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PHENOLOGY AND BIOCHEMICAL ANALYSIS OF SPONTANEOUS *PRUNUS SPINOSA* L. POPULATION IN THE VICTINITY OF BELGRADE

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Abstract: The aims of the research were to determine the variability of the phenological patterns of natural populations of *Prunus spinosa* L. in the ecotones of the southeastern part of the Balkan Peninsula and to create an informational database regarding the impact of air temperature and precipitation on the phenology of this species, based on research conducted during the period from 2007 to 2024. The obtained data were analysed using descriptive and multivariate statistical methods. The results showed that the onset of spring phenological phases has been significantly accelerated by the increase in air temperature, especially in 2024, when flowering started 31 days earlier, the flowering phase lasted 13 days longer, and fruit ripening occurred 65 days earlier compared to the 2007–2023 period. The content of phenols, flavonoids, anthocyanins, and antioxidant activity in fruits was determined in distilled water and 70% ethanol. The analysed extracts contained a high percentage of phenols and demonstrated significant antioxidant activity. The obtained results are the starting point for studying changes in the phenological patterns of blackthorn in response to climate change under the agro-ecological conditions of the southwestern suburb of Belgrade. The study confirmed the adaptability of blackthorn, except for the vulnerability of its flowering phenophase to late spring frosts, as observed in 2023. The resilience of the species to climatic challenges was further confirmed by its drought tolerance in 2024 and a significant correlation between fruit ripening and high air temperatures during the growing season over 18 consecutive years of research.

Key words: blackthorn, adaptability, functionality, antioxidant capacity of fruits, ecosystem services, heat waves, ecotones.

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Introduction

Climate change, with increasingly intense extreme events, is a limiting factor for the use of woody plants in the green infrastructure of urban and suburban areas, especially near roads (Rahman et al., 2017). Research confirms that taxa reach the upper limit of plasticity in the conditions of the temperate continental climate in Central Europe (LWG, 2018). Plasticity, or changes in morphology, phenological patterns, and bioactive components in response to environmental conditions (Price et al., 2003), can be assessed through the benefits of ecosystem services. Plant attributes that influence condition, growth, reproduction, survival, and ecosystem services are functional plant traits classified as morphoanatomical, bioactive, and phenological, and have been confirmed to be influenced by climate (Floret et al., 1990; Ocokoljić et al., 2023a). For future challenging environmental conditions, according to Klein (2014), plants that grow in conditions of seasonal drought have a greater tolerance to rainfall deficits compared to taxa from wetter habitats. In addition, there are specific morphological traits and phenological patterns that have evolved to enable survival (Roloff et al., 2013; Ocokoljić et al., 2023b, Petrov et al., 2024), as well as theoretical estimates aimed at identifying the sensitivity of woody plants based on knowledge of environmental conditions in natural and semi-natural habitats (Sjöman et al., 2012).

Blackthorn is an ornamental species commonly used in the design of hedges as an element of green infrastructure. However, it is also important for ecosystem services as it provides nectar for early pollinators in spring. Its thorny branches (in young plants) offer safe nesting sites for birds, as well as shelter and food for small mammals. Particularly in semi-natural ecosystems, its dense and thorny canopy in oak forest zones creates an impenetrable barrier through which large herbivores find it difficult to pass and thus serves to protect young, palatable seedlings such as oak saplings (Ružičkova et al., 1996; Ocokoljić and Petrov, 2022). Like most species of this genus, blackthorn exhibits a gametophytic self-incompatibility system (GSI), and a high degree of intra and interspecies hybridisation. Alongside with cherry plum – *Prunus cerasifera* – it is considered as one of the progenitors of the European plum (*Prunus domestica*) (Crane and Lawrence, 1952). Fully ripe fruits are a valuable source of natural antioxidants due to their high content of vitamin C and phenolic acids (Ruiz-Rodriguez et al., 2014).

Therefore, the paper analyses the responses of the flowering and fruiting phenophases of blackthorn (*Prunus spinosa* L.) to climatic factors during the 2007–2024 period in agroforestry ecotones near roads. *Prunus spinosa* L. belongs to the *Prunus* genus of the *Rosaceae* family, with its range extending across much of southern and central Europe, reaching northwards to the southern part of the Scandinavian peninsula (Ocokoljić and Petrov, 2022). The paper analyses: (1) the phenological responses of blackthorn to climatic variables at the population level over 18 consecutive years, and (2) the fruits as a source of bioactive compounds, with the aim of promoting the species as a source of safe natural antioxidants.

Material and Methods

Study site

The experimental area is located in Ostružnica, on the right bank of the Sava River, in a suburban zone within the Belgrade municipality of Čukarica, in the settlement of Ostružnica, as part of the local green road infrastructure on flat, unexposed terrain. By identifying the ecotone *in situ*, the conventional landscape pattern of the experimental area was completed, as the remote sensing bypasses transitional zones (Arnot et al., 2004). Seven linear agroforestry ecotones were selected, dominated by blackthorn and characterised by a uniform topography, with the soil classified as eutric brown (eutric cambisol) according to Tanasijević et al. (1963). The structure, composition, and diversity of the ecotones have changed significantly over the 18 years of research, and the presence of woody species (with the clustering of individual trees) revealed an inequality in tree distribution, although the blackthorn remained stable and occupied a larger area within the ecotones.

During 2024, which has been recorded as the warmest year since measurements started, the highest yielding blackthorn population was selected, covering an area of 124 m², and centred at coordinates 44°43'36.80"N and 20°19'35.91"E, at an altitude of 112 m.

Phenology, yield and meteorological data

The phenological patterns during the 2007–2024 period were classified according to the identification keys of BBCH phenological stages for stone fruit (plum, genus *Prunus* L.) as a mean value at the population level (Meier, 1997). Observations were made every other day, at the same time in all ecotones, recording: the onset of flowering (BF) – the day when more than 10% of the flowers on the bush were open, full flowering (FF) – the day when more than 50% of the flowers were open, the end of flowering (EF) – when no more flowers were open, and fruit ripening (RP) – the day when the fruits acquired the characteristic colour of ripe fruit.

