

MAHONIA AQUIFOLIUM (PURSH) NUTT.: VARIETIES GLOBOSA AND TEARS IN THE URBAN DENDROFLORA UNDER CLIMATE CHANGES

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Abstract. *Mahonia aquifolium* (Pursh) Nutt. (MA), from the *Berberidaceae* family, is an indigenous species of North America that was introduced to Europe for ornamental purposes. With the expansion of the food industry, it has been classified as a non-traditional crop for industrial use. Chemical analyses of its fruit mesocarp confirmed significant quantities of bioactive compounds, suggesting broader potential applications. This study analyzed the genetic structure and variability of secondary populations, as well as the species' role in providing ecosystem services and contributing to the stability of urban parks, supporting the diversity of shrub-dominated tree flora. An integrative approach, focusing on fruiting patterns and climatic variables, was used. From 30,816 phenological observations, the sustainability of the new varieties was confirmed, with earlier fruit ripening occurring within the appropriate accumulated heat sums under climate change conditions. Biochemical studies confirmed the significant role of both varieties in preserving urban health, revealing substantial levels of bioactive compounds and antioxidant capacity. Ethanol proved to be a more effective solvent for extracting phenols, tannins, flavonoids, and anthocyanins, with antioxidant properties strongly linked to phenolic compounds. The vitamin C content was higher in the *globosa* variety, highlighting the importance of genotypic and morphological traits. Both varieties serve as multifunctional fruits for consumption and medicinal use. The presence of anthocyanins makes the berries suitable for extracting natural food colors. Regarding ecosystem services, both MA genotypes, as adaptive taxa, provide shelter, nesting, and resting sites for birds. Their fruits play a vital role in the food chain, particularly in Important Bird Areas.

Keywords: *genetic diversity, phenological patterns, morphometry analysis, fruit, bioactive compounds, antioxidant activity*

Introduction

Mahonia aquifolium (Pursh) Nutt. (MA) is an invasive evergreen shrub native to western North America. It has spread from the Pacific coast to Montana and Idaho (Ross and Auge, 2008), as well as to Australia and Europe since 1822, when it was introduced for horticultural purposes (Ross, 2009). *M. aquifolium* used in landscape design and for medicinal purposes (Auge and Brandl, 1997;), particularly in areas with heavy traffic or other pollutants due to its high resistance to contamination (Samecka-Cymerman and Kempers, 1999). It thrives best in rich, well-drained soils with a pH of 6–8, but can also grow successfully in poor soils (Ocokoljić and Petrov, 2022). It is drought-tolerant and resistant to strong sunlight and extremely low temperatures (Hudek, 2005). Although Serbia is not a center of origin for MA, the species has been identified in gardens, parks, and elements of urban green infrastructure along roadways.

MA contains secondary metabolites in all parts: leaves, flowers, fruits, seeds, roots, and bark (Mathew and Abraham, 2006), as well as other pharmacological compounds that

make it useful in traditional medicine (Rackova et al., 2004). Research on the bioactive components of MA has been conducted on its roots, seeds, and bark (Košťálová et al., 1981, 1986), as well as on raw flowers (Slavík et al., 1985). The species is a source of phenols, flavonoids, anthocyanins, and antioxidants (Pyrkosz-Biardzka et al., 2014; Andreicut et al., 2018), which exhibit antimicrobial and antibacterial properties (Slobodniková et al., 2004), anticancer effects (Damjanović et al., 2020), anti-mutagenic activity (Čerňáková et al., 2002), anti-inflammatory effects (Andreicut et al., 2018, 2019), and benefits for skin disorders (Gieler et al., 2009).

Due to its abundant fruiting, MA provides significant ecosystem services and serves as a food source for birds (Houtman et al., 2004). Its fruits are used in the food industry (Ocokoljić and Petrov, 2022), and in North America, it is valued as a berry fruit species with a long tradition of use in the diets of Indigenous communities (Abrams, 1950). Considering all the above, as well as the fact that exogenous antioxidants support the body's fight against free radicals, the polyphenols in its fruits are highlighted for their health-promoting properties (Liu, 2003). Additionally, in the food industry, there is a growing focus on less-known fruits with unique flavors, rich in antioxidants, and natural anthocyanin-based colors (Andreicut et al., 2018).

Given that, to our knowledge, the morphology, content of bioactive compounds, and antioxidant activity of *Mahonia aquifolium* (MA) fruits at the genotype level are still relatively unknown (Pyrkosz-Biardzka et al., 2014), and that varieties or lower taxonomic units have not been distinguished, the main objectives of this study are: (1) in situ conservation of adaptive MA genotypes at two locations in the urban dendroflora of Serbia and (2) defining phenological fruiting patterns of MA genotypes for predictive understanding of phenological changes as elements of vulnerability or sustainability under climate challenges.

The aim of the study is also to investigate the content of total phenols, tannins, anthocyanins, flavonoids, vitamin C, and antioxidant properties of raw MA fruit extracts at the genotype level for tears and globosa, to determine the functional significance of their fruit extracts in the urban dendroflora of Serbia.

Materials and methods

Study areas

The study areas are central urban parks, as elements of green infrastructure of Belgrade (Location 1 – L1) and Novi Sad (Location 2 – L2) in Serbia. One park over 100 years old was selected at each location, where MA, commonly known as Oregon Grape due to its widespread presence in Oregon, was identified (Ocokoljić and Petrov, 2022).

Through monitoring conducted from 2007 to 2024, genotypes with fruits of different morphological shapes, consistent over all 18 consecutive years, were identified and defined in the study as tears and globosa. At L1, MA was located along the designed approach to the Temple of Saint Sava in Karadjordjev park, in the Belgrade district, within the municipality of Vračar, which has an urbanization rate of 95.33% (<https://land.copernicus.eu/local/urban-atlas/urban-atlas-2018>, accessed on 25 November 2024). MAbg tears was georeferenced at coordinates 44°47'54.87"N and 20°27'57.78"E, and MAbg globosa at 44°47'52.87"N and 20°28'01.26"E, both at an altitude of 132 m, on flat, unexposed terrain and anthropogenized soil of the Haplic Fluvisol type (Škorić et al., 1985).

At L2, MA was located along a path in Danube park, near Ignjat Pavlas Street, in the South Bačka district, within the municipality of Novi Sad and the local community of Stari

Grad, which has an urbanization rate of 80.4% (<https://land.copernicus.eu/local/urban-atlas/urban-atlas-2018>, accessed on 25 November 2024). *Mans tears* was georeferenced at coordinates 45°15'18.77"N and 19°50'58.32"E, and *Mans globosa* at 45°15'20.78"N and 19°51'01.09"E, both at an altitude of 80 m, on flat, unexposed terrain influenced by a high groundwater level and anthropogenized alluvial loamy soil (Škorić et al., 1985; Živković et al., 1972; Ćirić, 1984; Obrknežev et al., 2009).

Phenological data

Over 18 successive years, elements of the phenological fruiting patterns were recorded by observing every other day simultaneously at both locations throughout the calendar years. The dates were automatically converted to Days of the Year (DOY) using software. According to the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scale (Meier, 1997), the DOY of mature fruit occurrence (89 BBCH) was recorded, along with their accumulated growing degree days (GDD), calculated by summing air temperatures from 1 January to the beginning of 89 BBCH in each of the 18 years (Lalić et al., 2021). The elements of the phenological fruiting patterns were determined at both L1 and L2, as the fruiting occurred simultaneously in the *tears* and *globosa* varieties. The fruit yield was assessed at the genotype level by quantifying phenological observations (Stilinović, 1985) on a five-point scale: 0 - no yield (0% of branches with fruits), 1 - very low yield (<20%), 2 - low yield (>20 – <40%), 3 - moderate yield (>40 – <60%), 4 - abundant yield (>60 – <90%), 5 - maximum yield (>90%).

