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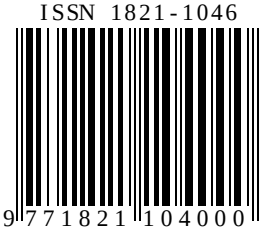

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EFFECT OF FERTILIZATION ON LEAF MORPHOMETRIC CHARACTERISTICS OF *Paulownia elongata* S. Y. Hu AND *Paulownia fortunei* Seem. Hemsl. IN THE SECOND YEAR OF GROWTH

Suzana MITROVIĆ[1], Milorad VESELINOVIĆ[1], Snežana STAJIĆ[1], Zoran PODUŠKA[1], Vanja STOJANOVIĆ[1], Natalija MOMIROVIĆ[1], Marija MILOSAVLJEVIĆ[2]*

Abstract: *This paper presents the results of an analysis of the effect of fertilization in the second year after planting on the morphological characteristics of Paulownia leaves. The results are continuation of the research and possibility of introduction and adaptation of paulownia to different habitats in Serbia. Obtaining of the results regarding quality of the plant leaves in the second year in relation to different fertilization treatments is significant for the technology of cultivation of this species on determined soil types. The research is carried out on two sites. Experimental fields on sites in Obrenovac and Pambukovica have been established with species Paulownia elongata S. Y. Hu. and Paulownia fortunei Seem. Hemsl.. Collecting of leaf material for the analysis in laboratory conditions was carried out within the experimental fields. Measuring of the following morphometric characteristics of leaves were carried out: leaf area, leaf circumference, leaf blade length, central veine length, leaf width on the widest part of leaf blade, leaf width at 1 cm from the leaf base, petiole length, distance between 3rd and 4th nerve, number of nerves on the left side of the central veine, and number of nerves on the right side of the central veine. The obtained results of morphometric measurings of leaves are statistically processed in the program Statgraphics. Morphometric analysis of leaves shows structural-functional connections, i.e. more detailed indicators of the adaptability of the species. Based on these measuring results it was determined that fertilization in the second year of plant development after planting has a positive effect on the size of leaves of analysed species of paulownia.*

Keywords: fast-growing species, adaptability of species, plant growth and development, introduction of woody species, soil type.

UTICAJ ĐUBRENJA NA MORFOMETRIJSKE KARAKTERISTIKE LISTOVA VRSTA *Paulownia elongata* S. Y. Hu. I *Paulownia fortunei* Seem. Hemsl. U DRUGOJ GODINI RASTA

Sažetak: *U radu su prikazani rezultati analize uticaja prihranjivanja biljaka u drugoj godini nakon sadnje, na morfološke karakteristike listova. Ovde su prikazani rezultati*

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koji su nastavak istraživanja u okviru istraživanja mogućnosti introdukcije i adaptacije paulovnija na različita staništa u Srbiji. Dobijeni rezultati o kvalitetu listova biljaka u drugoj godini razvoja biljaka nakon sadnje u odnosu na na različite tretmane prihranjivanja su značajni za tehnologiju gajenja ove vrste na određenim tipovima zemljišta. Istraživanja su sprovedena na dva lokaliteta. Ogledna polja na lokalitetima u Obrenovcu i Pambukovici su osnovana sa vrstama Paulownia elongata S. Y. Hu. i Paulownia fortunei Seem. Hemsl.. U okviru oglednih polja vršeno je prikupljanje lisnog materijala za analizu u laboratorijskim uslovima. Izvršena su merenja morfometrijskih karakteristika listova: površina lista, obim lista, dužina lisne ploče, dužina centralnog nerva, širina lista na najširem delu lisne ploče, širina lista na 1 cm od osnove lista, dužina peteljke, razmak između 3. i 4. nerva, broj nerava na levoj strani od centralnog nerva, i broj nerava na desnoj strani od centralnog nerva. Dobijeni rezultati morfometrijskih merenja listova statistički su obrađeni u programu Statgraphics. Morfometrijska analiza listova pokazuje strukturno – funkcionalne veze, odnosno detaljnije pokazatelje adaptibilnost vrste. Na osnovu ovih rezultata merenja utvrđeno je da prihranjivanje u drugoj godini razvoja biljaka nakon sadnje ima pozitivan uticaj na veličinu listova analiziranih vrsta paulovnije.

Ključne reči: brzorstuće vrste, adaptabilnost vrsta, rast i razvoj biljaka, introdukcija drvenastih vrsta, tip zemljišta.

1. INTRODUCTION

Anthropogenic influences and the speed of change in the environmental conditions of habitat influence that not all autochthonous plant species can adapt to the new conditions (Aragao et al., 2008; Betts et al., 2008; Innes et al., 2009; Lavadinović et al., 2010). That leads to changes in their growth and development, their vitality, and in some cases to the loss of species in certain area. The efforts to preserve autochthonous species are object of many studies, but most often their survival in certain area does not guarantee that they still have the same characteristics and provide the same ecosystem services. That is why the selection of adequate species for some area represents the greatest challenge, since it is necessary to preserve the areas covered with forests, and also to carry out reforestation where they are lost. In this sense, many studies are carried out which deal with examining the possibility of introduction of different species and their adaptation to new environmental and climate changes. The confirmation of choice of certain species through different research processes is essential for success in trying to find the right solutions for reduction of negative effects caused by anthropogenic and climate changes.

Plantation forestry and energy plantations of fast-growing woody species are the response of today's society to the growing need for wood as a raw material and wood biomass as an energy source. This study aims to harness the potential of fast-growing species as a means of mitigating excessive forest exploitation through the introduction of new species for plantation establishment in non-forested or long-deforested areas. Also, the recultivation of areas degraded by various surface extractions of mineral and other raw materials may provide suitable sites for establishing such plantations, whose utilization can have both ecological and economic benefits.

