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COMPARATIVE ANALYSIS OF THE ANATOMICAL STRUCTURE OF HEARTWOOD AND SAPWOOD SELECTED *GYMNOCLADUS CANADENSIS* LAM. TREES IN SRPSKA CRNJA

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Abstract - This paper shows the results obtained from the study of the macroscopic-microscopic structure (capillary system) in the growth stem of *Gymnocladus canadensis* Lam. originating from North America, which grows in "Muzljanski rit" in the area of Srpska Crnja. *Gymnocladus canadensis* Lam. falls under the ring-porous species according to its porosity, with large tracheas in its early zone. The early zone trachea lumens, contained in the sapwood, reach dimensions of up to 160 μm, while early zone trachea lumens in the growth stem rings of the sapwood reach dimensions of up to 120 μm. Examination of the microscopic structure of this tree show good properties of the tree.

Key words: Iron wood, anatomical properties, capillary elements, sapwood, heartwood, vessel, cultivation

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INTRODUCTION

Iron wood (*Gymnocladus canadensis* Lam.), more commonly known as *Gymnocladus dioicus* (L.) K. Koch, belongs to the deciduous species of trees from the family Fabaceae. The name originates from the Greek words *gymnos*, which means naked, and *klados*, which means branch. The name dioecious translated into Serbian means *episkopalan*, which actually means that this species has both male and female boles (dioecious type).

The natural habitats of the iron tree are the eastern and central parts of the North American continent. Optimal development and growth (primary and secondary) of the tree is provided for on wet soils, valleys and mild slopes (Elias, 1980). It also grows on dry, compact and alkaline soils, as well as in exposed locations. It can bear pH values of soil ranging from 4.5 to 8.5. It can sustain temperatures as low as -34°C. It belongs to heliophyte species. This tree can be reproduced generatively (from seed) and vegetatively (from root propagation). According to Jovanović (1971), the tree grows up to 18.3-21.3 m and belongs to a group of second class trees. This species is particularly resistant to heartwood decay, especially in contact with earth (Harry, 1955).

According to research conducted by Petrović (1951), iron wood was most commonly planted in Vojvodina, in green areas and tree alleys. The oldest trees in Serbia are more than 100 years old (Karas et al., 2003). Well-preserved trees of iron wood are located in Fruška Gora, in the area of Forestry Directorate of Erdevik. The trees from Fruška Gora, which are 78 years old, reach 36.5 m in height with a trunk diameter of 48.8 cm (Bobinac, 2007, 2008). Based on morphometric research conducted by Bobinac in 2008, we may conclude that this species belongs to first class trees in Serbia.

Iron wood is used to meet numerous human needs. Its first use dates back to the period of intensive settlements of the North American continent, when fruits of this tree were used as a substitution for coffee. Nowadays, iron wood is widely used in civil engineering, medicine, recultivation of degraded habitats, spatial and urban planning, and so on.

The anatomical properties of iron wood have not been researched in Serbia. The aim of this paper was to determine the anatomical properties of the capillary elements – trachea (lumen width and number of trachea per area unit) in the sapwood and heartwood. Also, one of the aims of this research is to determine what happens to trachea lumen width and number of trachea per mm^2 in heartwood whose physiological function is fading away.

MATERIALS AND METHODS

The wood samples used for the research of the macroscopic and microscopic structure of a tree originate from "Muzljanski rit", located in the area of Srpska Crnja. These iron wood trees have been growing in a community with acacia, as well as with mixed types of acid wood and common hackberry. Acacia is of sprout origins. The stand containing mixed iron wood trees is 28 years old (in decomposition phase).

Samples were taken from the tree trunk, and their length was the same as trunk diameter, with 10 mm in width. In order to enable cutting, the wood was boiled-softened in a solution of water, glycerin and ethyl alcohol, 1:1:1 proportion, before it was cut.

The samples were used to make preparation cuts, 16 μm thick, using a "Reichert" sliding micro-motor. The cuts (transversal (TS); tangentially-longitudinal (TLS) and radial-longitudinal (RLS)) served for measurements, i.e. statistic data processing and for the development of micro-photographs.

Each ring of the growth stem (from the heartwood to the bark) was measured at its transversal cut, where 30 tracheas were found in each of them, both in early and mature tree zones. Trachea widths were measured in radial (R) and tangential (T) directions. Also, each ring of the stem was measured in early and mature zones, where again 30 tracheas were measured on 1 mm^2.