The fruit abundance of blackthorn in the ecotones was assessed at the population level by quantifying phenological observations according to Stilinović (1985) on a five-point scale, where 0 stands for no yield (0% of branches with fruits); 1 – very small yield (<20%); 2 – small yield (20–40%); 3 – moderate yield (40–60%); 4 – abundant yield (60–90%); and 5 – maximum yield (>90%).

The sum of degree days, or accumulated heat (GDD), was determined for the key phenological stages of flowering and fruiting according to Lalić et al. (2021). Dates are presented as DOY, i.e., day of the year, with January 1st as DOY 1. Climatic variables were analysed based on hourly and daily values obtained from the main meteorological station Surčin (φ44º47'54.44"N; λ20º27'53.35"E, Figure 1). Bearing in mind that the fruits were under the influence of climatic parameters before sampling, the air temperature and precipitation after their ripening were analysed in relation to the reference period 1991–2020 (RHMZ, 2024; OGI, 2024).

Chemical analysis

In August 2024, physiologically mature fruits from the southern part of the bush of the selected genome (rating 5) were collected for mesocarp analysis to determine the impact of high air temperatures and drought on the total content of polyphenols and the antioxidant activity of blackthorn fruits in a laboratory oven at 80°C.

Five grammes of the fruit mesocarp were weighed and dried to constant weight and extracted with 70% ethanol and distilled water (10 ml) in a ratio of 1:50. The samples were then kept in a dark place for 24 hours, and centrifuged at 10,000rpm for 15 minutes. The content of total phenols and total tannins was determined using a Folin-Ciocalteu reagent (Nagavani and Raghava Rao, 2010). The absorbance at λ=730 nm was read using a Thermo Scientific Evolution 220 spectrophotometer. The total phenol and tannins were expressed as mg of quercetin equivalents/g of fresh weight. The method for determining the amount of total flavonoids was based on the properties of flavonoids to build appropriate metal complexes with metal ions (Markham, 1989). The absorbance at λ=430 nm was read on a spectrophotometer. The content of total flavonoids in the tested extracts was calculated using the calibration curve of the quercetin standard and expressed as mg of quercetin equivalents/g of fresh weight. The qualitative determination of total anthocyanins was based on the property of anthocyanins to reversibly change their structure when the pH of the medium changes, whereby the absorption spectrum changes. The content of monomeric anthocyanins was determined by the pH differential method, based on the properties of the monomeric anthocyanins at pH 1.0 in the form of oxonium ions (red coloured), while at pH 4.5 anthocyanins were in the semi-metallic form (colourless). The concentration of total anthocyanins was expressed as mg of cyaniding-3-glucoside/g of fresh weight.

The antioxidant activity of the blackthorn fruit extracts was evaluated using the FRAP test (reducing power) and DPPH and ABTS tests (radical scavenging).

The FRAP method was determined as previously reported by Benzie and Strain (1996) with adaptations by Kolarov et al. (2021). The calibration curve was constructed using a series of dilutions of FRAP in water. After 30 minutes, the absorbance was read at λ=593 nm. The total FRAP reducing capability was expressed in mg of trolox equivalents/g of fresh weight. The ability of the extracts of the tested plant species to neutralise the radical cation 2.2′-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid), $ABTS^{\cdot+}$, was measured according to the method of Re et al. (1999), with slight modifications. The absorbance at λ=734 nm was measured on a spectrophotometer. The calibration curve was constructed using a series of dilutions of trolox in water. The $ABTS^{\cdot+}$ radical scavenging activity was expressed in mg of trolox equivalents/g of fresh weight. The spectrophotometric determination of the "skewing" activity of the extracts studied was based on monitoring the transformation of $DPPH^{\cdot}$ radicals (2,2-diphenyl-1-picrylhydrazyl radicals) into reduced form (DPPH-H) (Przybylski et al., 1998). The $DPPH^{\cdot}$ radical itself was purple in colour and had an absorption maximum at 517 nm. By reacting with antioxidants, it bound the hydrogen atom to its unpaired electron and thus turned into the yellow DPPH-H. The absorbance at λ=517 nm was read on a spectrophotometer. The calibration curve was constructed using a series of dilutions of trolox in water. After 30 minutes, the absorbance was read at λ=517 nm. The $DPPH^{\cdot}$ radical scavenging activity was expressed in mg of trolox equivalents/g of fresh weight.

Statistical analysis

To assess statistical significance, the non-parametric Mann-Kendall test (Kendall's tau) was applied, which, according to the WMO, is suitable for trend analysis in environmental time series data (Rustum et al., 2017). The Spearman rank correlation coefficient (ρ) was also used, as it indicates whether there is a consistently increasing or decreasing relationship between variables, and because it does not require any assumptions about the frequency distribution of the variables (Quade, 1974). The range of ρ is from -1 to 1, with the sign determining the strength and direction of the relationship. For data interpretation, the scale of Horvat and Mijoč (2012) was used to interpret the data: 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). Coefficients with statistically significant correlations were interpreted with a probability of $p < 0.05$. The data processing and figure preparation were performed using the software packages XLSTAT 2020, Past 4.11, ArcGIS 10.8/ArcMap 10.8, and Google Earth Pro.

Results and Discussion

Relationship between phenophases and climate variables

The average monthly and annual air temperatures and average monthly, annual and total precipitation were calculated for the reference and research periods (Table 1).

Table 1. Climate variables for the reference period (1991–2020), the research period (2010–2024) and 2024 and deviations in 2024 compared to the reference period and the 2007–2023 period, according to the MMS Surčin data.

