

THE RELATIONSHIP BETWEEN THE END OF THE VEGETATION  
PERIOD AND FRUIT PROPERTIES IN THE WALNUT  
POPULATION IN EASTERN SERBIA

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**Abstract:** The main objective of the study was to examine the walnut population from the region of Eastern Serbia for determining the relationship between the end of the vegetation period and fruit properties (fruit weight, kernel percentage, oil and crude protein contents). The walnut trees showed highly significant differences in the tested parameters. The walnut population was dominated by trees with a medium end of the vegetation period, moderate resistance to low temperatures (39.5%) and very small fruits (23.5%). The fruits of these trees had a high kernel percentage (15.8%) and moderate oil and crude protein contents (25.8% and 38.3%, respectively). Contrary, the walnut population comprised the fewest trees of late ending of the vegetation period, with extremely large fruits (0.1%), exceptional kernel percentage (0.2%) and pronouncedly high oil and crude protein contents (0.07% and 0.5%, respectively). The studied walnut trees show great variability in the date of the end of vegetation and fruit properties, which indicates a high potential of the population in the selection of new genotypes, and the necessity for their conservation for further breeding, propagation and commercial cultivation.

**Key words:** *Juglans regia* L., end of the vegetation period, fruit characteristics.

### Introduction

In Serbia, the natural walnut populations are highly diverse since walnut has been propagated by seeds for years. Generative propagation accounts for the populations with pronounced biotype polymorphism, which makes the walnut a monoecious, anemophilous and dichogamous fruit species. The existence of natural walnut populations is very important, because it enables the collection, selection and hybridisation of genotypes, which therefore ensures a high quality selection aimed at breeding new cultivars.

Within phenological studies, the end of the growing season is the one of the most significant phases in the life of the walnut and is closely associated with the

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period of dormancy. A favourable time of the end of the growing season ensures good plant performance during dormancy and its resistance to low winter temperatures (Miletić, 2009). Korać and Cerović (1980) reported that in the walnut population there is a positive correlation between the time of leaf bud burst and the time of leaf fall, as well as a correlation between the end of the growing season and resistance to low winter temperatures. Therefore, the end of vegetation before the first autumn frost is an important trait for all plant populations, including walnut (Korać et al., 1990).

Additionally, high variability in fruit characteristics has been recorded in the walnut populations. Zeneli et al. (2005) and Sharma et al. (2010) noted a high range of variability observed for various fruit characters in native walnut populations. Also, Cerović et al. (2017) found that walnut populations in various parts of the world show not only great variability in the duration of the vegetative period and the time of leaf fall, but also in fruit weight, kernel percentage, etc.

Given the above, the objective of this investigation was to determine the relationship between the end of the vegetation period and major fruit characteristics (fruit weight, kernel percentage, oil and crude protein contents) in walnut seedlings from the natural population.

### Material and Methods

The study was carried out on five localities (Zaječar, Knjaževac, Negotin, Mali Izvor and Sokobanja) in Eastern Serbia, during 2008–2010. Over 1,464 walnut trees of generative origin were randomly collected. All the trees studied were of seedling origin.

The paper shows the end of the vegetation period of walnut and the main fruit properties. The description of the fruit characteristics is given based on the descriptor for the walnut from the International Union for the Protection of New Varieties of Plants (UPOV, 1999).

Fruit weight was classified in five categories: very small (>8.0 g), small (8.1–10.0 g), mid-large (10.1–12.0 g), large (12.1–14.0 g) and very large (<14.1 g), while kernel percentage was presented in seven categories: extremely low (>30%), very low (30.1–35.0%), low (35.1–40.0%), medium (40.1–45.0%), high (45.1–50.0%), very high (50.1–55.0%) and extremely high (<55.1%). Fruit weight was measured by using a 'Mettler' technical scale. Kernel percentage was calculated based on fruit and kernel weight.

The kernel oil content was determined by the NMR (nuclear magnetic resonance) method (AOAC, 1995) and presented in four categories: low (>50%), medium (50.1–60.0%), high (60.1–70.0%) and very high (<70.1%). The crude protein content was identified by the micro-Kjeldahl method (Jung et al., 2003), and the samples were classified in three categories: kernel with low (up to 15.0%), medium (15.1–20.0%) and high crude protein content (>20.1%).

The time of the end of the growing season is presented in three categories: early (1), medium (2) and late (3). The resistance of walnut trees to low winter temperatures was determined based on the percentage of frozen buds during the winter. The assessment of the damage caused by the low winter temperatures included examining of the top and lateral buds, plant monitoring before the growing period onset, and examining the results during the growing period onset (the number of inactivated buds). According to the results, walnut trees are described as those with high resistance (1), moderate resistance (2), and low resistance (3), respectively, to low winter temperatures.