Climatic data

To determine the impact of climatic parameters, hourly and daily data from the Republic Hydrometeorological Service of Serbia (RHMZ) were used for the reference period (1991–2020) and the study period (2007–2024). These data were obtained from the Republic Hydrometeorological Service of Serbia (RHMZ) (https://www.hidmet.gov.rs/ciril/meteorologija/klimatologija_godisnjaci.php and <https://www.ogi-met.com/synopsc.phtml.en>, accessed on 30 September 2024) from the Main Meteorological Station Belgrade (44°47'54.44"N; 20°27'53.35"E; altitude: 132 m) for L1 and Rimski Šančevi (45°19'19.97"N; 19°49'48.01"E; altitude: 86 m) for L2. Climatological standard normals of the analyzed parameters for the reference period 1991–2020 were determined to enable the application of statistical climatological methods, including percentiles and associated terciles, for the study period. According to RHMZ, the *n*-th percentile is the value below which *n* percent of data, arranged in ascending order, is located. The categorization of maximum and average daily and monthly air temperatures according to percentiles implies the following categories (RHMZ): EW - Extremely warm (>98 perc), VW - Very warm (>90 – ≤98 perc), W - Warm (>75 – ≤90 perc), N - Normal (≥25 – ≤75 perc), C - Cold (≥10 – <25 perc), VC - Very cold (≥2 – <10 perc), EC - Extremely cold (<2 perc), and according to terciles 1 - Warm (≥66 perc), 0 - Normal (<33 – > 66 perc), -1 - Cold (≥33 perc). Categorization of monthly precipitation according to percentiles includes categories (RHMZ): EW - Extremely wet (>98 perc), VW - Very wet (>90 – ≤98 perc), W - Wet (>75 – ≤90 perc), N - Normal (≥25 – ≤75 perc), D - Dry (≥10 – <25 perc), VD - Very dry (≥2 – <10 perc), ED - Extremely dry (<2 perc), and according to terciles: 1 - Wet (≥66 perc), 0 - Normal (<33 – >66 perc), -1 - Dry (≥33 perc). Certain percentiles of daily maximum air temperatures for the reference period 1991–2020 were used to determine the intensity of

heat waves in 2024 because, according to WMO (2023), every additional 0.5°C of global warming causes an increase in the intensity, frequency and duration of heat waves. By definition, a heat wave is a continuous series of five or more days when the maximum daily air temperature is according to percentiles in the categories of very warm and extremely warm (RHMZ). Each day of the heat wave must have a Tmax greater than the 90th percentile in relation to the same date of the reference period. To determine the intensity of the heat wave, that is, the sum of deviations Tmax for all days in the heat wave, the normals (mean values) of the same days for the series 1991-2020 are also necessary. In this study, heat wave intensity was used, which according to RHMZ was determined as the sum of all Tmax deviations from normal for each day of the heat wave.

Plant material

MA fruits were sampled at full maturity on 30 August 2024 from the southern, sunlit parts of the plants simultaneously at L1 and L2. The total sample for morphometric analysis consisted of 400 berries, with 100 from each of the four genotypes: MAbg tears, MAbg globosa, MAns tears, and MAns globosa. Using a digital caliper with an accuracy of 0.01 mm, the fruit length (FL) and fruit width (FWI) were measured. The number of seeds (NS) was recorded for each fruit, and the weight of individual fruits (FWE) was determined using a laboratory scale with a precision of 0.01 g. The color of ripe fruits was defined using the RGB (Red/Green/Blue) color system.

Biochemical characteristics

When determining the biochemical characteristics, the total number of technical repetitions was 192, considering that fruits with four genotypes (two from each location) were collected and analyzed. For each of the elements, there were 6 repetitions per reagent used for extraction and 6 repetitions for determining the amount of vitamin C. The extraction of the juicy parts of the fruit was performed using two analytical-grade reagents: 70% ethanol and distilled water (10 ml) in a 1:50 ratio. The research followed the official analytical protocol and methods (Helrich, 1990) which imply the use of several solvents, therefore distilled water was also used, which can rarely extract better than other solvents (Kolarov et al., 2021). Following a standard procedure, after centrifugation (10,000 rpm, 15 min) and filtration, the samples were transferred to new test tubes. Total phenols and tannins were determined using the Folin-Ciocalteu reagent following the standard methods (Singleton et al., 1999). Total flavonoids were determined by forming complexes with Al₃ (Markham, 1989). Total anthocyanins were determined using the pH differential method, with measurements at pH 1 and pH 4.5 at $\lambda = 520$ nm and $\lambda = 700$ nm. FRAP method was used to evaluate the total reducing capacity of the samples (Benzie and Strain, 1996). The antioxidant capacity was assessed by determining the radical scavenging activity of the samples using the ABTS^{•+} method (Re et al., 1999). Additionally, the DPPH method was applied to determine the transformation of DPPH radicals (Przybylski et al., 1998), to highlight the efficiency of natural antioxidants in the tested samples. The content of vitamin C (ascorbic acid) in raw MA fruits was determined using the 2,4-dinitrophenylhydrazine (DNP) method, as described by Al-Ani et al. (2007).

Processing of data

Descriptive statistics and Sen's slope in conjunction with the Mann-Kendall test to analyze trends were used for phenological patterns of fruiting because it is applied in

various fields, including meteorology, hydrology and environmental science. Namely, Sens slope is often used in conjunction with the Mann-Kendall test to determine both the presence and magnitude of a trend. A positive Sens slope indicates an increasing trend, while a negative slope indicates a decreasing trend. These methods, as well as Spearman rank test were chosen for their broader interpretation compared to the linear correlation coefficient, as they indicate whether a consistently increasing or decreasing relationship exists between variables. Furthermore, they do not require assumptions about the frequency distribution of variables. The value and sign (ρ ranging from -1 to 1) determine the strength and direction of the relationship. The strength of correlation was interpreted using the scale (Horvat and Mijoč, 2012): 0 (no correlation), 0–0.24 (very weak), 0.25–0.49 (weak), 0.50–0.74 (moderate), 0.75–0.99 (strong to very strong) and 1 (perfect). Only significant correlations with a probability of $p < 0.05$ were analyzed. The characteristics of the investigated fruits were analyzed using descriptive statistics, Tukey's HSD test, Duncan's test, analysis of variance (ANOVA), Spearman rank test, LS means tests and dendrogram (cluster) analysis.

Data processing was performed using the software packages XLSTAT 2020, Past 4.11, ArcGIS 10.8/ArcMap 10.8, Google Earth Pro, and The STATISTICA 13 (TIBCO Software Inc, 2020).

Results

Phenological characteristics and climatic predictors

The primary response of the investigated mahonia varieties to changes in air temperature and precipitation was earlier fruit maturation. During this period, the earliest fruit maturation was recorded in 2024 at DOY 238 for L1 and DOY 243 for L2, while the latest maturation occurred in 2011 and 2013 at DOY 331 for L1 and DOY 336 for L2 (*Fig. 1A*). The average accumulated growing degree days (GDD) required for fruit maturation were 3492.8°C at L1 and 3199.5°C at L2 (*Fig. 1B*). The lowest GDD was recorded in 2024 (L1 = 3151.4°C, L2 = 3057.1°C), while the highest was recorded in 2012 (L1 = 3783.1°C) and 2018 (L2 = 3458.3°C). In 2024, fruit maturation occurred 72 days earlier at L1 and 84 days earlier at L2 compared to the average DOY for the 2007–2023 period (*Fig. 1*).