Species of the genus *Paulownia* Sieb. & Zucc. represent a great potential in the area of their range for obtaining of biomass and biofuel due to their sprout power and rapid growth (Ivetić & Vilotić, 2014; Mishra et al., 2010; Lucas-Borja et al., 2011; Yadav et al., 2013). Leaves of young paulownia trees (in juvenile stage of development) have a wavy rim with pronounced lobes, they are extremely large and can reach a length of up to 90 cm. The fact that the size of the leaf is important for photosynthetic processes and production of nutrients is in direct correlation with production of entire biomass of plants. Leaf litter of this species has a pronounced meliorating role in improving quality of the soil around the tree thanks to the large leaf mass rich in nitrites. Because of these properties, the leaves are made into green manure which is used in some areas in China to improve soil-ecological properties of the soil. Dense hairs that cover both sides of leaves, as well as their size, contribute to the important role of crown in purification of air.

That is why this species was the subject of our research. The soils on which the afforestation is carried out are in most cases poor in nutrients, so it is necessary to add different fertilizers. Providing plants with the availability of necessary assimilatives should ensure formation of biologically healthy material, resistant to new environmental conditions (Jacobs et al., 2005; Mitrović et al., 2012; García-Morote et al. 2014). First of all, the choice of type, quantity and time of use of fertilizers depends on pedological condition, but also on biological properties (Óskarsson and Brynleyfsdóttir, 2009; Tucović and Simić, 2002; Güsewell et al., 2003; Hawkins et al., 2005). The aim of this paper was to determine how different fertilization treatments affect ten key leaf morphometric characteristics in the second year of development of the studied *Paulownia* species, using a modified protocol based on the Assessment of oak leaf morphology (Kremera et al., 2022).

2. MATERIAL AND METHODS

Experimental fields on the site in the village Veliko Polje near Obrenovac (site I) and in village Pambukovica near Ub (site II) – which have different orographic traits, climate conditions and physical-chemical characteristics of soil, were established by planting seedlings of two species of paulownia: *Paulownia elongata* and *Paulownia fortunei*.

The experimental field on site 1 is located in the village Veliko Polje, municipality Obrenovac on the left bank of the Kolubara River at an altitude of 74 m. The plot on which the experimental field is located is flat and has no slope. The rows of plants are oriented in the northwest-southeast direction. The experimental field on site II is located in the village Pambukovica near Ub and it belongs to Kolubara District in Tamnava microregion. The experimental area is located on the hill Jastrebovac, municipality Ub. The plot on which the experiment is located is undulating, whereby the lowest point is at 162.60 m, and the highest at 176.11 m above sea level. The relief of this area is flat and hilly with small differences in altitude. The plot has south-eastern exposure for the most part and north-western exposure on one smaller part. It is surrounded by beech and oak forests. The rows with plants are oriented in the direction north-south.

The seedlings were produced from the seeds which had been collected from well adapted genotypes of two species of paulownia, from the experimental field in

Bela Crkva. The seedlings were produced in containers and used as a starting material in establishing experimental plantations.

The seedlings were planted in rows, at the distance of 4x4 m. In each row, 25 plants were planted, out of which in 12 rows seedlings of the species *Paulownia elongata* and in 12 rows of the species *Paulownia fortunei*. The seedlings were planted manually in dug pits 30x30 cm in 12 rows each.

Due to heavy mechanical composition and acidic reaction of the soil, chicken manure – Fertor (http://www.mrf-garden.com) produced from 100% chicken manure which decomposes well in contact with water was used on the site in Obrenovac (I) and in Pambukovica (II). Other organic matters of plant origin were added to it, in order to increase the nutritional value of the fertilizer. This is a type of slow-release fertilizer where one part of the macroelements and microelements is easily accessible and immediately available to plants, while the other part is gradually released.

During planting, each of the sample plots was divided in six treatments (for each of the treatments 4 rows of 25 plants), which differ by the quantity of added fertilizer (Fertor) and control without fertilization. Fertilizer was added to plants in quantities of 240 gr per plant (T1) and 120 gr per plant (T2). On control areas (T3) fertilization of plants was not carried out.

Collection of the leaf material in the field was carried out using the sample method at the end of the second growing season, whereby the leaves were collected from the same part of the crown, i.e. from the same nodes. The collected leaf material was herbarized and scanned (Figures 1 and 2). Measurements were conducted using AutoCAD (Autodesk, version 2020). Morphometric characteristics were measured with the precision of 1 mm. On the site in Obrenovac (I) and in Pambukovica (II) the size of the sample was 150 plants per site. From each of the plants whose morphometric characteristics were measured 5 leaves were taken for morphometric analysis, which amounts to 750 leaves per site, i.e. 1500 leaves in total for both sites according to the previously determined terminology (Mitrovic et al., 2022).

Ten basic characteristics of the leaves were measured: total area of the leaf without petiole in cm^2 (AREA); total circumference of the leaf without petiole in cm (PERI); leaf blade length, from the base of the leaf to the tip of the leaf in cm (LL); the length of a central veine (CN); maximum width of the leaf in cm (MWL); leaf width at 1 cm from the leaf base in cm (LW); petiole length, from the leaf base to the petiole tip in cm (PL); the distance between 3^{rd} and 4^{th} nerve in cm (HL); number of nerves on the left side of the leaf (NLL); and number of nerves on the right side of the leaf (NLR) (Mitrovic et al., 2022) (Figure 3).