The paper presents the average three-year results, including the share (%) of each trait in the total number of trees. The data obtained were statistically processed by analysis of variance and the Duncan's multiple range test.

### Results and Discussion

Non-uniform flowering time and inflorescence development encourage cross-pollination in the walnut, which induces the development of an abundant and heterogeneous population exhibiting different pomological properties (Miletić, 2004). Regardless of the pronounced heterogeneity within the walnut population, there is a considerable relationship among particular phenophases, given that the span of the growing season may be the limiting factor to the overall performance of the walnut tree (Miletić, 2009; Miletić et al., 2010). The studied population was dominated by trees with a medium time of leaf fall (52.39%), then with an early time of leaf fall (34.97%), whereas a late time of leaf fall was the least prevalent (12.64%). These data are consistent with the results of Korać et al. (1990), who found that walnut populations are dominated by trees with a medium end of the growing season (55%), followed by those with an early period (28%), while trees with the late end of vegetation are the least prevalent (17%). Moreover, Cerović et al. (2017) indicated that the walnut population in the Western Balkans has the most genotypes of the early time of leaf fall (about 51%), and the fewest genotypes with a late time of leaf fall (about 17%).

Table 1. The relationship between the end of the growing season and the resistance to low winter temperatures.

End of vegetation	Trees in the population (%)			Total (%)
	High resistance	Moderate resistance	Low resistance	
Early	1.09	25.68	8.2	34.97
Medium	0.89	39.50	12.0	52.39
Late	0.14	5.30	7.2	12.64
Total (%)	2.12	70.48	27.4	100.0

With regard to low winter temperatures, the fewest were the trees exhibiting high resistance to low winter temperatures (2.12%), followed by low-resistant trees (27.4%), whereas there were mostly trees of medium resistance (70.48%). Generally, in the walnut population, the most prevalent genotypes were those with the medium end time of the vegetation and moderate resistance to low winter temperatures, while the smallest number of trees were those with the late end of the vegetation and high resistance. Agro-environmental conditions have a major impact on walnut performance in natural populations (Korać, 1998). A new set of genes is thus created and populations change to adapt to the existing environmental conditions (Borojević, 1986).

Fruit quality in walnut populations is quite non-uniform, which is the result of un-cultivated production. In the present study, the fruits were mainly very small and small, whereas those with large and very large fruits were in the minority (Table 2).

Table 2. The relationship between the end of the vegetation period and the fruit weight of the walnut (number of trees in the population in %).

End of vegetation	Fruit weight (g)					Total (%)
	>8.0	8.1–10.0	10.1–12.0	12.1–14.0	<14.1	
Early	16.0	12.4	5.6	1.8	1.6	37.4
Medium	23.5	16.0	7.5	2.0	1.0	50.0
Late	7.2	3.9	0.9	0.5	0.1	12.6
Total (%)	46.7	32.3	14.0	4.3	2.7	100.0

Trees of the medium ending of the vegetation had very small (23.5%) and small fruits (16.0%), while the percentage of large and very large fruits was significantly lower (2.0% and 1.0%, respectively). In trees that early end the vegetation period, the percentage of small and very small fruits (16.0% and 12.4%, respectively), as well as of large and very large fruits (1.8% and 1.6%, respectively), was higher than in the mid-season trees. The lowest percentage of very small and small fruits (7.2% and 3.9%, respectively), and of large and very large fruits (0.5% and 0.1%, respectively) was recorded in trees whose vegetation period end quite late. A considerable percentage of trees with small and very small fruits is indicative of population specificity and its high variability, which is comparable to the research by Korać et al. (1990), who reported that the walnut population grown on Fruška Gora is dominated by trees with small fruits (2.2%), while those with very large fruits are the fewest (7.2%). Also, Cerović et al. (2017) observed that most genotypes in the walnut population have very small (28.6%) and small (32.2%) fruit sizes. Namely, Zeneli et al. (2005) indicated that, in native trees which were of seedling origin, significant genetic variation was found in pomological characteristics, with fruit weights ranging from 3.8 to 21.1 g.

In terms of kernel percentage, the data in Table 3 reveal that fruits with medium, high and low kernel contents (29.0%, 28.7% and 16.0%, respectively) were predominant in the population studied, whereas the share of fruits with extremely low kernel content was the lowest (2.2%). Fruits with medium kernel percentage were mostly found in trees with the early ending of vegetation, while trees with mid-season ending were dominated by fruits with medium, low and high kernel percentages. Kernel percentage in walnut trees with the late ending of the growing season varied from 0.2 to 3.9%.