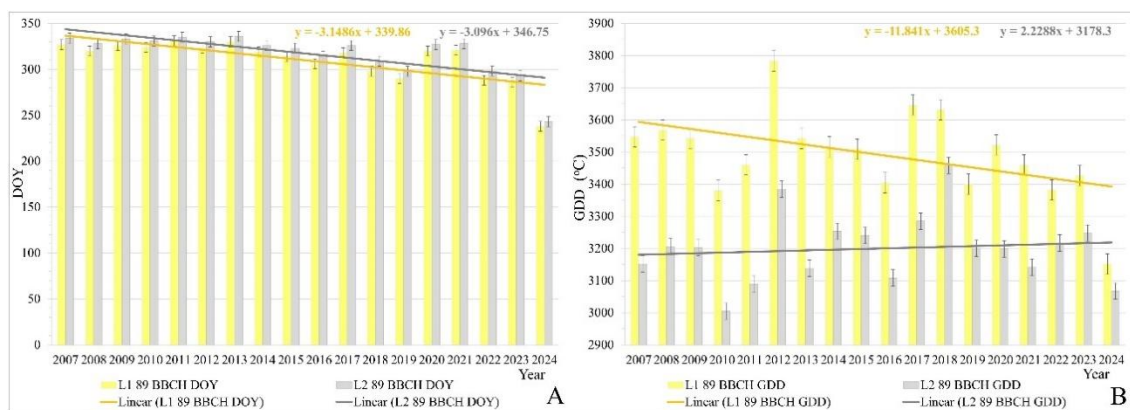


Figure 1. Elements of phenological monitoring of mahonia fruit maturation for the 2007–2024 period for the investigated locations: A - DOY (day of year) and B - GDD (growing degree days). Data are expressed as the mean $\pm$ SD ($n = 18$). L1 – location 1 (Belgrade), L2 – location 2 (Novi Sad), BBCH – the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry scale, 89 - mature fruit occurrence

Statistical analysis of average daily air temperatures and phenological observations determined that the mean daily air temperature for the period from 1 April (the start of the vegetation period) to 89 BBCH in 2024 was 23.2°C at L1 and 22.6°C at L2. Compared to the mean daily temperatures for the 2007–2023 period (L1: 1.4–25.8°C; L2: 1.4–30.8°C), the values were higher by 2.3°C at L1 and 2.9°C at L2. Comparative analysis of these differences between L1 and L2 highlights specific climatic events during 2024. Considering this analysis, along with the fact that 2024 recorded the earliest fruit maturation, deviation charts were created to illustrate the maximum air temperatures during heatwaves and cumulative precipitation during the summer months compared to the reference period 1991–2020 (Figs. 2 and 3).

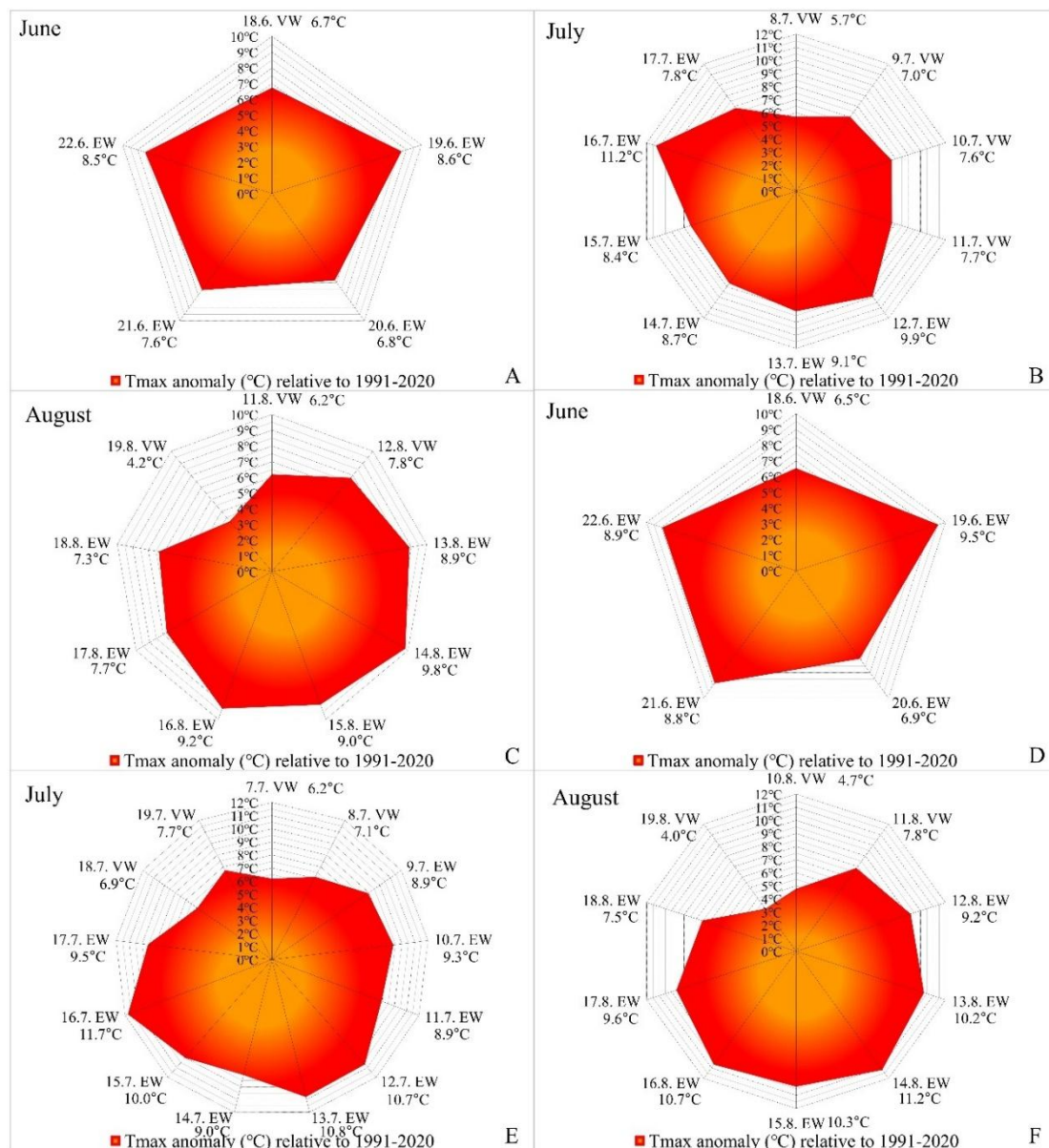


Figure 2. Deviations in mean daily maximum air temperatures during heatwaves at L1 - Belgrade (A - June, B - July, C - August) and L2 - Novi Sad (D - June, E - July, F - August) during summer 2024, based on RHMS data. The values above the axes are calculated deviations of Tmax, according to percentiles, for the specified date in relation to the reference period 1991–2020

