1992. Portugal	Quercus suber L.	355-648	397-671	22.1-22.6	20.0-22.9	15.5-16.2	15.3-15.8
Osborn i Taylor, 1990. S. Amerika	Quercus velutina Lam.	528	471				
Phelps, 1976. Missouri USA	Quercus rubra L.	355					
Phelps, 1976. Missouri USA	Quercus alba L.	302					
Phelps, 1976. Missouri USA	Quercus velutina L.	325					
Sha Valli Khan i sar., 1999. Luxenbo	Quercus robur L.	800		16-18			
present study 2008. Serbia	Quercus robur L.	257.2-352.4	262.3-352.7	24.9-26.6	24.2-26.4	19.9-20.6	19.1-20.4

and SSC, although in the majority of analyzed trees in all localities the stomatal density of sun-exposed leaves was lower than the stomatal density of shade leaves (Table 2). The dimensions of sun-exposed leaf stomata were larger, which confirmed the results of previous studies of *Quercus robur* L (Table 3), and a statistically significant difference was obtained in that respect (Table 2). According to Dirk et al., 2006 the lower stomatal density was compensated by an increased stomatal aperture and conversely, increased stomatal density was compensated by reduced stomatal aperture.

In most of the analyzed trees in all localities, except for the Vršac locality, the stomatal density of the sun-exposed leaves was lower than the stomatal density of shade leaves. The detailed analysis of the Cork Oak (*Quercus suber)* stomatal traits from the area of Portugal did not confirm significant differences in density and stomatal dimension, neither in leaf crown position nor between the sun-exposed and shade leaves (Nobrega and Pereira, 1992). A slightly higher stomatal density and guard-cell length occurred in the upper part of the crown and in the sun-exposed leaves. The authors see the cause of this in the position of analyzed trees in the open, as well as in the low influence of shade. A considerably larger difference between the stomata of sun-exposed and shade leaves is stated by Osborn et al. (1990) in *Quercus velutina* in natural populations in the North American area. The same authors state that, with respect to leaf position, there was no difference in the size of guard cells. Contrary to these results, Bruschi et al. (2003) state that in *Quercus petraea* from the Italian area, a sun-exposed leaf had a lower stomatal density and larger guard-cell length in comparison to a shade leaf (Table 3). The *Quercus rubra* tree populations, exposed to different light intensities in the course of the vegetation season, did not statistically significantly differ with respect to stomatal density traits or stomatal index (Daly and Gastaldo, 2010).

Although the research confirmed a high intra-individual variation, there are studies in which it is not considered as statistically relevant at this level of variation. However, the inter-population variation of the same species has a different leaf trait variation pattern (Bruchi et al., 2003). Owing to the great diversity in the results of previous researches, the above-mentioned authors suggest a better leaf sampling strategy – that the following data must be clearly defined in every research: branch position in the crown, leaf position in relation to light, the height of the branch from which leaf samples were taken and, especially, determination of the cardinal point from which samples were taken, since it strongly influences variance among species within the subgenus *Quercus*.

On the basis of the analysis of the inter-populational differences of the pooled data (since a relevant difference with regard to leaf position was not obtained), Bojčinska šuma had a statistically lower stomatal density in comparison to the other populations (Fig. 2). A statistically significantly lower number of stomata at this locality could be a result of species adaptation to increased air pollution, since the locality is situated in the proximity of the thermo electric power plant Nikola Tesla (Hetherington et al., 2003; Van Hoof et al., 2006; Wagner et al., 2005; Balasooriya et al., 2009). The stomatal density decrease presents an indicator of air pollution. For instance, 59% stomatal density decrease and 40% stomatal size increase were obtained for the species *Ficus religiosa* in polluted areas in comparison to non-polluted areas, while the results obtained for the species *Thevetia nerifolia* (Verma and Singh, 2006, according to Balasooriya et al., 2009) were opposite to the results stated by Balasooriya et al., 2009 for the species *Taraxacum officinalis*. Therefore, the analysis of stomatal traits cannot only be an indicator of intra- and inter-variation in relation to habitat. Still, such studies can contribute to identifying species which can serve as reliable indicators of alterations of environment quality with respect to air pollution. Thereby they can contribute towards timely actions aimed at preserving the habitat and biodiversity.

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