Table 3. The relationship between the end of the vegetation period and kernel percentage in walnut fruits (number of trees in the population in %).

End of vegetation	Kernel percentage (%)						Total (%)	
	>30.0	30.1–35.0	35.1–40.0	40.1–45.0	45.1–50.0	50.1–55.0		<55.1
Early	0.8	1.7	5.1	11.1	11.3	6.1	1.3	37.4
Medium	0.9	3.5	7.0	14.6	15.8	8.1	2.0	51.9
Late	0.5	0.2	3.9	3.3	1.6	1.0	0.2	10.7
Total (%)	2.2	5.4	16.0	29.0	28.7	15.2	3.5	100.0

Cerović et al. (2017) reported that only one third of genotypes have a kernel percentage of 40% and above, while 9.3% have high and very high kernel percentages. According to Paunović and Miletić (2013), walnut populations consist of genotypes with kernel percentages ranging from 20.2% to 65.6%. Many studies have found similar variability in fruit weight and kernel weight, as well as kernel percentage in fruits from the natural walnut populations (Sharma and Sharma, 2001; Draganescu et al., 2001; Botu et al., 2001).

With respect to oil content, the obtained results show that there is a significant relationship between the end of the vegetation period and the oil content in the kernel (Table 4).

Table 4. The relationship between the end of the vegetation period and the oil content in the kernel (number of trees in the population in %).

End of vegetation	Oil content (%)				Total (%)
	up to 50.0	50.1–60.0	60.1–70.0	<70.1	
Early	1.8	17.7	15.4	0.5	35.4
Medium	2.4	25.8	22.9	0.6	51.7
Late	1.0	7.03	4.8	0.07	12.9
Total (%)	5.2	50.53	43.1	1.17	100.0

The results show that most of the fruits were evidenced with medium (50.53%) and high oil content in the kernel (43.1%), while fruits with low (5.2%) and very high oil content (1.17%) were fewer. The total oil content in the fruits

was the highest in trees of the medium ending of vegetation (51.7%), and the lowest in those of the late ending of vegetation (12.9%).

Regarding crude protein content, fruits with medium protein content predominated in all categories studied (Table 5). The highest total protein content in the kernel was found in trees of the medium ending of the vegetation season (53.6%), and the lowest in fruits with the late ending of vegetation (12.2%), which is consistent with the results of Ozkan and Koyuncu (2005), Sharma et al. (2014) and Jaćimović et al. (2020), who recorded that in the walnut population the average crude protein content in the kernels ranges from 13.6% to 19.2%, while the oil content varies from 62.0% to 70.9%. According to Miletić (2004), the oil content in the walnut kernel is higher in dry years and lower in years with heavier rainfall, while the opposite regularity is observed for the crude protein content in the kernel.

Table 5. The relationship between the end of the vegetation period and the crude protein content in the kernel (number of trees in the population in %).

End of vegetation	Protein content (%)			Total (%)
	up to 15.0	15.1–20.0	<20.1	
Early	8.8	23.4	2.0	34.2
Medium	12.6	38.3	2.7	53.6
Late	2.6	9.1	0.5	12.2
Total (%)	24.0	70.8	5.2	100.0

### Conclusion

The results of the present research reveal that there is no strict relationship between the end of the vegetation period and fruit characteristics. The walnut population was dominated by trees with the medium ending of vegetation and moderate resistance to low temperatures, which can be considered an important characteristic of the population. In this way, good conditions for winter dormancy and higher resistance to low winter temperatures are provided, preventing damage due to late autumn frosts.

In all studied categories, the fruits were predominantly very small and small, while large and very large fruits were found on a small number of trees. Fruits with high and medium kernel percentages accounted for 57.7% of the total number of trees, which is a very significant property and an important parameter of the quality of the walnut population, especially from the standpoint of economics.