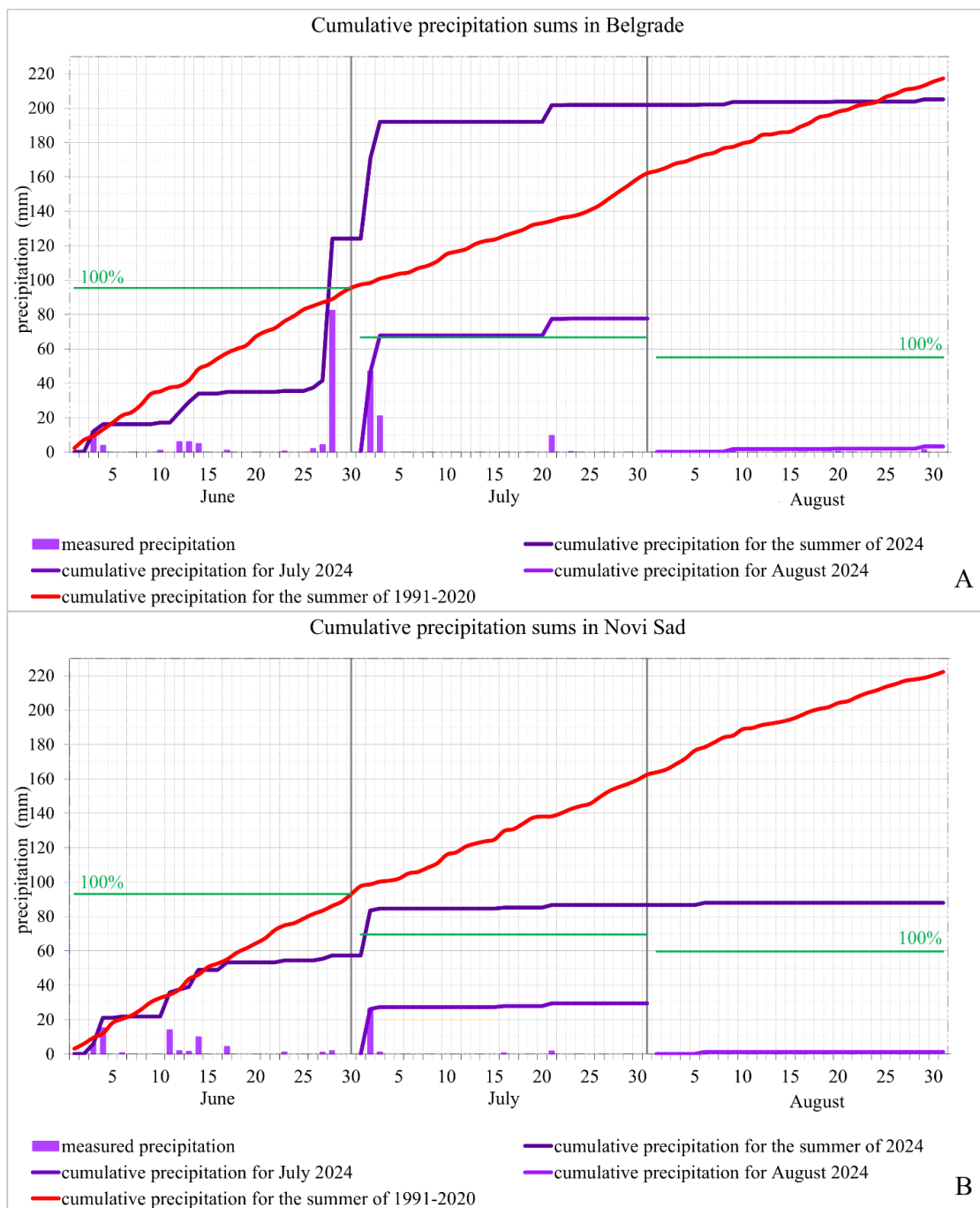


Figure 3. Precipitation amounts in summer 2024 compared to daily, monthly, and seasonal (summer) normals for the period 1991–2020: A for L1 (Belgrade) and B for L2 (Novi Sad)

The heatwaves recorded in mid-July and August were extremely intense, with deviations exceeding 10°C compared to the 1991–2020 normal. The number of tropical days during the summer of 2024 was 67 at L1 and 77 at L2, surpassing the maximum recorded in 2012 at both locations. The total heatwave intensity during the summer was 191.2°C at L1 (Fig. 2A, B, C) and 311.1°C at L2 (Fig. 2D, E, F). In August, when the fruits matured, the intensity reached 69.9°C at L1 (Fig. 2A) and 153.9°C at L2 (Fig. 2D), consistent with the previously reported mean daily temperatures. The total precipitation

during the summer of 2024, according to the percentile method, was categorized as normal at L1 (Fig. 3A) and extremely dry at L2 (Fig. 3B). A detailed overview of the monthly progression of air temperatures and precipitation from the beginning of the vegetation period to fruit maturation, along with deviations from the reference period normal, is provided in Table 1.

Table 1. Mean monthly air temperatures and monthly precipitation totals corresponding percentiles and terciles, and their deviations at L1 (Belgrade) and L2 (Novi Sad) for 2024 compared to the normals of the reference period 1991–2020, corresponding percentiles and terciles

L	Tmean (°C)	Perc. Cat.*	Tmean (°C) 1991-2020	Deviation (°C)	1991-2020	1991-2020	1991-2020	Terciles**
		1991-2020			33.-Perc.	50.-Perc.	66.-Perc.	Cat.
April								
1	16.3	VW	13.6	2.7	12.8	13.5	14.2	1
2	15.3	VW	12.4	2.9	11.8	12.6	13.1	1
May								
1	19.0	N	18.2	0.8	17.5	18.2	19.1	0
2	18.9	W	17.3	1.6	16.9	17.3	17.8	1
June								
1	25.2	EW	21.9	3.3	21.1	21.8	22.4	1
2	24.3	EW	20.9	3.4	20.1	20.6	21.5	1
July								
1	27.6	EW	23.8	3.8	23.1	23.7	24.3	1
2	26.9	EW	22.5	4.4	21.9	22.2	22.9	1
August								
1	28.4	EW	23.8	4.6	22.6	24.1	24.8	1
2	27.5	EW	22.4	5.1	21.5	22.3	23.0	1
Monthly precipitation								
L	Sum (mm)	Perc. Cat.*	Normal (mm) 1991-2020	Deviation (mm)	1991-2020	1991-2020	1991-2020	Terciles**
		1991-2020			33.-Perc.	50.-Perc.	66.-Perc.	Cat.
April								
1	28.4	D	51.5	-23.1	38.1	52.1	59.5	-1
2	21.5	D	46.6	-25.1	30.3	43.2	54.0	-1
May								
1	111.3	VW	72.3	39.0	50.2	62.1	79.6	1
2	78.0	N	78.2	-0.2	59.4	74.0	84.8	0
June								
1	124.2	N	95.6	28.6	55.7	83.0	127.0	0
2	57.3	D	93.0	-35.7	65.0	94.2	107.0	-1
July								
1	77.7	N	66.6	11.1	35.3	43.4	58.2	1
2	29.4	D	69.5	-40.1	39.9	59.8	78.3	-1
August								
1	3.3	ED	55.1	-51.8	42.8	47.6	64.5	-1
2	1.2	VD	59.7	-58.5	30.1	46.6	78.8	-1

*Extremely warm (EW), Very warm (VW), Warm (W), Normal (N), Cold (C), Very cold (VC), Extremely cold (EC), **Warm (1), Normal (0), Cold (-1), ***Extremely wet (EW), Very wet (VW), Wet (W), Normal (N), Dry (D), Very dry (VD), Extremely dry (ED), ****Wet (1), Normal (0), Dry (-1), categorization according to RHMZ categorization according to RHMZ

The statistical significance of deviations of analyzed elements was determined by Mann Kendall and Sen's slope tests, for DOY and GDD phases of 89BBCH (full mature fruits) mahonia, at two locations, during 18 years (*Table 2*). Before 2024, significant downward trends of DOY, i.e. early fruit ripening, were confirmed at both locations for the period 2007-2023, but for GDD there were no statistically significant trends, although they are visible in *Figure 1B*. When the globally warmest year 2024 in Belgrade (L1) is added to the series, a statistically significant trend for GDD with a negative sign of Sen's slope is also confirmed. In Novi Sad (L2), even when 2024 is included, no statistically significant trend was determined for GDD. The obtained results indicate, as well as the previous analysis, that air temperatures and precipitation during 2024 influenced the change of the accumulated sums of heat (GDD) for fruit ripening because there was no trend in this parameter for the previous 17 years. Spearman's correlation coefficients confirm a very strong correlation (0.99535) between L1 89 BBCH DOY and L2 89 BBCH DOY. Other correlations were not statistically significant.

Table 2. Results of Mann-Kendall and Sen's Slope Tests for the occurrence of full mature fruits (89BBCH) at L1 (Belgrade) and L2 (Novi Sad) for DOY (day of the year) and GDD (accumulated sum of heat) for the periods 2007-2023 and 2007-2024, based on own phenological observations and RHMS data. BBCH – the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry scale, 89 - mature fruit occurrence

Parameter/test	Kendall's tau	p-Value*	Sen's slope**
2007-2023			
L1: 89BBCH DOY	-0.583	0.001	-2.183
L2: 89BBCH DOY	-0.573	0.002	-2.071
L1: 89BBCH GDD	-0.309	0.091	-7.511
L2: 89BBCH GDD	0.176	0.343	5.509
2007-2024			
L1: 89BBCH DOY	-0.630	0.000	-2.636
L2: 89BBCH DOY	-0.620	0.000	-2.563
L1: 89BBCH GDD	-0.386	0.028	-9.443
L2: 89BBCH GDD	0.059	0.762	2.808

*As the computed p-value is greater than the significance level 0.05, the null hypothesis H0 cannot be rejected. H0: There is no trend in the series. **A positive Sens slope indicates an increasing trend, while a negative slope indicates a decreasing trend. ***Bold values indicate the existence of a statistically significant trend at the $p < 0.05$ level

The fruit yield of all selected individuals was maximal (rating 5) over the 18 consecutive years of observation (*Fig. 4*). Selected genotypes were located in secondary populations of 11 (MAbg tears), 15 (MAbg globosa), 14 (Mans tears) and 17 (Mans globosa) individuals.

Fully mature fruits with a white wax cover have an overall sky-blue color (RGB 85/101/114), while fruits without the white wax cover appear dark blue-purple (RGB 47/36/40). The color spectrum lies between these extremes, depending on the thickness of the white wax cover.