Mean air temperature (°C)													
Months Period	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$
$\bar{x}$ 2024	2.9	10.1	11.6	15.7	18.6	24.7	26.8	27.4	19.9	14.4	5.7	3.2	15.1
$\bar{x}$ 2007/2023	2.0	4.2	8.1	13.3	17.8	22.1	24.2	24.0	18.8	13.2	8.3	3.4	13.3
$\bar{x}$ 1991/2020	1.0	3.0	7.5	12.9	17.6	21.4	23.2	23.2	18.0	12.8	7.4	2.2	12.5
Deviation 2024(%) from normal value 1991–2020	1.8	7.1	4.2	2.8	1.0	3.3	3.6	4.2	1.9	1.6	-1.7	1.1	2.6
Deviation 2024(%) from normal value 2007–2023	0.9	5.9	3.6	2.4	0.9	2.6	2.6	3.4	1.1	1.2	-2.5	-0.2	1.8
Total and mean precipitation amounts (mm)													
Months Period	1	2	3	4	5	6	7	8	9	10	11	12	∑
$\bar{x}$ 2024	37.1	4.8	27.7	23.3	99.8	92.5	69.4	6.5	86.8	44.0	45.6	63.2	600.7
2007/2023	46.5	39.1	47.3	41.5	82.1	75.4	50.6	46.2	52.0	48.1	51.1	48.9	628.9
$\bar{x}$ 1991/2020	42.4	34.0	41.7	47.4	68.1	80.1	58.2	54.0	56.0	50.7	45.5	48.3	626.4
Deviation 2024(%) from normal value 1991–2020	-5.3	-29.2	-14.0	-24.1	31.7	12.4	11.2	-47.5	30.8	-6.7	0.1	14.9	-25.7
Deviation 2024(%) from normal value 2007–2023	-9.4	-34.3	-19.6	-18.2	17.7	17.1	18.8	-39.7	34.8	-4.1	-5.5	14.3	-28.2

Based on the analysis of the parameters, it can be seen that the mean annual air temperature for the reference period (1991–2020) was 12.5°C, 13.3°C for the research period (2007–2023), and 15.1°C for 2024. The mean annual rainfall for the reference period (1991–2020) was 626.4mm, 628.9mm for the research period (2007–2023), and 600.7mm for 2024. The deviations are shown in Table 1. The mean air temperatures and precipitation amounts by month and on an annual basis for the research period (2007–2024) are shown in Figure 1.

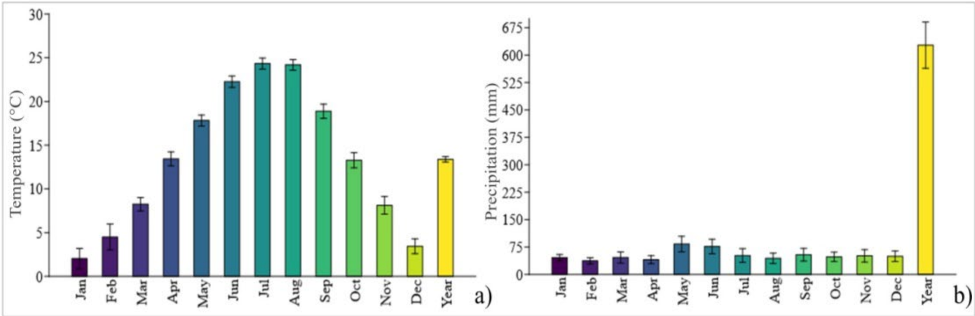

Figure 1. Mean air temperatures (a) and precipitation amounts (b) by month and on an annual basis for the research period (2007–2024), according to the MMS Surčin data. Data are expressed as the mean ± SD (n=18).

Descriptive statistics were used to assess the variability of the climate variables (Tables 2 and 3). The standard deviation and other deviation parameters indicate the variation of mean air temperatures and precipitation during the 18 years of research.

Table 2. Descriptive statistics for mean monthly and annual air temperatures for the 2007–2024 period according to MMS Surčin.

Parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Min	-4.40	-4.00	6.00	10.00	15.00	20.40	22.40	21.90	15.70	9.90	3.30	0.00	12.30
Max	6.30	10.10	11.60	17.40	20.80	24.70	26.80	27.40	21.70	17.00	12.10	6.40	15.10
Sum	36.60	80.90	148.5	241.7	320.8	400.7	438.0	435.4	339.9	239.0	146.2	61.90	240.9
Mean	2.03	4.49	8.25	13.43	17.82	22.26	24.33	24.19	18.88	13.28	8.12	3.44	13.38
Std. error	0.59	0.75	0.39	0.41	0.32	0.33	0.32	0.31	0.42	0.44	0.52	0.43	0.15
Variance	6.34	10.25	2.73	2.99	1.89	2.01	1.84	1.73	3.13	3.55	4.80	3.33	0.42
Stand. dev	2.52	3.20	1.65	1.73	1.38	1.42	1.36	1.32	1.77	1.88	2.19	1.83	0.65
Median	2.10	4.75	8.30	13.50	17.60	21.95	23.60	24.20	19.20	13.65	8.40	3.80	13.30
25 percentiles	0.20	2.88	6.80	12.60	16.80	20.88	23.35	23.40	17.25	11.38	6.95	2.03	13.10
75 percentiles	3.88	6.53	9.53	14.25	18.83	23.68	25.45	25.13	20.10	14.53	9.25	4.75	13.80
Skewness	-0.69	-0.88	0.35	0.16	0.19	0.23	0.44	0.38	-0.10	-0.02	-0.65	-0.33	0.78
Kurtosis	1.27	1.98	-0.74	1.05	0.34	-1.49	-1.13	1.08	-0.87	-0.53	0.76	-0.65	2.22
Geom. mean	0.00	0.00	8.10	13.32	17.77	22.22	24.30	24.16	18.80	13.15	7.77	0.00	13.37
Coeff. var	123.8	71.24	20.02	12.88	7.72	6.37	5.58	5.44	9.37	14.19	26.98	53.10	4.82

Based on the analysed data, the trends of increasing air temperature and decreasing precipitation can be observed. However, the Mann-Kendall trend tests only confirmed a statistically significant increase (significance level: $p<0.05$) in mean annual air temperatures ($S=71$, $Z=2.6733$, $p=0.00751$). Statistical significance was not confirmed for monthly air temperatures and total precipitation, which indicates extreme climatic events (WMO, 2021; RHMZ, 2024).