The data of morphometric measurements of the leaves were statistically processed in the program STATGRAPHICS (Statistical Graphics Corporation, USA). The design of the experiment factor A (site) with two levels: site I (Obrenovac) and site II (Pambukovica), factor B (species) with two levels: species 1 (*P. elongata*) and species 2 (*P. fortunei*) and factor C (treatment) with three levels: treatment 1, treatment 2 and treatment 3 corresponds to three-factor and two-factor analysis of variance: ANOVA III.

3. RESULTS

The soil that represents the profile on the site in Obrenovac (I) is well permeable to both water and air and has sufficiently high capacity to retain accessible water. According to its textural composition it belongs to sandy loams. The surface layer of soil on this site belongs to class of sandy loams, and deeper analysed layer belongs to the loam class. Although they belong to different texture classes, physical properties of both analysed layers are similar, i.e. there is no strong differentiation of profiles by textural composition. The content of individual textural fractions in both layers are close to the limit values between sandy loam and loam. This soil is well permeable to water and well aerated throughout the depth of the solum.

Table 1. *Physical properties of soil*

Site	Depth of profile (cm)	Coarse sand	Fine sand	Powder	Clay	Total		Textural class
						Sand	Clay	
		%						
I	0-20	4.50	48.62	20.58	26.30	53.12	46.88	Sandy loam
	20-40	4.20	46.60	22.30	26.90	50.80	49.20	Loam
II	0-20	0.60	44.50	29.00	25.90	45.10	54.90	Loam
	20-40	0.40	42.20	25.70	31.70	42.60	57.40	Sandy clay loam

The surface layer of the soil on the site in Pambukovica (II) belongs to class of loam and it is well permeable to water and air. With the depth of soil solum the content of clay and fine sand increases in textural composition and textural class changes to sandy clay loam, which is somewhat less permeable to water and air (Table 1).

Table 2. *Chemical properties of the soil*

Site	Depth of profile (cm)	pH		Adsorption complex					Total		C/N	Accessible	
		H_2O	KCl	T	S	T-S	V	Y1	humus	N		P_2O_5	K_2O
				equ.m.mol/100g			%	cm^3	%	%		mg/100g	
1	0-20	5.91	4.74	32.50	24.94	7.56	76.74	11.63	2.55	0.19	7.59	23.55	19.39
	20-40	5.87	4.77	32.90	25.27	7.63	76.80	11.74	2.03	0.12	10.13	21.63	17.45
II	0-20	5.35	3.84	32.13	19.56	12.57	60.87	19.34	1.47	0.15	5.87	<LD	8.76
	20-40	5.41	3.94	32.66	21.51	11.15	65.86	17.15	0.98	0.13	4.41	<LD	7.94

On the site I in Obrenovac the reaction of the soil solution is moderate. The total capacity of adsorption is quite high, due to the high proportion of clay in textural composition. According to the content of total humus both analysed layers belong to soils with low content of humus. A narrow C/N ratio is favourable for mineralization of the organic matter. The provision of easily accessible forms of phosphorus is good throughout the depth of the solum, and the provision of easily accessible forms of potassium is medium. The reaction of soil solution on site II is very acidic throughout the depth of the solum. Total adsorption capacity is high. According to the content of total humus surface layer belongs to the soils of low humus content, while deeper analysed layer is on the limit between soils with low and very low content of humus. The content of total nitrogen is low and the ratio of carbon to nitrogen is narrow. In both analysed layers the amount of phosphorus accessible to plants is below the limit of detection for Al–method, which means that this soil is extremely poorly provided by forms of phosphorus easily accessible to plants. The amounts of forms of

potassium easily accessible to plants are within the limits of poor provision throughout the depth of the profile (Table 2).

Figure 1. *Leaves of paulownia seedlings from the experimental field in Obrenovac at the end of second growing season*

Figure 2. *Leaves of paulownia seedlings from the experimental field in Pambukovica at the end of second growing season*

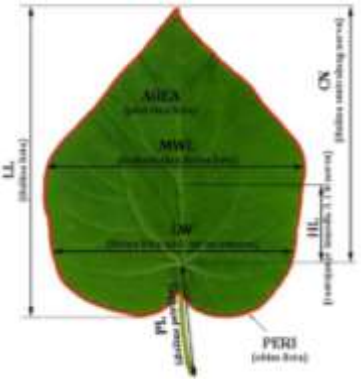

Figure 3. *Schematic representation of analysed measurement parameters of paulownia leaves (Mitrovic et al., 2022)*

The results of the statistical analysis of ten selected parameters of leaf are presented in tables 3 and 4. For the characteristic leaf area in the second growing season (Table 3), as well as in the first, there is a difference in mean values of the groups (measurements) depending on the factors site, species and treatment. The seedlings on the site in Obrenovac (I) have significantly higher mean leaf area values (75.36 cm^2) then mean values of seedlings from the site in Pambukovica (II) (43.98 cm^2). There is statistically significant difference between species. The seedlings of the species *Paulownia elongata* have higher mean values of leaf area (64.69 cm^2) than seedlings of the species *Paulownia fortunei* (54.66 cm^2). Statistically significant difference exists among all three treatments, whereby the seedlings from the treatment which was fertilized with larger quantity of fertilizer have higher mean values of the characteristic leaf area (80,61 cm^2). They are followed by the seedlings from the treatment fertilized by smaller quantity of fertilizer (54.05 cm^2), and the seedlings from the control treatment had the lowest mean values (44.36 cm^2). All interactions are statistically significant.