Morphometry analysis of fruits

The morphometric parameters of fruits sampled from selected plants in secondary MA populations within the urban dendroflora at L1 and L2 are shown in *Figure 5*. The

mean values for fruit length and width were as follows: MAbg tears – 12.30 mm and 7.78 mm, MAns tears – 10.09 mm and 7.82 mm, MAbg globosa – 8.23 mm and 8.11 mm, and MAns globosa – 7.12 mm and 6.61 mm. The raw fruit weight and seed number per fruit were: MAbg tears 2.85 g and 3.15, MAns tears 2.09 g and 6.61, MAbg globosa 1.67 g and 6.16, and MAns globosa 1.58 g and 4.81. The highest variability, expressed through the coefficient of variation (%), was confirmed for the parameter “number of seeds per fruit”: 43.60% (MAbg tears), 32.46% (MAns globosa), 19.81% (MAbg globosa), and 18.85% (MAns tears). MAbg tears stands out with the highest mean fruit weight (2.85 g), the greatest fruit length (12.30 mm), and the lowest number of seeds per fruit (3.15).

Table 3 presents the results of the ANOVA analysis, which show statistically significant differences in morphometric parameters concerning fruit shape, location, and the interaction between fruit shape and location. Statistically significant differences were observed for all parameters except NS (number of seeds) concerning fruit shape (A).

Table 3. ANOVA analysis (*F*-test) of morphometric characteristics of *M. aquifolium* fruits: fruit length (FL), fruit width (FWI), weight of individual fruits (FWE), and number of seeds per fruit (NS)

Trait	Fruit shape (A)		Location (B)		Interaction A × B	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
FWE	455.98*	0.00*	113.66*	0.00*	70.02*	0.00*
FL	1014.39*	0.00*	224.54*	0.00*	24.43*	0.00*
FWI	24.99*	0.00*	69.74*	0.00*	76.63*	0.00*
NS	0.63 ^{ns}	0.42 ^{ns}	139.71*	0.00*	209.68*	0.00*

(ns): non-significant; (): significance at the level of $p < 0.05$



Figure 4. Visual aspect of the maximum yield of mahonia (>90%, grade 5) on L1 (Belgrade) - A and varieties at mature fruit occurrence (89BBCH): globosa - B and tears - C

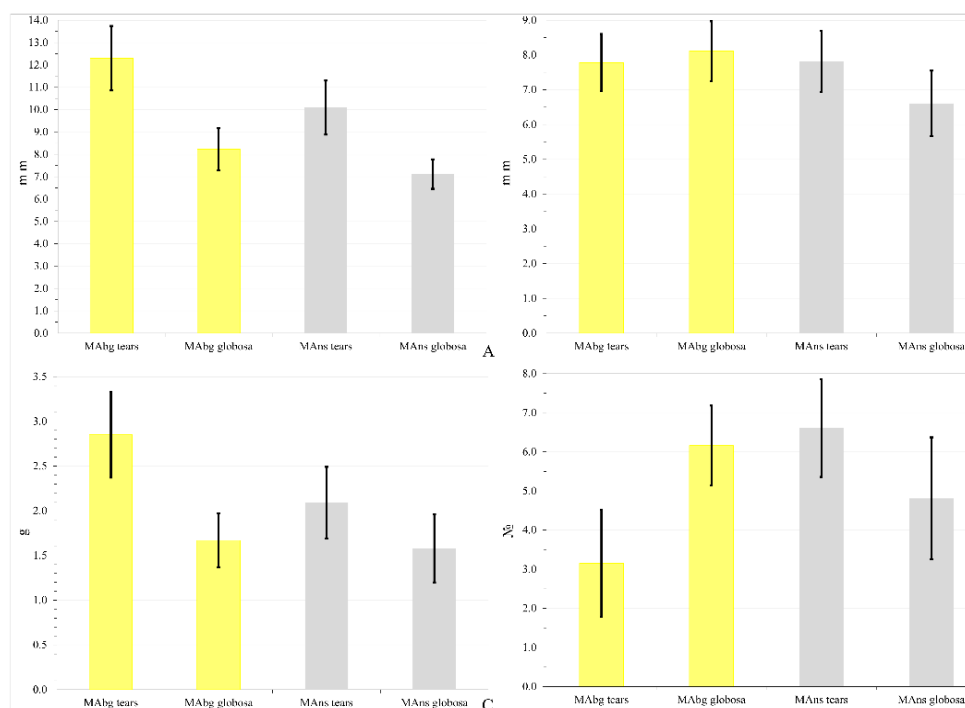


Figure 5. Morphometric parameters of fruits from the tears and globosa genotypes in the urban dendroflora at L1 (Belgrade) and L2 (Novi Sad): A – fruit length (FL), B – fruit width (FWI), C – weight of individual fruits (FWE), and D – number of seeds per fruit (NS). Data are expressed as the mean $\pm$ SD ($n = 100$). MAbg – *Mahonia aquifolium* Location Belgrade, MAns – *Mahonia aquifolium* Location Novi Sad

Using Tukey's HSD test, statistically significant differences were confirmed for all fruit parameters between the tears and globosa varieties at L1 and L2 (Table 4).

Table 4. Tukey's HSD test of morphometric characteristics of *M. aquifolium* fruits: fruit length (FL), fruit width (FWI), weight of individual fruits (FWE), and number of seeds per fruit (NS)

Genotype	FWE	Group	FL	Group	FWI	Group	NS	Group
MA _{bg} tears	2.85	a*	12.30	a	7.78	a	3.15	a
MA _{bg} globosa	1.67	b	8.23	b	8.11	b	6.16	b
MA _{ns} tears	2.09	a	10.09	a	7.82	a	6.61	a
MA _{ns} globosa	1.58	b	7.12	b	6.61	b	4.81	b

*a and b indicate statistically significant differences between groups at a significance level of $p < 0.05$

The Spearman correlation coefficients (ρ) confirm the following: a) For MAbg globosa, an almost perfect positive correlation (0.96113) between fruit length and weight, a very strong positive correlation between fruit width and weight (0.82866) and between width and length (0.84198; b) For MAbg tears, a very strong positive correlation (0.75507) between fruit weight and length; c) For MAns globosa, a very strong positive correlation (0.86796) between fruit weight and the number of seeds, moderate positive correlations between fruit weight and width (0.74003), width and length (0.61594), width and the number of seeds (0.61064); d) For MAns tears, a very strong positive correlation

(0.90406) between fruit width and the number of seeds. Other correlations were not statistically significant (Fig. 6).

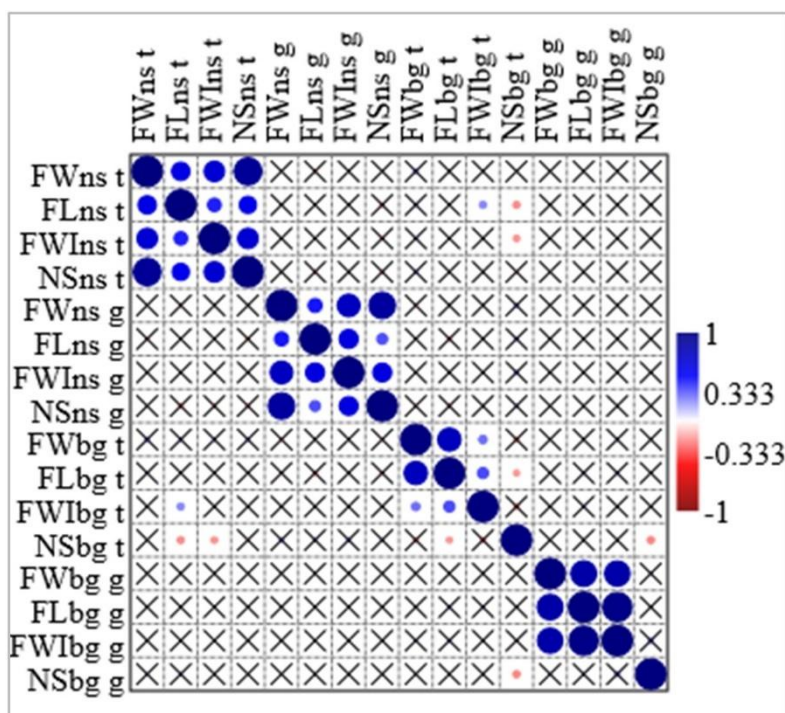


Figure 6. Graphic representation of Spearman's correlation coefficients for fruit length (FL), fruit width (FWI), weight of individual fruits (FWE), and number of seeds per fruit (NS), in the period 2007-2024. *Coefficients that are not statistically significant crossed ($p > 0.05$).