Table 3. Descriptive statistics for average monthly and annual rainfall for the 2007–2024 period according to MMS Surčin.

Parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Min	18.8	4.8	2.5	5.9	32.1	15.8	6.5	3.6	10.1	9.7	1.6	2	378.3
Max	89.6	68.9	110.3	86.9	233.4	156	181	126.6	161	97.4	152.7	125.5	937.3
Sum	827.6	669.9	832.4	728.1	1496	1375	929.5	791.5	971	861.3	914.8	895.1	11292
Mean	45.98	37.22	46.24	40.45	83.1	76.36	51.64	43.97	53.94	47.85	50.82	49.73	627.3
Std. error	4.395	4.359	7.739	5.696	10.94	10.23	9.575	7.218	8.797	6.507	8.703	7.418	32.31
Variance	347.7	342	1078	584	2154	1882	1650	937.8	1393	762.1	1363	990.5	18785
Stand. dev	18.65	18.49	32.84	24.17	46.41	43.38	40.62	30.62	37.32	27.61	36.92	31.47	137.1
Median	43.2	41.35	37.4	39.35	73.65	80.3	42.45	43.25	52.05	46.3	45.7	50.5	620.6
25 percentiles	33.55	20.2	24.6	21	58.2	33.93	21.25	20.5	22.65	17.03	27.18	30.63	519.3
75 percentiles	59.95	52.08	68.7	58.13	95.75	103.4	70.13	61.83	78.9	69.25	65.15	65.2	734.6
Skewness	0.571	-0.34	0.729	0.233	2.17	0.274	1.979	0.917	1.298	0.226	1.341	0.503	0.295
Kurtosis	0.269	-0.99	-0.52	-0.71	6.22	-0.99	5.419	1.727	2.726	-0.99	2.38	0.829	0.312
Geom. mean	42.34	30.74	33.33	31.45	74.02	62.9	39.39	31.36	42.05	38.83	36.12	33.74	613
Coeff. var	40.55	49.69	71	59.74	55.85	56.81	78.67	69.64	69.18	57.69	72.65	63.29	21.85

Descriptive statistics were used to assess the impact of climatic variables on the adaptability of blackthorn (Table 4). The standard deviation and other deviation parameters indicate a shift in the key phenophases of blackthorn.

Table 4. Descriptive statistics for the studied phenological parameters of blackthorn for the 2007–2024 period.

Parameters	BF DOY	FF DOY	EF DOY	RP DOY	BF GDD	FF GDD	EF GDD	RP GDD
Min	56.0	64.0	83.0	227.0	141.3	167.3	287.7	2697.6
Max	98.0	101.0	112.0	319.0	270.0	303.6	434.1	3333.2
Sum	1344.0	1408.0	1662.0	4893.0	3412.7	3890.7	5711.6	52275.0
Mean	79.06	82.82	97.76	287.82	200.75	228.86	335.98	3075.00
Std. error	2.74	2.68	1.87	5.34	9.06	8.16	9.01	34.71
Variance	127.68	122.03	59.57	484.90	1394.21	1133.26	1380.26	20476.74
Stand. dev	11.30	11.05	7.72	22.02	37.34	33.66	37.15	143.10
Median	82.00	87.00	98.00	289.00	203.40	233.30	343.00	3086.40
25 percentiles	68.50	71.50	92.00	273.50	173.90	202.50	303.55	3021.85
75 percentiles	87.50	91.50	103.50	305.50	225.35	243.20	355.10	3172.30
Skewness	-0.51	-0.36	-0.18	-1.14	0.22	0.55	0.91	-0.95
Kurtosis	-0.42	-0.97	-0.31	2.51	-0.28	0.82	1.64	2.26
Geom. mean	78.25	82.10	97.47	286.98	197.46	226.58	334.13	3071.77
Coeff. var	14.29	13.34	7.89	7.65	18.60	14.71	11.06	4.65

Considering the pronounced variability of the elements of the phenological patterns during the research period, the mean daily air temperatures were determined for the corresponding periods. To assess the impact of air temperature on the elements of the phenological patterns, statistical parameters were used to indicate the variation in the number of days within the phenological phases and the mean values of air temperatures during the relevant periods (Table 5).

Table 5. Descriptive statistics for the investigated phenological parameters and Tmean in the relevant elements of the phenological pattern of blackthorn (2007–2024).

Parameters	№ days BF-FF	Tmean BF-FF	№ days FF-EF	Tmean FF-EF	№ days BF-EF	Tmean BF-EF	№ days EF-RP	Tmean EF-RP
Min	2	6.8	9	6.5	12	7.3	142	17.9
Max	8	13.3	24	16.1	30	14.4	218	21.8
Sum	64	176.5	254	191.2	334	188.4	3248	337.1
Mean	3.76	10.38	14.94	11.25	19.65	11.08	191.06	19.83
Std. error	0.41	0.42	1.19	0.67	1.23	0.52	4.76	0.27
Variance	2.82	3.01	24.06	7.57	25.74	4.63	385.81	1.26
Stand. dev	1.68	1.73	4.90	2.75	5.07	2.15	19.64	1.12
Median	3.00	10.60	14.00	11.10	18.00	11.10	195.00	19.70
25 percentiles	3.00	9.20	11.50	9.05	16.50	9.50	177.00	18.90
75 percentiles	4.50	11.50	18.00	13.55	22.50	13.00	205.50	20.90
Skewness	1.41	-0.39	0.84	0.07	0.93	-0.20	-0.77	-0.05
Kurtosis	1.71	0.13	-0.39	-0.67	-0.02	-0.86	0.95	-0.86
Geom. mean	3.47	10.24	14.25	10.92	19.08	10.88	190.05	19.80
Coeff. var	44.58	16.71	32.83	24.47	25.82	19.41	10.28	5.67

Mann-Kendall trend tests confirmed a statistically significant earlier fruit ripening with a decrease in accumulated heat, as well as a reduction in the number of days from the end of flowering to fruit ripening, and an increase in the average daily air temperature during the EF-RP period (Table 6).