For the characteristic leaf circumference in the second growing season (Table 3), there is a difference in mean values of the groups depending on the factors site, species and treatment. The seedlings from the site in Obrenovac (I) show significantly higher values of leaf circumference (33.54 cm) compared to the mean values of seedlings at the site in Pambukovica (II) (25.99 cm). There is statistically significant difference in mean values of circumference between species, whereby the seedlings of the species *Paulownia elongata* show significantly higher mean values (31.33 cm) then the seedlings of the species *Paulownia fortunei* (28.19 cm). The difference in mean values of circumference among seedlings within different

treatments is statistically significant. The seedlings from the treatment which was fertilized with the larger quantity of fertilizer showed the highest mean values (34.20 cm), and the lowest were in the control treatment (25.90 cm). For the characteristic leaf circumference in the second growing season, all interactions are significant.

For the characteristic leaf blade length in the second growing season (Table 3) there is a difference in the mean values of the groups depending on the factors site, species and treatment. The mean values of the leaf blade length of the seedlings on the site in Obrenovac (I) differ significantly compared to the seedlings on the site in Pambukovica (II), whereby the seedlings on the site (I) have higher mean values of the leaf length (33.54 cm) then the seedlings on the site (II) (25.99 cm). The seedlings of the species *Paulownia elongata* have significantly higher mean values of the leaf length (31.33 cm) compared to the seedlings of the species *Paulownia fortunei* (28.19 cm). The difference between mean values of the leaf blade length of seedlings within different treatments is also significant, whereby the seedlings from the treatment which was fertilized with larger amount of fertilizer have the highest mean value of the length (10.39 cm), and the lowest was from the control treatment (7.91 cm). The interaction between species and treatments is not statistically significant. For the characteristic leaf blade length in second growing season, the interactions between factor site and factor species (AxB) and between factor site and factor treatment (AxC) were statistically significant.

For the characteristic the central veine length in the second growing season (Table 3) there is a difference in mean values of the groups depending on the factors site, species and treatment. The seedlings on the site in Obrenovac (I) have significantly higher mean values of the central veine length (8.23 cm), compared to the seedlings on the site in Pambukovica (II) (6.67 cm). The seedlings of the species *Paulownia elongata* have statistically significantly higher mean value (7.82 cm) compared to the seedlings of the species *Paulownia fortunei* (7.08 cm). The seedlings within the treatment fertilized by larger quantity of fertilizer have significantly higher (8.57 cm) mean values than the seedlings from the treatment fertilized by smaller amount of fertilizer (7.28 cm) and from the control treatment (6.50 cm). For the characteristic the central veine length in the second growing season, the interactions between factors site and species (AxB) and factors site and treatment (AxC) were statistically significant.

For the characteristic leaf width in the second growing season (Table 3) there is a difference in mean values of groups depending on the factors site, species and treatment. There is a significant difference in mean values of leaf width of the seedlings on the site in Obrenovac (I) and in Pambukovica (II), whereby it is larger in seedlings on the site (I) (9.42 cm) then in seedlings on the site (II) (7.07 cm). The seedlings of the species *Paulownia elongata* have significantly higher mean values of the characteristic (8.76 cm) than seedlings of the species *Paulownia fortunei* (7.74 cm). Mean values of leaf width are significantly different in seedlings within treatments, whereby the seedlings from the treatment which is fertilized with larger amount of fertilizer have the highest (9.70 cm) mean value of the characteristic leaf width, and seedlings from the control treatment have the lowest mean value (6.94 cm). All interactions are statistically significant.

Table 3. *Basic parameters of descriptive statistics and three-way ANOVA for the leaf characteristics: leaf width at 1 cm from the base (cm), petiole length (cm), the distance between the 3rd and the 4th nerve, the number of nerves on the left side and number of nerves on the right side; for seedlings on sites in Obrenovac (I) and in Pambukovica (II), at the end of the second growing season*

Factor	Level	Leaf width at 1 cm from the leaf base	Petiole length	Distance between the 3rd and the 4th nerve	Number of nerves - left	Number of nerves - right
Site (A)	Site I	8.76(3.64)^{b}	6.37(2.53)^{b}	2.09(1.03)^{b}	7.82(1.00)^{a}	7.86(0.98)^{a}
	Site II	6.71(2.81)^{a}	4.87(2.17)^{a}	1.76(1.29)^{a}	8.029)^{b}	7.99 (0.83)^{b}
		$F_{1.1490}$=205.16*	$F_{1.1490}$=170.25*	$F_{1.1490}$=34.95*	$F_{1.1490}$=21.23*	$F_{1.1490}$=10.88*
Species (B)	*P. elongata*	8.22(3.39)^{b}	5.82(2.44)^{b}	2.06(1.41)^{b}	7.67(0.95)^{a}	7.68(0.95)^{a}
	P. elongata	7.26(3.36)^{a}	5.42(2.50)^{a}	1.79(0.86)^{a}	8.18(0.88)^{b}	8.16(0.80)^{b}
		$F_{1.1490}$=44.46*	$F_{1.1490}$=2.32*	$F_{1.1490}$=22.90*	$F_{1.1490}$=152.03*	$F_{1.1490}$=152.34*
Treatment (C)	Treatment 1	9.26(3.82)^{c}	6.06(2.55)^{b}	2.30(1.02)^{c}	7.56(0.93)^{a}	7.53 (0.92)^{a}
	Treatment 2	7.45(2,47)^{b}	5.97(2.11)^{b}	1.97(1.23)^{b}	8.2(0.86)^{b}	8.14(0.78)^{b}
	Treatment 3	6.50(3.20)^{a}	4.82(2.55)^{a}	1.51(1.13)^{a}	8.09(0.95)^{b}	8.09(0.90)^{b}
		$F_{2.1490}$=129.50*	$F_{2.1490}$=48.11*	$F_{2.1490}$=67.90*	$F_{2.1490}$=78.01*	$F_{2.1490}$=98.32*
***Interactions* (AXB)**		$F_{1.1490}$=15.12*	$F_{1.1490}$=8.93*	ns	c	ns
***Interactions* (AXC)**		$F_{2.1490}$=103.25*	$F_{2.1490}$=23.30*	$F_{2.1490}$=55.65*	$F_{2.1490}$=108.73*	$F_{2.1490}$=111.85*
***Interactions* (BXC)**		$F_{2.1490}$=29.97*	$F_{2.1490}$=14.79*	$F_{2.1490}$=5.76*	$F_{2.1490}$=51.41*	$F_{2.1490}$=43.46*

