Arch. Biol. Sci., Belgrade, 67(4), 1399-1404, 2015 DOI:10.2298/ABS150424118J

CORRELATIONS BETWEEN THE ANATOMICAL TRAITS OF *GYMNOCLADUS CANADENSIS* LAM. IN HEARTWOOD AND SAPWOOD OF EARLY- AND LATEWOOD ZONES OF GROWTH RINGS

Dušan Jokanović[1,*], Dragica Vilotić[1], Suzana Mitrović[2], Danijela Miljković[3], Milan Rebić[4], Dragica Stanković[1] and Vesna Nikolić[1]

[1] *Faculty of Forestry, University of Belgrade*, Belgrade, Serbia

[2] *Institute of Forestry*, Belgrade, Serbia

[3] *Department of Evolutionary Biology, Institute for Biological Research "Siniša Stanković", University of Belgrade*, Belgrade, Serbia

[4] *Provincial Secretariat for Agriculture, Water management and Forestry*, Novi Sad, Serbia

***Corresponding author**: dusan.jokanovic@sfb.bg.ac.rs

Abstract: This paper shows correlations between vessel characteristics and differences in growth-ring width in heartwood and sapwood. Analyzed samples were from an iron-wood tree (*Gymnocladus canadensis* Lam.) that grew in the Mužljanski Rit area, of the Srpska Crnja municipality in Serbia. According to previous research, it was deduced that *Gymnocladus canadensis* Lam. belongs to ring-porous species with big vessel lumen in the earlywood zone and thicker cell walls in the latewood. Vessels were more numerous in the latewood zone, and the same was true for heartwood and sapwood. For both layers, sapwood possessed a few more vessels than heartwood, and a statistically significant difference was confirmed by t-test during the early phase. The greatest negative value of correlation coefficient was between the number of vessels and growth-ring width during the early phase for sapwood. The number of vessels decreased in the wider growth rings. The correlation between growth-ring width and the area of vessels had a statistically significant positive value of correlative coefficient, which means that wider growth rings had larger vessel areas in the early phase for sapwood.

Key words: *Gymnocladus canadensis* Lam.; number of vessels; area of vessels; growth rings width; correlations

Received: April 24, 2015; **Revised:** May 19, 2015; **Accepted:** May 20, 2015

INTRODUCTION

Heartwood is defined as the inner layers of wood, which in a growing tree have ceased to contain living cells, and in which the reserve materials have been removed or converted into heartwood substance (Bass, 1985). In some species, heartwood may be distinguished from sapwood by a darker color, lower permeability and increased decay resistance (Taylor et al., 2002). Sapwood is defined by the International Association of Wood Anatomists (IAWA) as the portion of wood that in a living tree contains living cells and reserve materials.

As for wood quality, this characteristic and its stability commonly depend on its internal structure (Meerts, 2002). On a cross-section of wood, there are two layers forming the trunk and vary from each other in size and color. The external wood layers form the sapwood, while the inside part, located in the center of the trunk, represents the heartwood. The presence of heartwood is the main factor that determines wood quality. Heartwood and sapwood differ not only in color, but also in function, degree of humidity, and one of the important heartwood characteristics is the absence of cambial cells (Yang et al., 2004).

Sapwood determines the physiological properties of wood, while heartwood determines the hardness of wood. Ring-porous species like *Gymnocladus canadensis* Lam. (iron-wood) have normal distribution of vessels inside the growth rings with a bit brighter color in earlywood than in mature wood zone.

The aim of the paper was to determine relation between anatomical properties of vessel number and their area in both zones of iron-wood. Detailed analysis was performed in order todetermine whether the number of vessels and their area and growth-ring width vary between heartwood and sapwood. We also attempted to establish if there is some correlation between these characteristics and whether they are the same for both zones of wood.

MATERIALS AND METHODS

Study area

Iron-wood belongs to the family *Fabaceae*. It originates from North America, where it is located in large habitats. Optimal development and growth of the tree is provided by wet soils, valleys and mild slopes (Elias, 1980). In Serbia, its presence is connected mostly to Vojvodina. It is mainly found in green areas and tree alleys. There are also some mixed communities where *Gymnocladus canadensis* Lam. is found alongside acacia and common hackberry. There are well-preserved representatives of iron-wood in Fruška Gora, in the area of the Forestry Directorate of Erdevik.