Table 6. Results of the Mann-Kendall trend test for statistically significant elements of the phenological patterns of blackthorn (RP DOY, RP GDD, number of days from EF to RP, and Tmean during EF-RP), at the population level, for the study period 2007–2024.

Phenological observation	RP DOY	RP *GDD*
S*	-61	-64
Z	-2.4737	-2.5951
p	0.013374	0.0094554
Phenological observation	№ days EF-RP	Tmean EF-RP
S*	-68	50
Z	-2.5799	2.0253
p	0.0057818	0.042834

*It has a negative sign (–) when the trend is decreasing, a value of (0) if there is no trend, and a positive sign (+) for an increasing trend.

The values of the Spearman correlation coefficient (ρ) for GDD and DOY, for the relevant flowering phenophase periods, were statistically significant for DOY, confirming a very strong and consistently increasing relationship between BF DOY and FF DOY, BF DOY and EF DOY, and FF DOY and EF DOY. This indicates that changes in the DOY of the onset and full flowering influenced the onset and end of other key stages in the flowering phenological pattern. Strong and consistently increasing positive correlations were observed between BF GDD and FF GDD, as well as between RP DOY and RP GDD, which suggests that an increase in accumulated heat for the onset of flowering affected the accumulated heat for full flowering, and that an increase in RP DOY influenced a higher accumulation of heat for fruit ripening.

Spearman's correlation coefficient indicates a very strong positive correlation between the number of days from full flowering to the end of flowering and the total duration of the flowering phenophase. It also shows that the average air temperature in this interval also rose with the number of days from full flowering to the end of flowering (Figure 2b). The moderate negative correlation between Tmean FF-EF and Tmean BF-EF confirms that if the average temperature from full flowering to the end of flowering is lower, the average temperature from the beginning to the end of flowering will also be lower (Figure 2b).

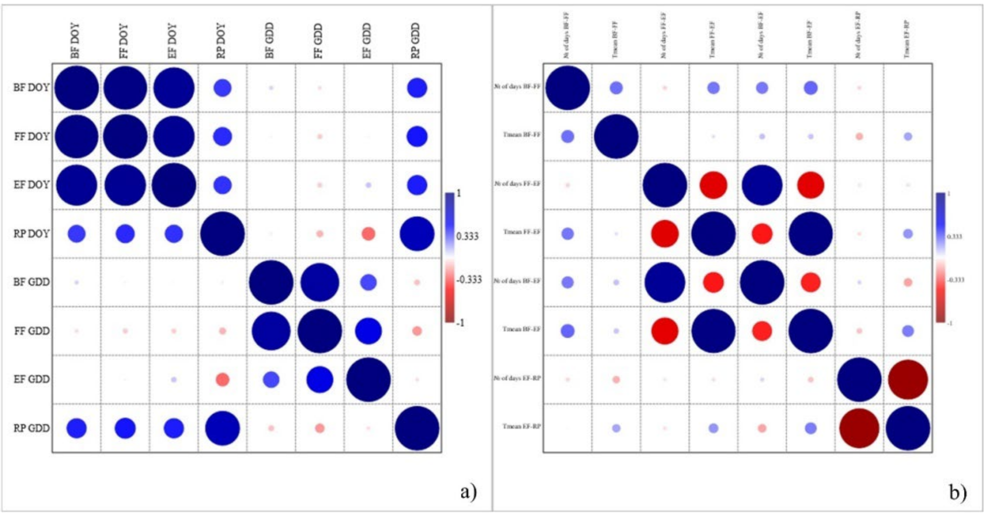

Figure 2. Graphical representation of Spearman's correlation coefficients: a) for the investigated phenological parameters of blackthorn (BF DOY, FF DOY, EF DOY, RP DOY, BF GDD, FF GDD, EF GDD, RP GDD), and b) for the investigated phenological parameters (number of days BF-FF, number of days FF-EF, number of days BF-EF, number of days EF-RP) and Tmean in the relevant elements of the phenological pattern of blackthorn (Tmean BF-FF, Tmean FF-EF, Tmean BF-EF, Tmean EF-RP) during the study period (2007–2024).

Strong and constantly increasing positive correlations were recorded between BF GDD and FF GDD and RP DOY and RP GDD, suggesting that an increase in accumulated heat for the beginning of flowering affected an increase in accumulated heat for full flowering, as well as that an increase in RP DOY affected a greater heat accumulation for fruit ripening (Figure 2a). Other correlations were not statistically significant.

The key phenological stages of blackthorn flowering during 2024 were recorded after the heat accumulation (Figure 3) at 183.3°C (BF), 249.3°C (FF), and 370.6°C (EF), which is approximately in line with the average values for the 2007–2023 period: 204.8°C (BF), 231.5°C (FF), and 334.9°C (EF).