A three-factor analysis of variance (ANOVA III). Factor A (Site) with 2 levels: site 1 (Obrenovac) and site 2 (Pambukovica); Factor B (Species) with 2 levels: species 1 (*P. elongata*) and species 2 (*P. fortunei*); Factor C (Treatment) with 3 levels: treatment 1 (larger amount of fertilizer), treatment 2 (smaller amount of fertilizer), and treatment 3 (control), and their interactions. The size of the pooled sample (number of elements of the pooled sample), n=1500 (2 sites x 2 species x 3 treatments x 125 = 1500). A = mean value (standard deviation); B= F-test indicator with the number of degrees of freedom; ns = non-significant difference between mean values of populations (P□ 0.05); * = statistically significant difference (P□ 0.05).

For the characteristic leaf width at 1 cm from the base in the second growing season (Table 4) there is a difference in mean values of groups depending on the factors site, species and treatment. The seedlings on the site in Obrenovac (I) have statistically significantly higher mean values of the leaf width (8.76 cm) compared to the seedlings on the site in Pambukovica (II) (6.71 cm). The seedlings of the species *Paulownia elongata* have significantly higher (8.22 cm) mean values of leaf width than the seedlings of the species *Paulownia fortunei* (7.26 cm). Among the treatments there is a significant difference whereby the seedlings within the treatment fertilized with larger amount of fertilizer have statistically significantly higher (9.26 cm) mean values of the characteristic compared to the seedlings from the treatment that was fertilized with less fertilizer (7.45 cm) and control treatment (6.50 cm). All interactions of factors are statistically significant.

For the characteristic petiole length in second growing season (Table 4) there is a difference in mean values of the groups depending on the factors site, species and treatment. The seedlings on the site in Obrenovac (I) have significantly higher mean values of petiole length (6.37 cm) than mean values of the characteristic in seedlings at the site in Pambukovica (II) (4.87 cm). The seedlings of the species *Paulownia elongate* have statistically significantly higher (5.82 cm) mean values of petiole length than the seedlings of the species *Paulownia fortunei* (5.42 cm). The mean value of the characteristic petiole length differs significantly in seedlings within different treatments that were fertilized (no statistically significant difference between them) compared to the seedlings from the control treatment. The seedlings from the treatment fertilized with the larger amount of fertilizer have the highest mean value of petiole length (6.06 cm), and the seedlings from the control treatment have the smallest (4.82 cm). All interactions are statistically significant.

For the characteristic the distance between the 3rd and the 4th leaf nerve in the second growing season (Table 4) there is a difference in mean values of the groups depending on the factors site, species and treatment. The seedlings on the site in Obrenovac (I) show significantly higher mean values of the distance between the 3rd and the 4th leaf nerve (2.09 cm) compared to the mean values in seedlings on the site in Pambukovica (II) (1.76 cm). The seedlings of the species *Paulownia elongata* have significantly higher mean values of the characteristic (2.06 cm) compared to the seedlings of the species *Paulownia fortunei* (1.79 cm). The difference in mean values of the distance between the 3rd and the 4th leaf nerve in seedlings within different treatments is statistically significant. The highest mean value of this characteristic is in the seedlings from the treatment which was fertilized by the larger amount of fertilizer (2.30 cm), and the smallest is in seedlings from the control treatment (1.51 cm). For the characteristic the distance between the 3rd and the 4th leaf nerve in the second growing season, the interactions of the factors site and treatment (AxC) and factors species and treatment (BxC) are statistically significant.

For the characteristic number of nerves on the left side of the leaf in the second growing season (Table 4) there is a difference in the mean values of the groups depending on the factors site, species and treatment. The seedlings on the site in Pambukovica (II) have significantly higher mean values of the number of nerves on the left side of the leaf (8.02) than the mean values of the characteristic in seedlings on the site in Obrenovac (II) (7.82). The seedlings of the species *Paulownia fortune* have statistically significantly higher (8.18) mean values of the number of

nerves on the left side of the leaf than the seedlings of the species *Paulownia elongata* (7.67). In seedlings within different treatments there is no statistically significant difference in the mean value of number of nerves. The highest mean values of the number of nerves on the left side of the leaf appears in seedlings from the treatment which is fertilized by smaller amount of fertilizer (8.12), then from the control treatment (8.09) and the lowest mean values from the treatment fertilized by larger quantity of fertilizer (7.56). For the characteristic number of nerves on the left side of the leaf in the second growing season, the interactions of factors site and treatment (AxC) and factors species and treatment (BxC) are statistically significant. Interaction of factors site and species has no statistical significance.