Laboratory work

Wood samples for analysis originated from iron-wood trees located in the Mužljanski rit area. Parts of the wood were transported into the Laboratory of the Institute of Forestry in Belgrade, where all microscopic analyses, including all preparations for making permanent wood samples, were performed. Samples of wood parts were prepared with a width of 8 mm. These parts were taken in a north-south and east-west direction, respectively. Prepared material was fixed in formaldehyde-acetic:acid:ethyl alcohol in a ration of 1:1:18. Before making transversal, tangential and radial wood sections, samples were cooked in water in order to soften at 100°C. After softening, blocks of samples with a length of 18 mm in a radial direction were made. Each block was numbered from 1 to n, going from pith to bark. Sections of samples in all directions (transversal, tangential and radial) were made using a Reichert sliding-microtome. Thickness of all sections was 17-18 µm. Samples were colored with a combination of safranin and aniline for about

Table 1. Pearson's correlation coefficients (r) of anatomical traits are presented for *Gymnocladus canadensis* Lam tree. The values of correlation coefficients (as p-value) are shown for sapwood and heartwood separately. Above the diagonal are correlations coefficients for the early phase and below for the late phase. Underlined values indicate statistically significant correlations between analyzed traits.

	Sapwood	**Early**		
		Number of vessels (#/mm^2)	**Area of vessels** (mm^2)	**Width of growth rings** (mm)
Late	**Number of vessels** (# /mm^2)		-0.4130	**-0.7724**
	p		0.0501	0.0001
	Area of vessels (mm^2)	-0.0304		**0.4340**
	P	0.8905		0.0001
	Width of growth rings (mm)	-0.4238	-0.0726	
	P	0.4390	0.3978	

	Heartwood	**Early**		
		Number of vessels (# /mm^2)	**Area of vessels** (mm^2)	**Width of growth rings** (mm)
Late	**Number of vessels** (# /mm^2)		0.4163	0.5453
	P		0.5837	0.4547
	Area of vessels (mm^2)	-0.3451		0.2953
	p	0.6549		0.1615
	Width of growth rings (mm)	0.3637	0.1101	
	p	0.6363	0.6081	

5 min, and then transferred through alcohol solutions of different concentrations (50%, 70%, 96% and 100%) (Vilotić, 1992). The samples treated with different concentrations of alcohol solutions were conserved with Canada balsam. The last step was drying of the samples at a temperature of 60°C.

Measurements

Each growth ring was measured at its transversal cut, from pith to the bark: the width was measured and 30 vessels were found in both in the early- and latewood zones. Vessel width was measured in tangential and radial sections (Vilotić et al., 2011). The area of vessels was approximately determined by multiplying its width, which was measured in the two perpendicular directions. Apart from measuring the width of each growth ring, the width of both zones was measured. The number of vessels per 1 mm^2 was counted (Table 1).

Statistical analysis

Statistical analysis was performed using the PROC MEANS procedure of the SAS statistical package (SAS Institute, 2003). In this study, the following characteristics were analyzed: vessel number (number/mm^2), area of vessels (mm^2) and growth-ring width (mm) for the early- and latewood zones in both parts of the xylem – heartwood and sapwood. Statistically significant differences in the mean values for wood anatomical traits between early and late zones and within xylem parts – heartwood and sapwood – were determined using the t-test. Pearson's correlation coefficients between pairs of analyzed traits were calculated by the PROC CORR option from the SAS statistical package (SAS, version 9.3).

RESULTS AND DISCUSSION

The mean values of wood anatomy traits, such as (A) number of vessels, (B) area of vessels and (C) growth-ring width for *Gymnocladus canadensis* Lam. are shown in Fig.1. For both xylem parts, the pattern of number of vessels was the same; in the early zone, the number of vessels was lower than in the late zone. The differences between mean values of vessel number were statistically significant between the early and late zones within the heartwood (41.91 number/mm^2 vs. 106.61 number/mm^2; respectively) and sapwood (21.25 number/mm^2 vs. 95.00 number/mm^2; respectively): according the results of the t-test all were $p < 0.05$ (Table 1). The difference in number of vessels in the early zone between heartwood and sapwood was statistically significantly different (41.91 number/mm^2 vs. 21.25 number/mm^2; respectively) ($p < 0.05$, t-test), while in late zone that relation was similar (106.61 number/mm^2 vs. 95.00 number/mm^2).