The basic components of descriptive statistics were: mean value, standard error and coefficient of variation (CV %) for the anatomical properties of iron wood *Gymnocladus canadensis*. They were obtained by PROC MEANS procedure from the SAS program package. The analyzed properties of tree anatomy were: width of tracheas (T in tangential, R in radial direction), mean value of total trachea width in tangential and radial directions (T+R)/2 and number of tracheas per mm^2, cross-section of iron wood within early and mature growth phases within rings, for each part of the trunk, heartwood and sapwood. The statistic significance of discrepancies between the mean values of the anatomical properties of a tree between the parts of the tree cross-sections (sapwood and heartwood), as well as between the phases (early and mature) within each part of the trunk (sapwood and heartwood), was established through T-test (PROC GLM procedure, option T-test). Estimation of the statistic significance of various property variability sources (parts of the tree, rings, and phases within rings) was performed through variance analysis (PROC GLM in SAS program package).

RESULTS AND DISCUSSION

There is a difference between the color of the sapwood and heartwood ring-porous species with a higher share of early zone within the stem ring. The sapwood is narrow (four stem rings) and is light yellowish in color, while heartwood is dark brown.

Iron wood is remarkable for its darker zone in mature trees than in young trees. The central part of the tree, i.e. heartwood, is orange in color and is made of live parenchyma cells. Lignum strips are not visible with the naked eye (Fig. 1). The bark is ash-grey to brown-grey in color. It is deeply fissured while still a young tree, with a scaly surface (Vilotić, 2009).