Table 4. *Basic parameters of descriptive statistics and three-way ANOVA for the leaf characteristics: leaf width at 1 cm from the base (cm), petiole length (cm), distance between the 3rd and the 4th nerve, number of nerves on the left side and number of nerves on the right side; for the seedlings on sites in Obrenovac (I) and in Pambukovica (II), at the end of the second growing season*

Factor	Level	Leaf width at 1 cm from the leaf base	Petiole length	The distance between the 3rd and the 4th nerve	Number of nerves – left	Number of nerves - right
Site (A)	Site I	8.76(3.64)^{b}	6.37(2.53)^{b}	2.09(1.03)^{b}	7.82(1.00)^{a}	7.86(0.98)^{a}
	Site II	6.71(2.81)^{a}	4,87(2.17)^{a}	1.76(1.29)^{a}	8.02(0.89)^{b}	7.99(0.83)^{b}
		$F_{1.1490}$=205.16*	$F_{1.1490}$=170.25*	$F_{1.1490}$=34.95*	$F_{1.1490}$=21.23*	$F_{1.1490}$=10.88*
Species (B)	*P. elongata*	8.22(3.39)^{b}	5.82(2.44)^{b}	2.06(1.41)^{b}	7.67(0.95)^{a}	7.68(0.95)^{a}
	P. elongata	7.26(3.36)^{a}	5.42(2.50)^{a}	1.79(0.86)^{a}	8.18(0.88)^{b}	8.16(0.80)^{b}
		$F_{1.1490}$=44.46*	$F_{1.1490}$=12.32*	$F_{1.1490}$=22.90*	$F_{1.1490}$=152.03*	$F_{1.1490}$=152.34*
Treatment (C)	Treatment 1	9.26(3.82)^{c}	6.06(2.55)^{b}	2.30(1.02)^{c}	7.56(0.93)^{a}	7.53 (0.92)^{a}
	Treatment 2	7.45(2.47)^{b}	5.97(2.11)^{b}	1.97(1.23)^{b}	8.12(0.86)^{b}	8.14(0.78)^{b}
	Treatment 3	6.50(3.20)^{a}	4.82(2.55)^{a}	1.51(1.13)^{a}	8.09(0.95)^{b}	8.09(0.90)^{b}
		$F_{2.1490}$=129.50*	$F_{2.1490}$=48.11*	$F_{2.1490}$=67.90*	$F_{2.1490}$=78.01*	$F_{2.1490}$=98.32*
***Interactions* (AXB)**		$F_{1.1490}$=15.12*	$F_{1.1490}$=8.93*	ns	C	ns
***Interactions* (AXC)**		$F_{2.1490}$=103.25*	$F_{2.1490}$=23.30*	$F_{2.1490}$=55.65*	$F_{2.1490}$=108.73*	$F_{2.1490}$=111.85*
***Interactions* (BXC)**		$F_{2.1490}$=29.97*	$F_{2.1490}$=14.79*	$F_{2.1490}$=5.76*	$F_{2.1490}$=51.41*	$F_{2.1490}$=43.46*

A three-factor analysis of variance (ANOVA III). Factor A (Site) with 2 levels: site 1 (Obrenovac) and site 2 (Pambukovica); Factor B (Species) with 2 levels: species 1 (*P. elongata*) and species 2 (*P. fortunei*); Factor C (Treatment) with 3 levels: Treatment 1 (larger amount of fertilizer), treatment 2 (smaller amount of fertilizer), and treatment 3 (control), and their interactions'. The size of pooled sample (number of elements of the pooled sample), n=1500 (2 sites x 2 species x 3 treatments x 125 = 1500). A = mean value (standard deviation); B= F-test indicator with the numbers of degrees of freedom; ns = non-significant difference between mean values of populations (P□ 0.05); * = statistically significant difference (P□ 0.05).

For the characteristic number of nerves on the right side of the leaf in the second growing season (Table 4) there is a difference in mean values of groups depending on the factors site, species and treatment. The seedlings on the site in Pambukovica (II) have significantly higher mean values of the number of nerves on the right side of the leaf (7.99) than the mean values of the characteristic in seedlings on the site in Obrenovac (I) (7.86). The seedlings of the species *Paulownia fortunei* have statistically significantly higher (8.16) mean values of the number of nerves on the right side of the leaf than the seedlings of other species (7.68). The seedlings from the treatment that was fertilized by smaller amount of fertilizer have higher mean values of the characteristic (8.14) than the seedlings from the control treatment (8.09), and statistically they do not differ significantly. Significant difference exists in the mean value of the characteristic in seedlings from these two treatments compared to the seedlings from the treatment fertilized by larger amount of fertilizer, which has the lowest mean value (7.53). For the characteristic number of nerves on the right side of the leaf in the second growing season, interaction of factors site and treatment (AxC) and interaction of factors species and treatment (BxC) are statistically significant. Interaction of site and species is not statistically significant.

4. DISCUSSION

The analysis of the results in the second growing season shows that mean values of all morphometric parameters of the leaf are higher (Table 3; Table 4) compared to the first growing season (Mitrovic et al., 2022). The differences between morphometric characteristics of the leaf between the seedlings on the site in Obrenovac (I) and in Pambukovica (II), and between the seedlings within different species, as well as between the seedlings within the treatments in the second growing season followed the trends of morphometric characteristics of the leaf same as in the first year of the research.

The differences have statistical significance for all analysed parameters of the leaf. Mean values of the length (33.54 cm) and the width of the leaf (9.42 cm) and petiole (6.37 cm) of the seedlings on the site in Obrenovac (I) in the second year of the research are far below average values for these species (Graves, 1989; Šijačić-Nikolić et al., 2009). In seedlings on the site in Pambukovica (II) mean values of these parameters of the leaf are even lower and amount to 25.99 cm, 7.07 cm and 4.87 cm.