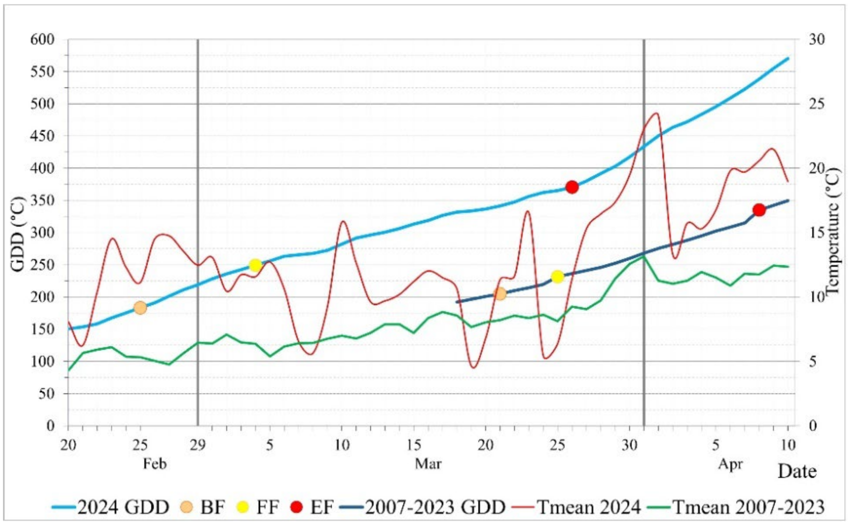

Figure 3. Graphical presentation of the temperature sums (GDD, °C) for the onset of flowering (BF), full flowering (FF), and the end of flowering (EF) of blackthorn (own observations), and the mean daily temperatures, in the study area, for 2024 and the average values, or normal values, for the 2007–2023 period (MMS Surčin). Legend: 2024 GDD (cumulative totals of GDD, with BF, FF and EF markers for 2024); 2007–2023 GDD (average values of cumulative sums of GDD, with BF, FF and EF markers for the 2007–2023 period); Tmean 2024 (mean daily air temperatures during the flowering period during 2024) and Tmean 2007–2023 (mean daily air temperatures during the flowering period during the 2007–2023 period).

The data obtained through descriptive statistics confirm that the temperature sums triggered flowering (Tables 1, 2, 3, 4 and 5). To compare the GDD and the mean daily air temperatures shown in Figure 3, both values during the flowering phenophase of *P. spinosa* L. in 2024 are presented graphically. The statistical analysis of the average daily air temperatures and the phenological observations revealed that: 1) the average value of the daily air temperatures for BF-FF during the 2007–2023 period was 11.1°C, 2) the average value of the daily air temperatures for BF-FF for 2024 was 10.8°C, 3) the average value of the daily air temperatures for the period from DOY 56 to DOY 86 for 2007–2023 was 7.2°C, and 4) the average value of the daily air temperatures for the period from DOY 80 to DOY 96 for 2024 was 15.0°C. It is evident that the average daily air temperatures during 2024 were higher by 3.6°C and 3.9°C, respectively, compared to the previous seventeen-year period. Additionally, an average annual air temperature of 13.3°C was determined for the research period , while it was 12.5°C for the reference period (1991–2020), indicating an increase in air temperature of 0.8°C. These findings are directly related to climate change (WMO, 2021).

Figure 4 shows the phenogram of blackthorn flowering for 2024 and the 2007–2023 period. The year of 2024 stands out clearly, with the flowering phase lasting 31 days, which is 13 days longer than in the 17-year period (2007–2023). Compared to the previous 17-year period, flowering began 24 days earlier.

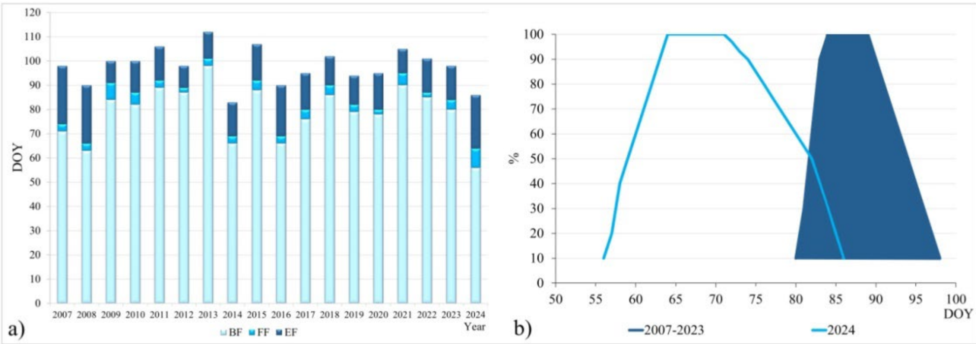

Figure 4. Phenological observations (a) DOY for the onset of flowering (BF), full flowering (FF), and end of flowering (EF) of blackthorn during the 2007–2024 period and (b) in 2024 and the average values for the 2007–2023 period.

The phenological patterns of fruit ripening in blackthorn are influenced by numerous ecological factors at the population level over 18 consecutive years. The most important factors are air temperature and precipitation, which affect ecosystem services and plant phenology (Cosmulescu and Ionescu, 2018; Ocokoljić et al., 2023b). The main response of blackthorn to changes in climatic variables is an earlier fruit ripening, which occurred 65 days earlier in 2024 compared to the average DOY for the 2007–2024 period (Figure 5). During this period, the earliest fruit ripening was recorded on DOY 227 (2024), and the latest on DOY 319 (2011). The average accumulated heat sum (GDD) for fruit ripening is 3075°C, with the lowest value recorded in 2024 (2697.6°C), and the highest in 2012 (3333.2°C).

Statistical analysis of the mean daily air temperatures and phenological observations revealed the following: 1) the average mean daily air temperature for EF-RP during the 2007–2023 period was 19.7°C, 2) the average mean daily air temperature for EF-RP in 2024 was 21.8°C, 3) the average mean daily air temperature for the period 56 to 227 DOY for 2007–2023 was 19.6°C, and 4) the average mean daily air temperature for the period 99 to 292 DOY in 2024 was 21.7°C. It is evident that the mean daily air temperatures from the end of flowering to fruit ripening in 2024 were higher by 2.2°C and 2.0°C compared to the previous seventeen-year period. A comparative analysis showed almost identical average air temperatures from the end of flowering to fruit ripening for the period 2007–2023 and 2024, regardless of DOY, confirming the thesis that phenophases occur within the corresponding accumulated heat sums.