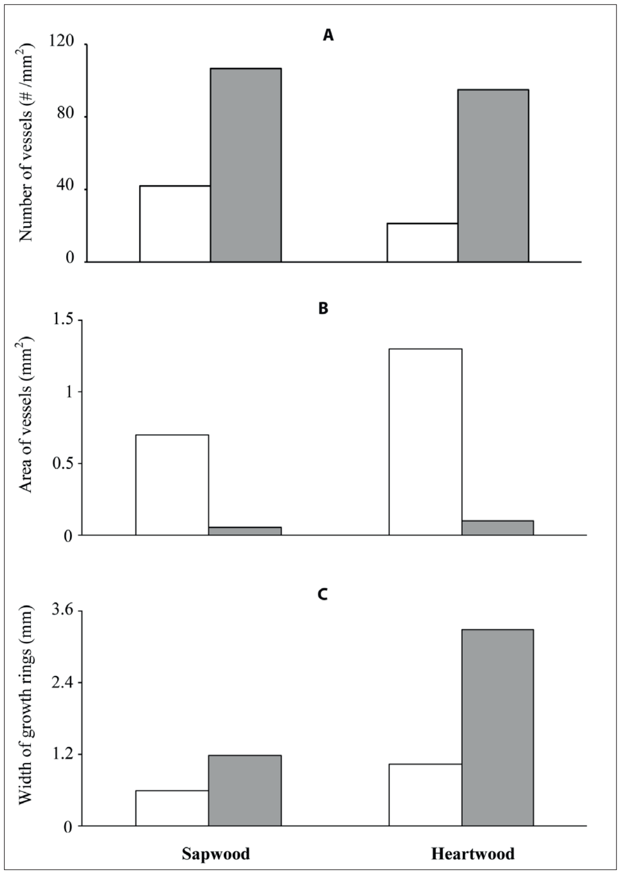

Fig.1. Histogram showing (A) mean value of number of vessels (number/mm^2) (Vilotić et al 2011), (B) area of vessels (mm^2) and(C) width of growth rings (mm) for sapwood and heartwood of a transverse section of *Gymnocladus canadensis* Lam. (open bars – early zone; dark bars – late zone)

The opposite pattern was observed for the area of the vessels in both parts of the xylem. In the late zone, vessels had a statistically significant smaller surface area (Table 1) in comparison with with that in the early zone for both parts of the xylem (a greater surface of vessel was observed in heartwood) (all comparisons p <0.05, t-test) (Table 1). The growth rings were wider in heartwood than in sapwood for both zones. The differences between zones within the xylem were statistically significant, according to t-test results (all p <0.05) (Table 1).

In Fig. 2, the cumulative contribution of each phase for both sapwood and heartwood is given where it can be concluded that the contribution of the late phase was greater than of that of the early phase in total growth-ring width for both parts of the trunk. The greatest negative value of correlation coefficient was between number of vessels and growth-ring width in the early phase for sapwood (r = -0.77; p = 0.0001) – the wider growth rings had a smaller number of vessels. The correlation between growth-ring width and area of vessels had a statistically significant positive value (r = 0.43; p = 0.001), which means that the wider growth rings had a greater vessel area in the early phase for sapwood (Table 1).

Difference in color and size of vessels enables the determining of the border between two zones. There are no cambial cells in heartwood, because its primary function is hardness of trunk. Therefore, heartwood is darker in comparison to sapwood, providing the outside layer of the trunk. For this reason, sapwood in ring-porous Angiosperms is usually bright (Vilotić, 2000). As for visibility of growth rings, they are very noticeable in *Gymnocladus canadensis* Lam., with a clear border between early- and latewood zones, which is a feature of other ring-porous species (Vilotić , 2000). Vessels of the early zone of *Gymnocladus canadensis* Lam. are oval with quite wide lumens distributed individually or 2-3 in tangential ranges. The vessels of the late zone have narrower lumens, distributed in groups, making wavy or slant tangential ranges (Vilotić et al., 2011). From the results of the research (Vilotić et al., 2011), it was concluded that the vessels in sapwood have wider lumens and brighter color and their cell walls are very thin. The main function of sapwood is to transport water and organic material, and the dominant elements for performing this process are the vessels of earlywood.