**Labels: bg (L1 - Belgrade), ns (L2 - Novi Sad), t (tears) and g (globe)

Biochemical characteristics of fruits

The results presented in Table 5 highlight variations in the total phenol content in MA fruits depending on the sampling location and the type of solvent, as analyzed using Duncan's test. The lowest total phenol content was recorded in samples from L2 extracted with water, particularly in the MAns globosa, while the highest phenol content was found in samples from L1 extracted with ethanol in the MAbg globosa. Samples from L1 generally had a higher total phenol content compared to those from L2, indicating a potential influence of ecological factors on phenol levels in the fruits.

The analysis results for the total phenol and tannin content in the fruits of MA varieties reveal similar trends depending on the sampling location (L1 and L2) and the type of solvent (water and ethanol). The content of both types of bioactive compounds is higher in ethanol-extracted samples compared to water-extracted ones, indicating the greater efficiency of ethanol in extraction. Duncan's test for both parameters shows consistent patterns of homogeneous groups, with water-extracted samples grouped with lower values and ethanol-extracted samples grouped with higher values. This further confirms the importance of the solvent and sampling location in determining the content of bioactive compounds in the fruits of MA varieties.

The results presented in Table 5 indicate significant differences in the flavonoid and anthocyanin content in the fruits of MA varieties, depending on the variety, sampling

location, and solvent (ethanol and water). Individuals from L1 generally showed higher flavonoid and anthocyanin content compared to those from L2. This may be linked to more favorable ecological conditions for the biosynthesis of these compounds at L1 or to higher stress levels, which can also influence their production.

Table 5. Results of Duncan's test for Values of polyphenolic compounds and antioxidant tests (FRAP, DPPH, ABTS) determined in the fruits at the level of genotype MAbg tears - *Mahonia aquifolium* var. tears Location Belgrade, MAbg globosa - *Mahonia aquifolium* var. globosa Location Belgrade, MAns tears - *Mahonia aquifolium* var. tears Location Novi Sad, MAns globosa - *Mahonia aquifolium* var. globosa Location Novi Sad

Genotype	Solvent	Total phenols ¹	Total tannins ¹	Total flavonoids ¹	Total anthocyanins ²	FRAP ³	DPPH ³	ABTS ³
MA _{bg} tears	Ethanol	19.83 ± 0.67 ^a	9.04 ± 0.53 ^a	6.16 ± 0.62 ^a	6.09 ± 0.50 ^a	59.16 ± 0.63 ^a	61.18 ± 1.73 ^a	106.02 ± 0.35 ^b
	Water	5.61 ± 0.24 ^c	3.73 ± 0.29 ^c	0.38 ± 0.14 ^c	0.25 ± 0.03 ^c	18.11 ± 0.53 ^c	15.02 ± 0.71 ^c	49.70 ± 0.75 ^c
MA _{bg} globosa	Ethanol	20.09 ± 0.56 ^a	7.85 ± 0.65 ^b	6.10 ± 0.37 ^a	3.43 ± 0.32 ^b	54.86 ± 0.56 ^b	50.07 ± 0.60 ^b	110.95 ± 0.65 ^a
	Water	5.83 ± 0.30 ^c	3.57 ± 0.35 ^c	0.47 ± 0.16 ^c	0.16 ± 0.03 ^c	16.99 ± 0.70 ^f	14.57 ± 0.22 ^c	49.79 ± 0.55 ^c
MA _{ns} tears	Ethanol	15.12 ± 0.73 ^b	5.28 ± 0.78 ^d	4.52 ± 0.31 ^b	3.22 ± 0.22 ^b	40.40 ± 0.85 ^c	44.96 ± 0.78 ^c	90.18 ± 0.79 ^c
	Water	4.78 ± 0.13 ^d	2.59 ± 0.26 ^f	0.31 ± 0.08 ^c	0.15 ± 0.04 ^c	13.12 ± 0.58 ^e	12.48 ± 0.41 ^f	37.37 ± 0.47 ^f
MA _{ns} globosa	Ethanol	14.91 ± 0.55 ^b	6.02 ± 0.49 ^c	4.85 ± 0.65 ^b	3.39 ± 0.24 ^b	39.24 ± 0.42 ^d	43.88 ± 0.88 ^d	89.01 ± 0.61 ^d
	Water	4.66 ± 0.16 ^d	2.22 ± 0.18 ^f	0.32 ± 0.10 ^c	0.16 ± 0.03 ^c	13.17 ± 0.48 ^e	12.26 ± 0.28 ^f	37.12 ± 0.49 ^f

¹Expressed as mg quercetin equivalents per g of raw weight

²Expressed as mg cyanidin-3-glucoside per g of raw weight

³Expressed as mg trolox equivalents per g of raw weight

The results for the three antioxidant tests – FRAP, DPPH, and ABTS – show similar trends depending on the sampling location, variety, and solvent, indicating a correlation between phenolic compound content and antioxidant properties. Ethanol-extracted samples, particularly those from varieties at L1, demonstrated the highest antioxidant capacity. The fruits of the MAbg tears extracted with ethanol exhibited high values across all antioxidant tests: FRAP (59.16 ± 0.63 mg/g), DPPH (61.18 ± 1.73 mg/g), and ABTS (106.02 ± 0.35 mg/g). These values correlate with the high content of total phenols (19.83 ± 0.67 mg/g) and tannins (9.04 ± 0.53 mg/g), suggesting that phenolic compounds are key contributors to antioxidant activity. A similar trend was observed in MAbg globosa (ethanol), with high values for FRAP (54.86 ± 0.56 mg/g), DPPH (50.07 ± 0.60 mg/g), and ABTS (110.95 ± 0.65 mg/g). Conversely, in samples from varieties at L2, where phenolic content was lower, antioxidant capacities were also reduced. The fruits of the MAns tears extracted with water showed low values for all three methods: FRAP (13.12 ± 0.58 mg/g), DPPH (12.48 ± 0.41 mg/g), and ABTS (37.37 ± 0.47 mg/g). These values correspond to the lower content of phenols (4.78 ± 0.13 mg/g) and tannins (2.59 ± 0.26 mg/g). These results suggest that phenolic compounds, including flavonoids and tannins, are significant factors contributing to the pronounced antioxidant capacity of MA fruits.

In addition to the content of polyphenolic compounds and the antioxidant capacity of MA fruits, the vitamin C content was determined in the fruits of all selected individuals. The average values of vitamin C ranged from 4.96 to 6.36 mg/g.

Considering the results of descriptive statistics and Duncan's test (least significant differences) to verify the claim that environmental conditions have a stronger influence on the content of metabolites, except for anthocyanins where the influence of genotype is also evident, ANOVA was performed between localities. The results shown in Table 6

confirm the statistical significance of the differences between the localities for all analyzed parameters and both solvents, except for anthocyanins and for the water solvent at the significance level of $p < 0.05$.

Table 6. ANOVA of measured fruit parameters of *M. aquifolium* for ethanol and water solvents: phenols, tannins, anthocyanins, flavonoids and antioxidant tests (FRAP, DPPH and ABTS) and vitamin C between L1 (Belgrade) and L2 (Novi Sad)

Solvent	Phenols		Tannins		Flavonoids		Anthocyanins		FRAP		DPPH		ABTS		Vitamin C	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Ethanol	353.60		73.95	0.0001	14.919	0.001	47.34	0.0001	568.63	0.0001	47.168	0.0001	517.96	0.0001	11.737	0.003
Water	115.78	0.0001	91.95	0.0001	11.717	0.003	3.830	0.064	223.07	0.0001	165.99	0.0001	2742.6	0.0001		

In order to identify the influence of location on the analyzed parameters of bioactive compounds and antioxidant capacity, as well as observed differences in previous tests, the LS means test (least square means) was also applied. Namely, LS values estimate the mean values that would be if the data were balanced and indicate the significance of the response of the analyzed parameter. Based on the results of the LS means of the investigated parameters (Table 7), only the significance of the differences for anthocyanins for solvent water between L1 (Belgrade) and L2 (Novi Sad) was not confirmed.