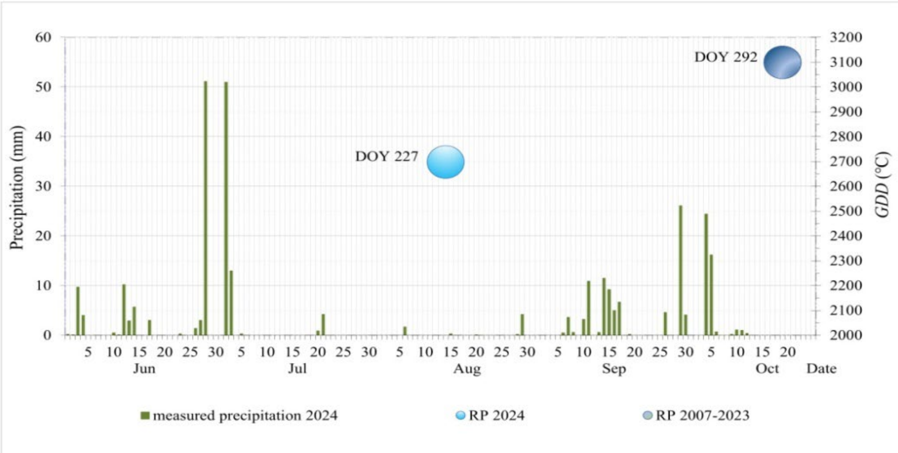

Figure 5. Phenomonitoring of blackthorn fruit ripening (RP) at the population level, DOY and GDD in 2024, and the mean values for the period 2007–2023, along with a graphical representation of the daily precipitation amounts during the summer of 2024 at the Surčin meteorological station.

The lowest and highest GDD values were recorded in the warmest years during the study period, which is correlated with the DOY being lower in 2024, following the warm 2023 and the hottest year of 2024. This is confirmed by the daily mean, maximum, and minimum air temperatures for Surčin, according to the corresponding percentiles. It can be observed that the mean, maximum, and minimum temperatures were above the multi-year average for most of the summer, apart from mid-June, early and late July, and early August, when temperatures were below normal. Heat waves were evident, with five consecutive days above the normal temperature threshold, and the most intense heat wave occurred over 8 days in August, during which fruit ripening was recorded on 227 DOY (14 August) in 2024. Combined with the air temperatures, the rainfall totals indicate specific patterns, such as the rainfall sum for July 2024, which, according to the percentile method, falls within the ‘normal’ category. A closer look at the distribution of daily rainfall amounts reveals that both July and August, as well as the entire summer of 2024, were dry in the study area (Figure 5). The monthly rainfall totals resulted from heavy showers and intense rainfall events, where the amount of rainfall in a single day was equivalent to the amount expected for a whole month in the reference period.

To the best of our knowledge, research on the phenology of *Prunus spinosa* L. is limited. The results of our 18-year study on phenological patterns are consistent with the findings of Réaumur (1735) and Ocokoljić et al. (2023), who stated that plant growth and development are proportional to accumulated thermal units (GDD), which measure the accumulation of heat above a threshold temperature over 24 hours, rather than to daily air temperatures during phenophases. Our results are also in line with the World Meteorological Organisation (WMO, 2021), which asserts that each taxon has an optimal period for its phenophases. Our findings are similar to those of studies in Romania, where a two-year study confirmed a correlation between flowering and spring air temperature (Cosmulescu and Gavrila Calusaru, 2020), and to those of Vander et al. (2016a), who highlighted that flowering occurred earlier in southern Europe compared to populations in Belgium. Additionally, they have noted that the timing of flowering is correlated with the local climate, suggesting that *Prunus spinosa* L. displays plasticity in response to rising air temperatures (Vander et al., 2016b). As in the study by Flynn and Wolkovich (2018), our research shows that in a moderately continental climate, phenological patterns were influenced by both air temperature and photoperiod, which interact to make plant responses complex and nonlinear. The start of flowering and subsequent phenophases in *Prunus spinosa* L. were correlated with temperature and precipitation from January to November. Increased heat accumulation has led to a longer flowering phase (particularly in 2024) and earlier fruit ripening, which aligns with the findings of Teskey et al. (2015). Given that our research has confirmed the adaptability of *Prunus spinosa* L. and its increased coverage in the studied ecotones, these findings are important for agriculture (beekeeping), landscape contexts aimed at creating a sense of place by predicting future changes in biodiversity, as well as for the conservation of habitats for wildlife such as the harvest mouse, great tit, blackbird, starling, tawny owl, and little owl, which exist in the study area (Batrićević and Batanjski, 2014).

The quality of the studied fruits

The comparative analysis of the phytochemical extraction efficacy between distilled water and 70% ethanol reveals significant differences in the content of total phenolics, tannins, flavonoids, anthocyanins, and antioxidant activities, highlighting the importance of solvent choice in phytochemical studies (Table 7).

Table 7. Total polyphenolic content and antioxidant activity of physiologically mature fruits of blackthorn in 2024.

	Total phenolic[1]	Total tannins[1]	Total flavonoids[1]	Total anthocyanins[2]	FRAP[3]	ABTS[3]	DPPH[3]
Distilled water	4.50 ± 0.21	2.30 ± 0.11	0.28 ± 0.04	0.13 ± 0.02	15.25 ± 0.73	34.09 ± 1.19	10.54 ± 0.42
Ethanol	9.99 ± 0.48	4.94 ± 0.50	2.44 ± 0.24	2.76 ± 0.51	31.18 ± 0.52	72.47 ± 1.38	33.86 ± 0.47

The data are mean values ± standard error; [1] Expressed as mg of quercetin equivalents/g of fresh weight; [2] Expressed as mg of cyaniding-3-glucoside/g of fresh weight; [3] Expressed as mg of trolox equivalents/g of fresh weight.