In this study, there were more vessels in sapwood than in heartwood. The physiological function of sapwood, particularly of its early phase, was confirmed by the presence of numerous vessels in that area. The total vessel area was bigger in the earlywood zone than in the latewood zone, both for sapwood and heartwood. This is confirmation that is valid for all ring-porous specie. where there is a regular distribution of vessels in comparison to diffuse-porous species with no expressed border between the early and mature phases (Vilotić, 2000). *Gymnocladus canadensis* Lam. has a brighter earlywood zone with bigger lumens of vessels, providing dor its bigger area, as in other ring-porous Angiosperms (*Quercus* sp., *Morus alba*, *Robinia pseudoacacia*, *Ulmus* sp.). Only in the early phase is there a statistically significant correlation between number of vessels and area.

Cochard and Tyree (1990), in their researth related to the dimensions of vessels of *Quercus* sp., considered its length and diameter (width). On the basis of this research, they concluded that latewood vessels made up the majority of vessels less than 4 cm long. In current-year shoots, there was no clear difference between earlywood and latewood vessels. The first-formed vessels nearest the pith were as small as the latewood vessels; vessels near the middle of the wood ring were largest. In all other rings, earlywood and latewood vessels were clearly distinguishable. In comparison with *Gymnocladus canadensis* Lam., the dimensions of *Quercus* sp. vessels – width and length – are much greater than those of *Gymnocladus canadensis* Lam., and, as a result, the approximate area of vessels based on these dimensions of *Quercus* sp. is much bigger than that of *Gymnocladus canadensis* Lam.. Vessels of *Quercus* sp. are particularly wide in the earlywood zone, and their number is lower than in the earlywood zone of *Gymnocladus canadensis* Lam.. Vessels in current-year shoots of *Quercus alba* and *Quercus rubra* L. were about the same, or up to 30% larger in diameter than vessels found in the third to fifth annual rings of *Acer saccharum* Marshall, *Rhizophora mangle* and *Cassipourea elliptica* (Sw.) Poir, (Sperry and Tyree, 1988; Sperry et al., 1988 a, 1988 b). In another study (Adamopoulos and Voulgaridis, 2002), the length and diameter (width) of vessels in *Robinia pseudoacacia* L early- and latewood were measured. For earlywood, vessel member length and diameter were 0.16 mm and 47μm, respectively. For latewood, vessel member length and diameter were 0.18 mm and 24 μm, respectively. The differences were statistically significant (t-test, $p < 0.05$) in all cases. With regard to other ring-porous species, a study of ash (*Fraxinus excelsior* L.) has shown that earlywood vessel members showed a similar pattern of length variation, but latewood vessel members did not change (Frey-Wyssling and Bosshard, 1959). In a radial direction, the vessel member diameter of earlywood increased in the first growth rings near the pith and then remained stable towards the bark. Other ring-porous species, such as oak (*Quercus petraea* Liebl.) and ash (*Fraxinus excelsior* L.) have also been reported to show a similar radial trend in earlywood vessel diameter (Helinska Raczkowska, 1994).

The distribution of vessels depends on growth-ring width, as well as on wood zone. Similar to the earlywood of *Quercus* sp., vessels are in tangential groups, while latewood vessels are located in radial direction (Gajić et al., 1992). On the other hand, earlywood vessels of *Gymnocladus canadensis* Lam. are in regular groups, while latewood vessels form nests. As regards diffuse-porous species like *Fagus* sp., vessels are located in regular groups with wider lumens in the earlywood zone compared to the latewood zone where distribution of vessels is irregular and its lumens narrower (Stojanović, 2005). On the other side, the number of vessels was bigger in the late than in the early zone. The conclusion confirmed results of previous research (Vilotić et al., 2011), with 78.43 vessels/mm^2 in the early zone, and 137.26 in the late zone, respectively, for *Gymnocladus canadensis* Lam.. As regards the contribution of both zones to total growth-ring width, there is apparently a bigger participation of the late phase. This is confirmed by the mechanical properties of *Gymnocladus canadensis* Lam. (Fig. 2).