Table 7. Results of LS means, bioactive compounds and antioxidant capacity of mahonia fruits, at the locality/group level (L1 - Belgrade and L2 - Novi Sad), at the level of significance $p < 0.05$

Location/Parameter	L1	L2	Pr > F(Model)	Significant	Pr > F(group)	Significant
Ethanol (Phenols)	19.995a*	15.011b	<0.0001	Yes	<0.0001	Yes
Water (Phenols)	5.668a	4.719b	<0.0001	Yes	<0.0001	Yes
Ethanol (Tannins)	8.449a	5.650b	<0.0001	Yes	<0.0001	Yes
Water (Tannins)	3.634a	2.405b	<0.0001	Yes	<0.0001	Yes
Ethanol (Flavonoids)	4.905a	3.305b	0.001	Yes	0.001	Yes
Water (Flavonoids)	0.212a	0.151b	0.003	Yes	0.003	Yes
Ethanol (Anthocyanins)	0.318a	6.155a	<0.0001	Yes	<0.0001	Yes
Water (Anthocyanins)	0.412a	4.684b	0.064	No	0.064	No
Ethanol (FRAP)	57.173a	39.818b	<0.0001	Yes	<0.0001	Yes
Water (FRAP)	17.524a	13.147b	<0.0001	Yes	<0.0001	Yes
Ethanol (DPPH)	56.196a	44.423b	<0.0001	Yes	<0.0001	Yes
Water (DPPH)	14.842a	12.375b	<0.0001	Yes	<0.0001	Yes
Ethanol (ABTS)	108.337a	89.590b	<0.0001	Yes	<0.0001	Yes
Water (ABTS)	49.733a	37.245b	<0.0001	Yes	<0.0001	Yes
Vitamin C	5.281b	6.148a	0.003	Yes	0.003	Yes

*Groups a and b represent the values of the LS means of the investigated parameters

Patterns of variability in fruit biochemical characteristics suggest adaptation to environmental conditions at two different locations.

Multivariate cluster analysis

Multivariate cluster analysis indicates the separation of individuals based on the sampled MA fruits. Two subclusters are observed (Fig. 7A). In the first subcluster, two subgroups are identified: the first includes the MAbg globosa and MAns globosa, which are closely related in terms of the parameters FWE, FWI, and NS. The second subgroup isolates the MAns tears, characterized by lower values for FWE, FL, and FWI compared to the other investigated genotypes. The second subcluster distinguishes the fruits of MAbg tears, which significantly differ in all morphometric parameters from the individuals in the first subcluster but are closely related to the MAns tears of the second subgroup in the first subcluster. Cluster analysis confirmed the differentiation and definition of the varieties of the investigated MA based on the morphological characteristics of the fruits.

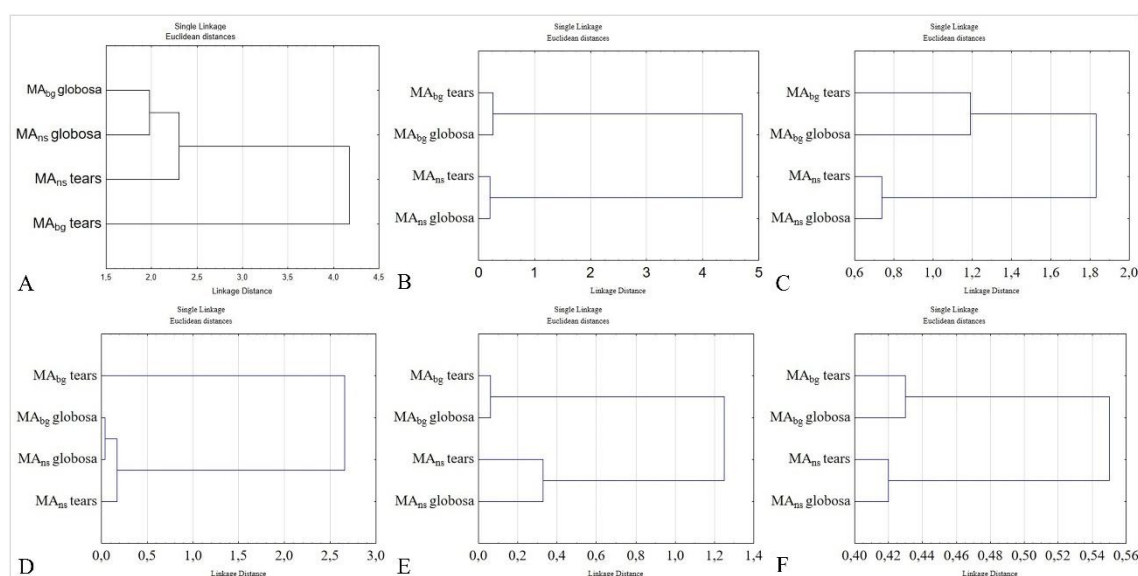


Figure 7. Dendrogram cluster analysis of measured fruit parameters of MA genotypes: A – morphometric parameters, B – phenols, C – tannins, D – anthocyanins, E – flavonoids and F – vitamin C. MAbg – *Mahonia aquifolium* Location Belgrade, MAns – *Mahonia aquifolium* Location Novi Sad

Based on the dendrograms (Fig. 7B, C, D, E), MA fruits were grouped according to the content of various metabolites (phenols, tannins, flavonoids, and anthocyanins), taking into account the variety (tears and globosa) and location (L1 and L2). Grouping of MA fruits by phenol, tannin, flavonoid, and anthocyanin content indicates different dominant factors influencing their chemical composition. For phenols, tannins, and flavonoids, location emerges as the key factor, as fruits from the same location (L1 or L2) are closer to each other regardless of the tears or globosa variety. This suggests that environmental conditions at a specific location significantly influence metabolite content. On the other hand, for anthocyanins, the variety plays a more dominant role in grouping, as fruits of the same variety are grouped together even when originating from different locations. This indicates that genetic or morphological traits specifically influence anthocyanin accumulation, independently of ecological conditions. Grouping of MA individuals by vitamin C content (Fig. 7F) shows that fruits from the same location

(MABg tears and MABg globosa, or MAns tears and MAns globosa) are more closely related, indicating a dominant influence of location. Fruits from L1 are distinctly different from those from L2, while the variety has minimal influence.

Discussion

Despite studies identifying correlations between factors and variations in phenological changes (Ge et al., 2015), fewer have focused on the mechanisms driving phenological pattern responses. Developing an understanding of current patterns of phenological shifts is an important step toward predicting future patterns of phenological changes (Chmura et al., 2019). Therefore, in our study, 18 successive phenological fruiting patterns of MA in the urban dendroflora were defined, contributing to a better understanding of interactions and the ability to predict phenological responses to current and future climate challenges.

The ripening of MA tears and globosa varieties in 2024 was recorded 72 days earlier at L1 and 84 days earlier at L2 compared to the average DOY of the previous 17 years of research. This aligns with findings (Li et al., 2016) that urbanization leads to changes in ecosystem services and (Jeong et al., 2019) that the degree of urbanization (95.33% at L1 and 80.4% at L2) affects regional and local climate changes. It also corresponds with phenological models (Prevéy et al., 2020) predicting earlier occurrence of 89 BBCH in MA. However, these models forecasted that by the mid-21st century, 89 BBCH in MA would occur 25 days earlier, and by the end of the century, 36 days earlier. As in studies of Ma and Zhou (2011) and Cosmulescu et al. (2010a, b), our 18-year research indicates that meteorological trends and climate challenges in the 21st century have led to significantly earlier fruit ripening in MA. Compared to the model, this occurred 47 and 36 days earlier at L1 and 59 and 48 days earlier at L2. It is evident that the urban environment has accelerated elements of MA phenological fruiting patterns. While DOY varied by year, the 89 BBCH phase occurred within specific growing degree day (GDD) requirements. While DOY varied by year, the 89 BBCH phase occurred within specific growing degree day (GDD) requirements, until 2024 when the GDD value was the lowest at both locations in the eighteen-year study. The result interacts with the climate parameters and the RHMZ's statement that 2024 is the absolute warmest year at all the main meteorological stations in Serbia since measurements have been taken. Earlier ripening of fruits is also explained by the hottest seasons (winter, spring and summer), as well as the hottest months of February, March, June, July and August, which, in combination with the deficit of precipitation (RHMZ), and especially the uneven distribution of precipitation, resulted in earlier ripening of fruits with lower GDD values. The influence of high air temperatures during 2024 in Serbia was also recorded in the phenological patterns of flowering of *Cornus mas* L. (Ocokoljić et al., 2025), *Rosa* 'Casino' (Skočajić et al., 2025), *Fraxinus ornus* 'Globosa' (Čukanović et al., 2024a), as well as in the phenological patterns of fruiting of *Prunus spinosa* L. (Čukanović et al., 2024b). Therefore, phenological patterns are one of the most evident tools for measuring plant responses to climate change, the impact of global warming, and urbanization (Ocokoljić et al., 2023). In the context of climate change and pollution challenges, ornamental plant like those of MA at L1 and L2 improve environmental quality while enhancing the aesthetics of urban parks (Radutoiu and Stefanescu, 2017).