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## References

- AOAC (1995). Official Methods of Analysis, 16th ed.; Association of Official Agricultural Chemists: Washington, DC, USA.
- Botu, M., Botu, I., Achim, G.H., & Godeanu, I. (2001). Genetic variability of the *Juglans regia* L. natural populations from Oltenia–Romania. *Acta Horticulturae*, 544, 149-154.
- Borojević, K. (1986). *Genes and populations*. Forum press, Novi Sad. (In Serbian).
- Cerović, S., Gološin, B., Bijelić, S., & Bogdanović B. (2017). Walnut biodiversity in the Western Balkans. *Agriculture & Food*, 5, 202-216.
- Draganescu, E., Nedelea, G., Mihut, E., & Blidariu, A. (2001). Researches concerning the germplasm variability of walnut (*Juglans regia*) existing in Banat, Romania. *Acta Horticulturae*, 544, 133-140.
- Jaćimović, V., Adakalić, M., Ercisli, S., Božović, Dj., & Bujdoso, G. (2020). Fruit quality properties of walnut (*Juglans regia* L.) genetic resources in Montenegro. *Sustainability*, 12, 9963.
- Jung, S., Rickert, D.A., Deak, N.A., Aldin, E.D., Recknor, J., Johnson, L.A., & Murphy, P.A. (2003). Kjeldahl and Dumas methods for determining protein contents of soybean products. *Journal of the American Oil Chemists' Society*, 80, 1169-1173.
- Korać, M., & Cerović, S. (1980). Resistance of walnut tree to frost under continental climatic conditions. *Journal of Yugoslav Pomology*, 14, 245-249. (In Serbian).
- Korać, M., Cerović, S., Gološin, B., & Miletić, R. (1990). Population variability of domestic walnut and selection results. *Journal of Yugoslav Pomology*, 24, 3-10. (In Serbian).
- Korać, M. (1998). *Walnut*. Prometej, Novi Sad. (In Serbian).
- Miletić, R. (2004). *Population and assortment of walnut trees in the region of Timocka Krajina*. Endowment Andrejevic, Belgrade. (In Serbian).
- Miletić, R. (2009). Phenological correlations in the walnut population. *Plant Science* 46, 214-218.
- Miletić, R., Mitić, N., & Nikolić, R. (2010). Fruit characteristics in walnut tree population in relation to growing season onset. *Genetika*, 42, 493-500.
- Ozkan, G., & Koyuncu, M.A. (2005). Physical and chemical composition of some walnut (*Juglans regia* L) genotypes grown in Turkey. *Grasas y Aceites* 56, 141-146.
- Paunović, M.S., & Miletić, R. (2013). *Walnut*. Fruit Research Institute, Čačak, Republic of Serbia. (In Serbian).
- Sharma, O.C., & Sharma, S.D. (2001). Genetic divergence in seedling trees of Persian walnut (*Juglans regia* L.) for various metric nut and kernel characters in Himachal Pradesh. *Scientia Horticulturae*, 88, 163-171.
- Sharma, O., Liaqat Ali, C., Ghanai, N.A., & Sandeep, K. (2010). Variability and nut characteristics of seedling walnuts (*Juglans regia* L.) growing in Ladakh Region of India. *Research Journal of Agricultural Science*, 1, 219-223.
- Sharma, M.R., Kour, K., Singh, B., Yadav, S., Kotwal, N., Rana, C.J., & Anand, R. (2014). Selection and characterization of elite walnut (*Juglans regia* L.) clone from seedling origin trees in North Western Himalayan region of India. *Australian Journal of Crop Science*, 8, 257-262.
- UPOV (International union for the production of new varieties of plants) (1999). *Walnut (Juglans regia L.) Guidelines for the Conduct of Test for Distinctness, Uniformity and Stability*; Int. Union Production New Varieties Plants: Geneva, Switzerland.
- Zeneli, G., Kola, H., & Dida, M. (2005). Phenotypic variation in native walnut populations of northern Albania. *Scientia Horticulture*, 105, 91-100.

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## ODNOS ZAVRŠETKA VEGETACIJE I OSOBINA PLODA U POPULACIJI ORAHA U ISTOČNOJ SRBIJI

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### R e z i m e

Osnovni cilj istraživanja bio je proučavije populacije oraha sa područja istočne Srbije radi utvrđivanja odnosa između završetka vegetacionog perioda i osobina ploda (masa ploda, sadržaj jezgre, sadržaj ulja i sirovih proteina). Stabla oraha su pokazala značajne razlike u ispitivanim parametrima. U populaciji oraha dominirala su stabla srednjeg vremena završetka vegetacije, umerene otpornosti prema niskim zimskim temperaturama (39,5%) i veoma sitnih plodova (23,5%). Plodovi ovih stabala imali su veliki randman jezgre (15,8%), i srednji sadržaj ulja (25,8%) i sirovih proteina (38,3%). Nasuprot tome, u populaciji je bilo najmanje stabala oraha poznog vremena završetka vegetacije, sa ekstremno krupnim plodovima (0,1%), visokim randmanom jezgre (0,2%) i izrazito visokim sadržajem ulja (0,07%) i sirovih proteina (0,5%). Proučavana stabla oraha pokazuju veliku varijabilnost u pogledu vremena završetka vegetacije i osobina ploda, što ukazuje na visok potencijal populacije u selekciji novih genotipova, kao i na neophodnost njihovog očuvanja u cilju daljeg oplemenjivanja, razmnožavanja i komercijalnog gajenja.

**Ključne reči:** *Juglans regia* L., završetak vegetacije, karakteristike ploda.

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