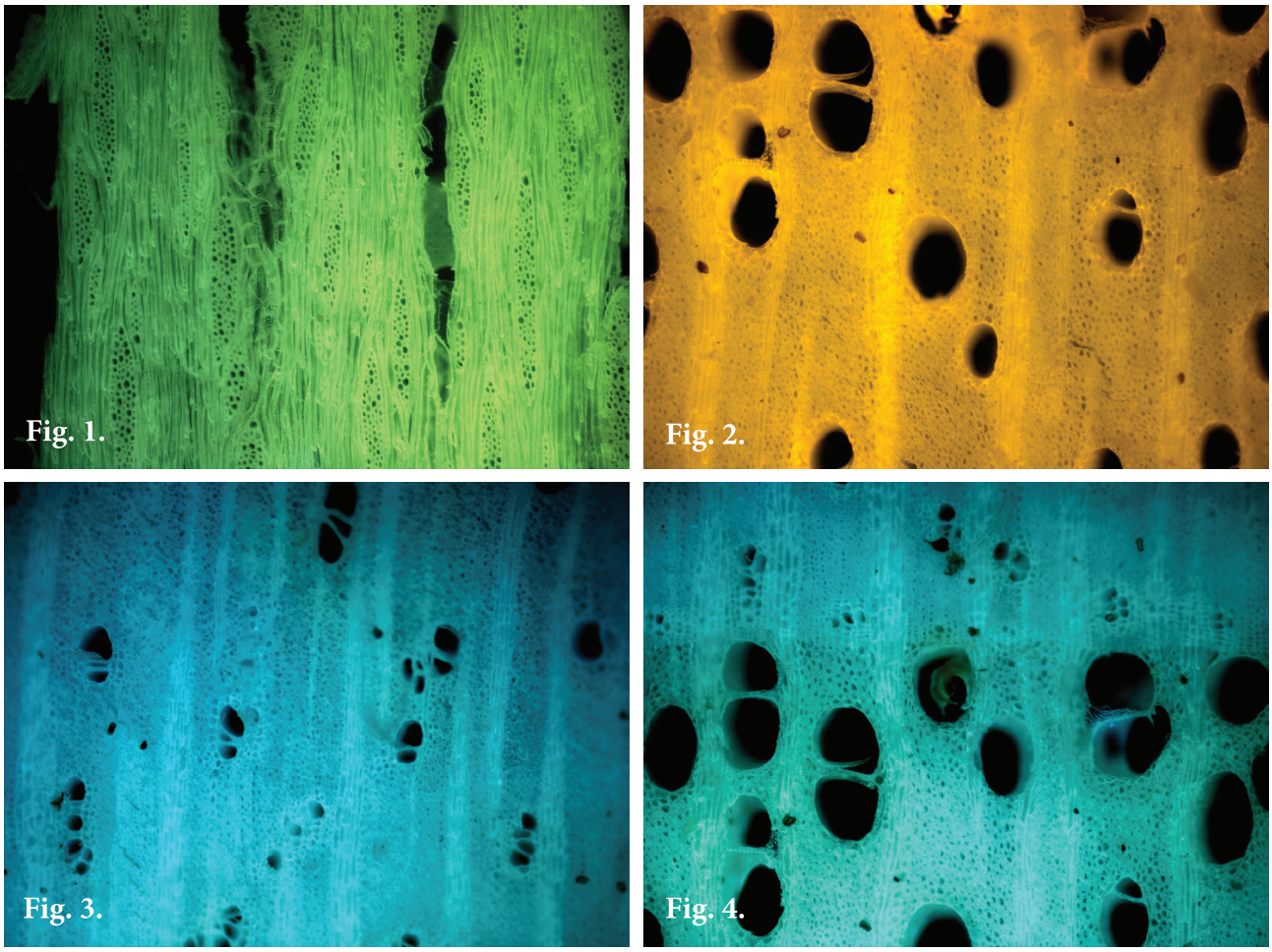

Fig. 1. Rays in tangential section, x 620., **Fig. 2.** Tracheas of the early zone, x 620
Fig. 3. Tracheas of the mature zone, x, **Fig. 4.** Transition from early wood to late wood zone, x

Tracheas of the early zone (Fig. 2) are oval, with quite wide lumens distributed individually or 2-3 in tangential ranges. The tracheas of the mature zone have narrower lumens, distributed in groups, making wavy or slant tangential ranges (Fig. 3).

The sapwood of iron wood is the outer layer of the xylem (trunk), which is located below the cambial ring, in which parenchyma cells live and through which water with dissolved mineral substances moves through the capillary elements (tracheas) from the roots towards the leaves. In addition to the capillary elements, the sapwood also makes radial and axial parenchyma which carries, processes and accumulates nutritive matters, and wooden fibers which have a mechanical function. The width of the sapwood in a 27-year-old tree is four stem rings. The average width of the trachea lumens in the stem rings of the sapwood early zone is 126.95 μm, while it is 29.02 μm in the mature zone. Sapwood early zone tracheas are quite large, so their number per area unit is smaller if we compare it to mature zone tracheas. The number of tracheas per mm^2 in the early zone amounts to 21.25, while this number for mature zone is 95.0.

Heartwood is the inner part of the xylem (trunk), located below the sapwood and has lost its physio-

Table 1. Basic statistics are shown through the following parameters: sample size (n), mean value, standard error (SE), coefficient of variation (CV(%)), as values of t-test, for the analyzed properties of iron wood *Gymnocladus canadensis* anatomy (T, R and (T+R)/2, two types (heartwood and sapwood), for early and mature phase within each of the types, as well as number of tracheas per mm^2).

Properties	n	Early phase			Mature phase			T-test
		mean	SE	CV%	mean	SE	CV%	
Heartwood								
T	690	69.76	1.23	46.92	22.02	1.07	127.43	-29.19****
R	690	85.15	1.64	50.58	24.80	0.40	43.89	-35.69****
(T+R)/2	690	77.46	1.34	45.62	23.41	0.64	71.82	-36.28****
Number of tracheas per mm^2	23	78.43	14.12	86.34	137.26	12.43	43.44	3.13***
Sapwood								
T	120	104.43	2.44	25.58	23.65	0.85	39.17	-31.30****
R	120	149.47	3.60	26.25	34.40	1.18	37.69	-30.51****
(T+R)/2	120	126.95	2.78	24.00	29.02	0.96	36.28	-33.27****
Number of tracheas per mm^2	4	21.25	1.93	18.18	95.00	20.07	42.26	-33.60**
T-test								
T			11.04****			0.63^{ns}		
R			15.30****			8.65****		
(T+R)/2			14.44****			3.54****		
Number of tracheas per mm^2			-4.01****			-1.35^{ns}		

* = significant at the 0.05 level, ** = significant at the 0.01 level,*** = significant at the 0.001 level, ns = non-significant

logical function. It is made by transforming one ring of growing stem sapwood into heartwood every year (Vilotić, 2000). The formation of heartwood occurs with the loss of the ability of the capillary elements (tracheas) to carry water and mineral matters and to accumulate organic matter. The average width of trachea lumens in the rings of a growing stem early zone is 77.46 μm, while this width is 23.41 μm in the mature zone. The number of tracheas per mm^2 is 78.43 in the early, and 137.26 in the mature zone, respectively (Fig. 4).