The study confirms that air temperature and precipitation significantly influence the phenological fruiting patterns and their duration but did not affect the fruit shape. Our

findings align with the results of a study Horčinová Sedláčková et al. (2022) conducted in Slovakia, which documented variability in fruit shape and color among genotypes using photographic evidence. However, their research was conducted only in 2018, and therefore, they did not analyze the possibility of identifying lower taxa. The variability in ripe fruit color reported by Horčinová Sedláčková et al. (2022) was determined in our study to be influenced by the presence or absence of the white wax cover and was defined using the RGB color system. This confirmed identical colors across all investigated genotypes.

Study confirmed the stability of fruit shape and color, leading to the identification of the new MA varieties, tears and globosa, which, to our knowledge, have not been previously taxonomically recorded. According to numerous studies on lower taxa of MA (Center for Applied Nursery Research, 2003; Natural Resources Conservation Service, 2003; Houtman et al., 2004; Teeling, 2010; Ocokoljić and Petrov, 2022), only cultivars have been identified based on habitus, autumn leaf color, and leaf coloration during the vegetation period. The tears variety was identified in 14.28 of the population at L1 and 28.57 at L2 within the secondary populations, from which MAbg tears and MAns tears were isolated. The globosa variety, MAbg globosa and MAns globosa, were selected from the gene pool of pure secondary populations of the globosa variety at both locations.

Our results for fruit length differ from those reported on the population level in Turkey (Marakoglu et al., 2010), particularly for the tears variety. At L1, the fruit length exceeded reported values by 20.59%, while at L2, it was slightly lower, with a reduction of 1.08% while the fruit width for the tears variety was smaller, with reductions of 9.01% at L1 and 7.4% at L2. Similarly, a comparison with findings from Uzbekistan (Chorshanbiev et al., 2022), where only elongated fruits were recorded (similar to our tears variety), revealed that fruit length at L1 was 23% greater, while at L2, it matched the reported values. The fruit width at both locations showed minimal differences, being slightly lower, around 1%. Despite these deviations, our results align with the ranges reported for genotypes (Horčinová Sedláčková et al., 2022), where fruit lengths were between 5.57–13.22 mm and widths between 0.98–11.00 mm. This, as stated by Teeling (2010) that mahonia is a diploid species ($2n = 28$) where high genetic diversity is expected, supports the importance of identifying distinct varieties. In our study, the tears variety had mean fruit lengths ranging from 10.09 mm (L2) to 12.30 mm (L1), with widths from 7.78 mm (L2) to 7.82 mm (L1). The globosa variety exhibited fruit lengths between 7.12 mm (L2) and 8.23 mm (L1), with widths ranging from 6.61 mm (L2) to 8.11 mm (L1). The average fruit weight of the tears variety was 2.85 g (L1) and 2.09 g (L2), which is 45.19% lower at L1 and 59.80% lower at L2 compared to the average weight of 5.2 g reported in Turkey [62] at the population level for MA. For the globosa variety, the fruit weight ranged from 1.67 g at L1 to 1.58 g at L2, representing deviations of 67.88% and 69.61%, respectively, from the same study's population-level data. At the individual level, our results for fruit weight are higher than those reported by Horčinová Sedláčková et al. (2022) in Slovakia, which ranged between 0.18 g and 0.50 g. Additionally, the number of seeds per fruit is a significant characteristic. According to our results, the number of seeds at L1 was 3.15 for var. tears and 6.16 for var. globosa, while at L2, it was 6.61 for var. tears and 4.81 for var. globosa. These findings align with the study at the population level in Turkey (Gunduz, 2013), which reported an average of 3.8 seeds per fruit. The findings of He and Mu (2015) in China, reporting between 1 and 7 seeds, and (Chorshanbiev et al., 2022) in Uzbekistan, reporting 2 to 8 seeds per fruit at the population level, are consistent with the seed counts observed for both varieties at L1 and L2. Seed counts at the genotype level,

ranging from 1.67 to 5.30 in Slovakia (Horčinová Sedláčková et al., 2022), align closely with our findings. This parameter in our study exhibited the highest coefficient of variation, ranging from 18.81% to 43.60%, which falls within the range of 15.19% to 66.67% reported in the genotype-level research in Slovakia (Horčinová Sedláčková et al., 2022). Although MA is primarily considered an ornamental species for use in urban parks, research on the morphometric characteristics of its fruits is important as MA is also a plant with edible fruits and can serve as a significant food source in urban green spaces (Sorokopudov et al., 2017). MA fruits have been used since ancient times in North America, known for their exceptionally high nutritional value (Baumann, 2008), as well as their medicinal properties. In this context, examining the biochemical composition of MA fruits from different genotypes is also of great importance.

The presented data underscores the significant influence of sampling location, solvent type, and genotype on the content of total phenols, tannins, flavonoids, anthocyanins, and antioxidant capacities in the fruits of the MA genotypes. Ethanol proved to be a more effective solvent than water for extracting phenolic compounds (Bisht et al., 2023; Domínguez et al., 2020). These results align with existing literature highlighting ethanol's superior ability to solubilize phenolic compounds due to its polarity (Kalaskar and Surana, 2014).

Fruits harvested during their peak phenolic production phase may yield significantly higher antioxidant capacities (Fischer et al., 2011). The importance of optimizing extraction parameters to fully capture bioactive compounds is reinforced by these findings, as water extracts consistently underperformed in TPC, flavonoids, and anthocyanins (Ben Ahmed et al., 2016; Prvulović et al., 2012; Prvulović et al., 2019).

A strong correlation between phenolic content and antioxidant capacity, demonstrated by FRAP, DPPH, and ABTS assays, underscores the pivotal role of phenolics, including flavonoids and anthocyanins, in antioxidant potential. Notably, MAbg tears and MAbg globosa genotypes exhibited superior antioxidant capacities, which were aligned with their higher phenolic content. This finding suggests that genetic traits significantly influence the synthesis and accumulation of bioactive compounds. Moreover, the potential synergistic effects between phenolics and other antioxidants, such as vitamin C, further amplify the fruits' antioxidant potential (Åkerström et al., 2010).

The role of ecological stressors, such as drought, UV radiation, and pathogen exposure, cannot be overstated in determining phenolic content. These stressors often trigger the upregulation of phenylpropanoid pathways, leading to increased synthesis of phenolic compounds (Calzone et al., 2019).

Differences also emerge as critical determinants of phenolic composition and antioxidant capacity. Among the genotypes, MAbg globosa consistently outperformed others in phenolic content and antioxidant metrics, highlighting its genetic predisposition toward bioactive compound synthesis. Breeding programs aiming to enhance the nutritional and functional properties of MA fruits may benefit from focusing on such high-performing genotypes. Additionally, morphological features, such as fruit size, skin thickness, and surface area, may influence the distribution and concentration of phenolic compounds, as these are often concentrated in the outer layers of the fruit. These findings suggest that to optimize the bioactive profile of MA fruits, emphasis should be placed on selecting genotypes like MAbg globosa, cultivating in ecologically favorable locations such as L1, harvesting at appropriate developmental stages, and employing ethanol-based extraction methods. The integration of genetic, environmental, and methodological considerations will be essential in maximizing the functional and nutritional potential of

MA fruits (Bošnjaković et al., 2012). This study highlights the intricate interplay of genetic and environmental factors, solvent effects, and their implications for the cultural and functional utilization of MA fruits, providing a roadmap for future research and practical applications in the field of functional food development.

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