Environmental conditions on experimental fields are unfavourable for the genus *Paulownia*. The shape and the structure of the leaves of *Paulownia elongata* compared to *Paulownia fortunei* significantly depend on numerous factors (Zhu et al., 1986; Bergmann, 1998; Popović 2005; Fender et al., 2011; Stojnić, 2013). The results of morphometric characteristics of leaves of seedlings on the sites are the consequence of adaptation to the unfavourable environmental conditions in which they grew (Yong-Hua et al., 2012; Mitrovic et al., 2022; Mitrovic et al., 2024). On the site in Pambukovica (II) the content of nitrogen, phosphorus and potassium in the soil (Table 2) was lower than on the site in Obrenovac (I), which is why the conditions for plant growth were less favourable, which also affected leaf growth.

The seedlings on the site in Pambukovica (II) showed greater lack of moisture in the soil although the average value of precipitation in the growing season

on this site is higher than for the site in Obrenovac (1). The reason for this is that the site is located on a slope, so the atmospheric precipitation quickly ran off the plot. Accordingly, the leaves on this site had smaller dimensions. Climatic factors significantly influence the size of the leaves, whereby as a rule the leaves are smaller in drier environmental conditions which is pointed out by numerous authors in their research (Pedrol et al., 2000; Otieno et al., 2005; Niinemets, 2001; Ozturk et al. 2014; Wright et al., 2001).

In accordance with the findings of other authors which indicate the variability of morphologic characteristics within the genus *Paulownia* (Zhu et al., 1986; Šilić, 1990; Bergmann, 1998; Cvjetićanin i Perović, 2009) the differences in morphometric characteristics of the leaf between the seedlings of analysed species are clearly confirmed. The seedlings of the species *Paulownia elongata* had higher mean values of all analysed parameters compared to the seedlings of *Paulownia fortunei* on both sites – in Obrenovac (I) and in Pambukovica (II), except for the characteristic number of nerves on left and right side of the leaf blade, where the differences were not significantly pronounced (Niinemets 2001; Wright et al., 2001).

The differences in morphometric parameters of leaves are clearly expressed through correlation with the results of fertilization of seedlings. The seedlings fertilized with a larger amount of fertilizer had the highest mean values of the majority of measured parameters, which indicates a direct relation between the availability of fertilizer and growth intensity. These findings are in accordance with the research of Adejobi et al. (2014), which showed that fertilization affects positively the increase of leaf area and dimensions. The exception is the number of nerves on the left and right side of the leaf blade, where the highest values were recorded in the treatment with the smaller amount of fertilizer and in the control variant, while the lowest values were recorded in seedlings fertilized with the larger amount of fertilizer.

5. CONCLUSION

Species *Paulownia elongata* S. Y. Hu. and *Paulownia fortunei* Seem. Hemsl., which were planted on different habitats and treated with different amounts of fertilizer, based on the analyses of the results of measurement of morphological parameters of leaves indicate that fertilization affects positively the leaf parameters and size in the second year after the planting. Also, the type of habitat affects morphological parameters of the leaf.

On the site in Obrenovac (I) the results of all measured parameters are statistically significantly higher than the values measured on the site in Pambukovica (II) which is in correlation with the physical properties of the soil which are better on the site in Obrenovac (I). Also, the soil on this site has greater content of humus and a more favourable ratio of carbon and nitrogen (C/N).

The size of the leaves of the plants in experimental fields is in direct correlation with the amount of fertilizer by which the plants are fertilized. In plants which were fertilized with the larger amount of fertilizer (240 g per plant) all analysed parameters of the leaf had statistically significantly higher values than the leaves of the seedlings which were fertilized with the smaller amount of fertilizer (120 g per plant) and leaves of the plants on the control plot where fertilization was

not carried out. Compared to the leaves from the control plot and all analysed parameters of the leaves of plants that were fertilized with the smaller amount of fertilizer were statistically significantly higher, except for the parameters number of nerves on the right side of the leaf and number of nerves on the left side of the leaf where fertilization did not have any effect.

The analysis of morphological parameters of the leaf between the researched species shows that in *Paulownia elongata* mean values are higher and they are statistically significant for the characteristics leaf area, leaf circumference, leaf blade length, the length of the central veine, leaf width, leaf width at 1 cm from the base, petiole length, the distance between the 3rd and the 4th nerve of the leaf, number of nerves on the left side of the leaf. For the characteristic number of nerves on the right side of the leaf in *Paulownia fortunei* mean values are higher and they are statistically significant.

Based on the obtained results, it can be concluded that particular attention should be paid to soil structure when introducing these species. The soil should be loose, well-aerated, and capable of supporting rapid early root development, as compact or poorly aerated soils may limit their growth potential. Given that paulownia is a fast-growing genus with high nutrient demands, fertilization practices must be carefully harmonized with the physical and chemical properties of the soil in order to ensure optimal nutrient availability and prevent physiological imbalance. The results also highlight a significant potential of these species for use in energy plantations, where fast biomass accumulation is a key requirement.

To fully evaluate and utilize their production capacity in such systems, further research is needed in later stages of plant development, including measurements up to harvest and across repeated post-harvest cycles typical of energy plantations. Continuous monitoring over multiple rotations will enable a complete valorisation of their biomass yield, resilience, and long-term suitability for sustainable energy production. These recommendations provide a practical framework for growers and land managers aiming to optimize cultivation, fertilization, and site selection for maximizing the performance of paulownia in energy-oriented plantations.