There is an obvious correlation between climatic parameters and activities of the cambium and formation of the xylem (Venugopal and Liangkuwang, 2007). According to the presented results, there is a remarkable increased participation of the late zone, which means that *Gymnocladus canadensis* Lam. is a hardwood with emphasized mechanical properties. The larger portion of latewood inside the growth ring makes this species for good for technical purposes, such as wood for constructions in civil engineering, and for the covering of roofs. The presence of wider late zone within the growth ring in comparison with the early zone provides better mechanical characteristics of *Gymnocladus canadensis* Lam. and, consequently, greater technical capacity. Lignified cell walls within a latewood zone are responsible for the mechanical and technical features of this species.

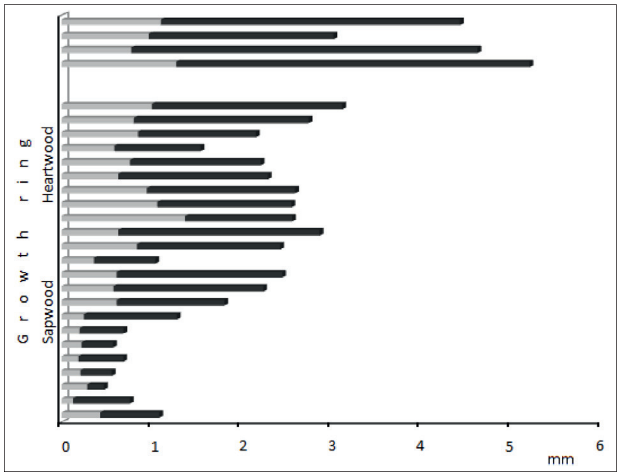

Fig. 2. Mean cumulative growth-ring widths on transect of *Gymnocladus canadensis* Lam. The first 23 growth rings represent sapwood, and the last 4 growth rings represent heartwood. Every bar refers to parts corresponding to the early phase (open bar), and parts corresponding to late phase within the growth rings (dark bars). Growth rings width is expressed in mm.

On the basis of our research, it can be concluded that the larger width of the late zone, particularly in heartwood (Fig. 1C and Fig. 2), contributes to the mechanical and technical characteristics of the *Gymnocladus canadensis* Lam. tree. This is related to the role of the heartwood in the trunk, which determines the hardness of the wood. In addition, the number of vessels and the area inside wood zones have reverse relations to each other. In the early zone, vessels are much bigger (with greater surface), but their number is lower in comparison to the late zone, which is characterized by more numerous vessels with a smaller surface (Fig. 1A and 1B).

Acknowledgments: This paper was done as part of the project entitled Establishing of Wood Plantations intended for Afforestation of Serbia (31041), financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia within the framework of technological development for the period 2011-2014.

Author's contribution: Dušan Jokanović wrote following chapters: Introduction, Materials and Methods, and Discussion. He also translated the whole paper into English. Dragica Vilotić designed the whole study. Danijela Miljković performed the statistical part of the paper, including all analyses of data. Suzana Mitrović and Milan Rebić did all the necessary measurements. Dragica Stanković and Vesna Nikolić did all the practical work related to outside research.

Conflict of interest disclosure: We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES

Adamopoulos, S. and *E. Voulgaridis (*2002). Within-tree variation in growth rate and cell dimensions in the wood of black locust (*Robinia pseudoacacia*). *IAWA J.* **23,** 191-199

Bass, P. (1985). A new multilingual glossary of terms used in wood anatomy. *IAWA J*. **6**, 83-83

Cochard, H. and *M.T. Tyree (*1990). Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiol*. **6**, 393-407

Elias, T.S. (1980). The Complete Trees of North America, Field Guide and Natural History, Van Nostrand Reinhold Co., 948, New York

Frey-Wyssling, A. and *H.H. Bosshard (*1959). Cytology of the ray cells in Sapwood and Heartwood. *Holzforschung*. **13**, 129-137