The anatomical properties of the trunk (xylem) are complex and they depend on a number of factors (Zhang, 1992; Vilotić et al., 1994; Vilotić, 2000; Hacke and Sperry, 2001). Esteban et al. (2010) state that ecological conditions of the environment have significantly affected the properties of the tree and tracheas. Venugopal and Liangkuwang (2007) state that there is an obvious correlation between climatic parameters and activities of cambium and formation of the xylem.

Habitat conditions with all their characteristics have a great influence on the macroscopic characteristics, microscopic structure, density, physical, mechanical and technological properties of a tree (Vilotić, 1994; Vilotić and Radošević, 2005; Šoškić and Popović, 2002).

The size of the sample (n), mean value and coefficient of variation (CV%), for trachea widths in tangential (T) and radial (R) directions, their mean value (T+R)/2 and number of tracheas per mm^2 are shown in Table 1. This table contains values of T-test of comparison between mean values and statistic significance of discrepancies. Mean values for all the anatomical properties of the iron wood *Gymnocladus canadensis* Lam., were smaller in the mature phase than in its early phase for both parts of

Table 2. The results of ANOVA (with parts of a tree, rings and phases found in rings) as factors which contribute to sources of variability in analyzed characteristics of iron wood *Gymnocladus canadensis* anatomy (T, R and (T+R)/2, number of tracheas per mm^2)

Characteristics / Sources of variability	T			R		(T+R)/2	
	df	MS(*10^3)	F-value	MS(*10^3)	F-value	MS(*10^3)	F-value
Parts of a tree	1	67.32	231.03****	279.22	733.53****	155.18	558.19****
Rings	25	25.56	87.72**	23.65	62.13****	17.13	61.62****
Phases (founds in rings)	27	53.67	184.19***	89.84	236.02****	70.29	252.82****
Error	1566	456.28		596.09		435.36	
Characteristics / Sources of variability	**number of tracheas (mm^2)**						
	df	MS(*10^3)	F-value				
Parts of a tree	1	16.85	4.66*				
Phases (rings)	1	50.3	13.91****				
Error	51	184.4					

the trunk – sapwood and heartwood. A statistically significant discrepancy was confirmed by the T-test. The number of tracheas had a reverse trend and was higher in the mature than in the early phase (137.26 vs 78.43, respectively) for heartwood and were statistically significant (t-value: 3.13, P<0.001). The same result was obtained for the sapwood: the early phase had fewer tracheas per mm^2 than the mature one, statistically significantly less, as well (t-value: 33.66, P<0.01).

Parts of the trunk (heartwood and sapwood) also exhibited statistically significant differences in the mean values of analyzed properties with respect to phases. For example, heartwood has statistically far smaller values of tree anatomy characteristics compared to the sapwood of the early phase (all t-values P<0.0001), while sapwood had statistically significantly lower value with respect to number of tracheas than the heartwood (21.25 vs 78.43; respectively, t-value: -4.01, P<0.001). In the mature phase, discrepancies between sapwood and heartwood were not so expressed: the only significant statistical discrepancy was obtained for the properties of R and (T+R)/2 (t-value: 8.65, P<0.001, and t-value: 3.54, P<0.001, respectively). The number of tracheas was also higher in the heartwood than in the sapwood (137.26 vs 95.00), but the statistical significance was not confirmed (t-value: -1.35).

Table 2 shows the results obtained in the analysis of variance for each of the analyzed properties. All the factors (parts of the trunk /heartwood, sapwood/, rings and phases /early, mature/) as possible sources of variability in this analysis of the anatomical properties of iron wood were statistically significant (all P < 0.0001). As for properties related to the number of tracheas, the analysis model for variances was different; it had only two factors – parts of the tree and phase – because values of trachea number were expressed with respect to rings. Each of the factors had a statistically significant share in the variation of the number of tracheas per mm^2.

CONCLUSIONS

Based on the conducted researches, the following may be concluded:

- there are differences in the width of the trachea lumens, as well as in the number of trachea per mm² between sapwood and heartwood;

- there are differences between trachea lumens and number of trachea in the early and mature zones of a tree, with respect to sapwood and heartwood as well;

- as a tree grows older, the widths of the trachea lumens increase, while their number per area unit (mm^2) decreases.

The first implemented analysis of the anatomical structure of iron wood contributes to the confirmation of its quality. The results obtained in research of the macroscopic and microscopic structures (capillary elements) of a tree show the good properties of the wood which supports the ideas of cultivating this species in Serbia. Bearing in mind that this species can propagate through vegetative and generative means, that it grows well (primary and secondary growth), that it is resistant to low temperatures and air pollution, we recommend it for the recultivation of degraded habitats.

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