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EFFECT OF FERTILIZATION ON LEAF MORPHOMETRIC CHARACTERISTICS OF *Paulownia elongata* S. Y. Hu AND *Paulownia fortunei* Seem. Hemsl. IN THE SECOND YEAR OF GROWTH

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Summary

Starting material for the production of seedlings of species *Paulownia elongata* and *Paulownia fortunei* of *Paulownia* Sieb. & Zucc. from the family *Paulowniaceae* was collected on plantation established in 1993 near Bela Crkva in Vojvodina. The species used within these studies should show whether the introduction of these species of paulownia is possible and justified within the research of adaptability of these species to environmental conditions of two different sites in Serbia. The introduction of new species in the territory of Serbia is significant if we have in mind the climate change which is evident, where many species are lost from their natural habitats, and the need for production of biomass is increasingly pronounced. The size and characteristics of leaves which are significant for photosynthetic processes and production of nutrients are in direct correlation with the production of total biomass of plants. Starting from the above-mentioned facts, a comparative analysis of ten basic morphological characteristics of leaf was carried out. Morphological analysis was carried out according to the modified protocol Assessments of Oak Leaf Morphology, with adjustments to the specificities of the species of the genus *Paulownia*. Morphological characteristics of the leaf were analysed depending on the pedological conditions and the regime of plant fertilization during the second year of growth, whereby different quantities of fertilizer were applied. The results of the analysis of the measurement data of leaf morphological parameters of species *Paulownia elongata* S. Y. Hu. and *Paulownia fortunei* Seem. Hemsl., planted on different habitats and treated by different amounts of fertilizers, indicate that the type of habitat has a significant effect on leaf morphological characteristics. Fertilization proved itself to be a factor which has a positive effect on the development of morphological parameters, as well as on overall size of the leaves in the first year after the planting. The obtained results indicate significant potential of the examined species of *Paulownia* genus, whereby greater adaptive and morphogenetic

potential was pronounced in the species *Paulownia elongata* compared to *Paulownia fortunei*. During the introduction and establishing of plantation of the species *P. elongata* it is necessary to take care of adjusting the quantity of nutrients introduced by fertilization to the chemical properties of the soil, in order to provide optimal values for growth and development of plants. Since it is a fast-growing species with high requirements in terms of aeration and permeability of the substrate, it is advisable to form plantations on loose, well-aerated soils rich in humus, which enables intensive vegetative growth and maximum use of species potential.

UTICAJ ĐUBRENJA NA MORFOMETRIJSKE KARAKTERISTIKE LISTOVA VRSTA *Paulownia elongata* S. Y. Hu I *Paulownia fortunei* Seem. Hemsl. U DRUGOJ GODINI RASTA

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Rezime

Polazni materijal za proizvodnju sadnica vrsta *Paulownia elongata* i *Paulownia fortunei* roda *Paulownia* Sieb. & Zucc. iz familije *Paulowniaceae* je sakupljen na plantaži koja je osnovana 1993. godine kod Bele Crkve u Vojvodini. Korišćene vrste u okviru ovih istraživanja treba da pokažu da li je introdukcija ovih vrsta paulovnija moguća i opravdana u sklopu istraživanja adaptibilnosti ovih vrsta na uslove sredine dva različita lokaliteta u Srbiji. Introdukcija novih vrsta na području Srbije je od značaja ako imamao u vidu klimatske promene koje su evidentne, gde se mnoge vrste gube sa svojih prirodnih staništa, a potreba za produkcijom biomase je sve izraženija. U dirktnoj korelaciji sa produkcijom celokupne biomase biljaka su veličina i karakteristike lista koje su od značaja za fotosintetičke procese i produkciju hranljivih materija. Polazeći od navedenih činjenica, izvršena je komparativna analiza deset osnovnih morfoloških obeležja lista. Morfološka analiza sprovedena je prema modifikovanom protokolu Assessments of Oak Leaf Morphology uz prilagođavanja specifičnostima vrsta roda *Paulownia*. Analizirana su morfološka svojstva lista u zavisnosti od pedoloških uslova i režima prihranjivanja biljaka tokom druge godine razvoja, pri čemu su primenjene različite količine đubriva. Rezultati analize merenih podataka morfoloških parametara listova vrsta *Paulownia elongata* S. Y. Hu. i *Paulownia fortunei* Seem. Hemsl., posađenih na različitim staništima i tretiranih različitim količinama đubriva, ukazuju da tip staništa ima značajan uticaj na morfološke karakteristike lista. Prihranjivanje se pokazalo kao faktor koji pozitivno utiče na razvoj morfoloških parametara, kao i na ukupnu veličinu listova u prvoj godini. Dobijeni rezultati ukazuju na značajan potencijal ispitivanih vrsta roda *Paulownia*, pri čemu je kod vrste *Paulownia elongata* S. Y. Hu. izražen veći adaptivni i morfogenetski potencijal u odnosu na *Paulownia fortunei* Seem. Hemsl. Prilikom introdukcije i podizanja zasada vrste *P. elongata* neophodno je voditi računa o usklađivanju količine hranljivih materija unetih đubrenjem sa hemijskim osobinama zemljišta, kako bi se obezbedile optimalne vrednosti za rast i razvoj biljaka. S obzirom na to da je reč o brzo rastućoj vrsti sa visokim zahtevima u pogledu aeracije i propustljivosti supstrata, plantaže je preporučljivo formirati na rastresitim, dobro aerisanim i humusom bogatim zemljištima, što omogućava intenzivan vegetativni porast i maksimalno iskorišćenje potencijala vrste.