Gajic, M., Kojic, M., Karadzic, D., Vasiljevic, M. and *M. Stanic (*1992). Vegetacija Nacionalnog parka Tara, Šumarski fakultet-Beograd, Nacionalni park-Tara, Bajina Bašta, 267-273

Helinska Raczkowska, L. (1994). Variation of vessel lumen diameter in radial direction as an indication of juvenile wood growth in oak (*Quercus petraea* Liebl.). *Ann.Sci.For*. **51**, 283-290

Meerts, P. (2002). Mineral nutrient concentrations in sapwood and heartwood: a literature review. *Ann.Sci.For*. **59**, 713-722

SAS Institute. (2003). SAS/STAT user's guide, version 9.1.3, SAS Institute Inc., Cary, N.C.

Sperry, J.S. and *M.T. Tyree (*1988). Mechanism of water stress-induced xylem embolism. *Physiol. Plant*. **88**, 581-587

Sperry, J.S., Donnelly, J.R. and *M.T. Tyree (*1988a). Seasonal occurrence of xylem embolism in sugar maple (*Acer saccharum*). *Am. J. Botany*.**75**, 1212-1218

Sperry, J.S., Tyree, M.T. and *J.R. Donnelly (*1988b). Vulnerability of xylem to embolism in a mangrove *vs*. an island species of *Rhizophoraceae*. *Physiol. Plant*. **74**, 276-283

Stojanović, Lj., Isajev, V., Karadzić, D., Bajić, V., Šoškic, B., Krstić, M., Medarević, M., Ranković, N. and *R. Cvjetićanin (*2005). Bukva u Srbiji, monografija, Udruženje šumarskih inženjera i tehničara, Beograd, Šumarski fakultet Univerziteta u Beogradu, Beograd , 373-382

Taylor A., Gartner B., J. Morrell (2002). Heartwood formation and natural durability: A review. *Wood Fiber Sci*. **34**, 587-611

Tešić, Ž., Vilotić, D. and *M. Gajić (*1992). Prilog rasprostranjenju i mikrosistematici kitnjaka *Quercus petraea* (Matt.) Liebl. In: Monography: „Vegetacija Nacionalnog parka Tara" (M. Gajić, M. Kojić, D. Karadžić, M. Vasiljević, and M. Stanić), 267-273. Šumarski fakultet - Beograd, Nacionalni park Tara - Bajina Bašta

Venugopal N. and *M.G. Liangkuwang* (2007). Cambial activity and annual rhythm of xylem production of elephant apple tree (*Dyllenia indica* Linn.) in relation to phenology and climatic factor growing in sub-tropical wet forest of north-east India. *Trees Struct. Funct*. **21**, 101-110

Vilotić, D. Anatomska građa stable virgilijskog hrasta (*Quercus virgiliana* /Ten/Ten.) na različitim staništima Deliblatske peščare. [dissertation]. [Belgrade]: Faculty of Forestry, University of Belgrade. 1992. 117 p

Vilotić D. (2000). Uporedna anatomija drveta. Faculty of Forestry, University of Belgrade.

Vilotić, D. and *G. Radošević (*2005). Anatomska građa stable bukve. In: Monography "Bukva u Srbiji" (Lj. Stojanović, V. Isajev, D. Karadzić, V. Bajić, B. Šoškić, M. Krstić, M. Medarević, N. Ranković, and R. Cvjetićanin), 373-382. Udruženje šumarskih inženjera i tehničara Srbije, Beograd

Vilotić, D., Šijačić-Nikolić, M., Miljković, D., Ocokoljić, M. and *M. Rebić (*2011). Comparative analysis of the anatomical structure of heartwood and sapwood selected *Gymnocladus canadensis* Lam. trees in Srpska Crnja. *Arch.Biol.Sci*. **63**, 831-836

Yang, J., Kamdem, J. D., Keathley, D. E. and *K. H. Han* (2004). Seasonal changes in gene expression at the sapwood-heartwood transition zone of black locust (*Robinia pseudoacacia*) revealed by cDNA microarray analysis. *Tree Physiol*. **24**, 461-474