The antioxidant capacity of blackthorn fruit (*Prunus spinosa* L.) extracts is notable, particularly when comparing the efficacy of distilled water and ethanol as solvents. Ethanol extracts exhibited a significantly higher total phenolic content (9.99 ± 0.48 mg/g) compared to distilled water (4.50 ± 0.21 mg/g), enhancing the release of bioactive compounds that combat oxidative stress. The elevated tannin further demonstrates the superior extraction capabilities of ethanol, as tannins are known for their antioxidant properties through metal chelation and radical scavenging. The total flavonoid content was also markedly higher in the ethanol extracts (2.44 ± 0.24 mg/g) than in water (0.28 ± 0.04 mg/g), contributing to cellular protection against oxidative damage. Additionally, total anthocyanin levels were significantly higher in ethanol (2.76 ± 0.51 mg/g) compared to water (0.13 ± 0.02 mg/g), underscoring the role of these pigments as powerful antioxidants. The antioxidant activities assessed through FRAP, ABTS, and DPPH assays confirmed the effectiveness of the ethanol extracts, with FRAP values of 31.18 ± 0.52 mg/g for ethanol versus 15.25 ± 0.73 mg/g for water.

The fruits of *Prunus spinosa* L. analysed in this study had a high content of phenolic compounds and a strong antioxidant capacity, which, according to Jiménez et al. (2017), is important for both the food chain and the pharmaceutical industry. The differences in the content of the analysed compounds, depending on the extraction medium used, are a result of the varying polarity of the organic solvents and their mixtures, which selectively extract individual compounds. The examined extracts showed a strong DPPH radical scavenging activity. Phenols, flavonoids, tannins, and anthocyanins in the ethanol extracts exhibited significantly higher values, similar to the findings of Veličković et al. (2014), who analysed sloe fruits collected in Serbia in October. The sloe fruits (*Prunus spinosa* L.) from the selected ecotone in Southeastern Europe, grown under climate challenges as part of the local roadside green infrastructure, which ripened in August, confirmed their high antioxidant capacity. This finding aligns with studies on sloe fruits in the Romanian flora from 2015 (Skrovankova et al., 2015) and in September 2019 (Andronie et al., 2019). These findings highlight blackthorn as a valuable source of natural antioxidants with potential health benefits (Pinacho et al., 2015), and provide researchers with more detailed information on blackthorn identification in

Southeastern Europe for multipurpose uses (cosmetics, phytotherapy, pharmaceuticals, and the food industry, for the development of new functional foods or the enhancement of existing products). The results support the further evaluation of the functional potential of sloe fruits, as studies from different parts of the world have demonstrated a high concentration of antioxidant compounds (Opriș et al., 2021). Previous studies on other species have revealed that antioxidant capacity is influenced by factors such as cultivar, genotype, geographical region, and stage of ripeness (Memete et al., 2023). However, genotype has proven to be the most important factor influencing the anthocyanin content and antioxidant capacity of sloe fruits in northeastern Turkey (Ilhan, 2023).

Conclusion

The findings provide a basis for studying changes in the phenological patterns of *Prunus spinosa* L. in response to climate change in the ecotones of the road green infrastructure. Furthermore, these results are significant for research in the fields of fruit growing, horticulture, landscape architecture, and for defining guidelines for landscape design aimed at enhancing the physiognomic composition of landscapes and promoting *Prunus spinosa* L. as a species for the future. It has been confirmed that blackthorn (*Prunus spinosa* L.) exhibits plasticity in key functional traits associated with drought tolerance. Fruit ripening shows a significant correlation with high air temperatures during the growing season and rainfall, as well as with extreme climatic events such as late frosts during flowering, making this phenophase useful for assessing the tolerance of the species to climate adaptation and plasticity in response to climate challenges. Understanding the functional traits of blackthorn is essential for directed selection to improve resilience to future climate changes and for the sustainable use of wild fruits. Based on the results, it is recommended to study the genetic variability of blackthorn and consider its introduction into cultivation, as it has been confirmed that, as a wild fruit, it is an exceptionally healthy food suitable for directed use and the creation of sustainable, climate-smart landscapes.

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FENOLOGIJA I BIOHEMIJSKA JEDINJENJA PLODOVA *PRUNUS SPINOSA* L. POD UTICAJEM TEMPERATURE VAZDUHA I PADAVINA

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R e z i m e

Fenologija je proučavanje periodičnih bioloških promena kroz koje biljke prolaze, pod uticajem geografskih i ekoloških uslova sredine, posebno klimatskih varijabli. Ciljevi istraživanja bili su utvrđivanje varijabilnosti fenoloških obrazaca *Prunus spinosa* L. prirodnih populacija u ekotonima jugoistočnog dela Balkanskog poluostrva i formiranje informacione baza podataka o uticaju temperature vazduha i padavina na fenologiju vrste na osnovu istraživanja u periodu 2007–2024. Dobijeni podaci analizirani su deskriptivnim i multivarijantnim statističkim metodama. Rezultati pokazuju da je početak prolećnih fenoloških faza značajno ubrzan porastom temperature vazduha posebno u 2024. godini, kada je fenofaza cvetanja počela 31 dan ranije, 13 dana duže trajala, a dozrevanje plodova bilo 65 dana ranije u odnosu na period 2007–2023. S obzirom na to da trnjina značajno doprinosi ekosistemskim uslugama kao medonosna i vrsta sa primenom u prehrambenoj industriji i fitoterapiji određen je sadržaj fenola, flavonoida, antocijana i antioksidativna aktivnost plodova u destilovanoj vodi i 70% etanolu. Rezultati sugerišu da etanol ne samo da efikasnije ekstrahuje bioaktivne komponente, već i pojačava njihov potencijal za neutralizaciju slobodnih radikala. Istraženi ekstrakti sadrže visok procenat fenola i pokazuju značajnu antioksidativnu aktivnost. Dobijeni rezultati su polazna osnova za proučavanje promena fenoloških obrazaca trnjine kao odgovora na klimatske promene u agroekološkim uslovima jugozapadne prigradske zone Beograda. Potvrđeno je da je trnjina adaptivna sa izuzetkom ranjivosti fenofaze cvetanja na pozne prolećne mrazeve, što je evidentirano 2023. godine. Plastičnost vrste na klimatske izazove potvrdjena je tolerancijom na sušu, posebno tokom 2024, ali i značajnom korelacijom dozrevanja plodova sa visokim temperaturama vazduha tokom vegetacionog perioda tokom 18 uzastopnih godina istraživanja.

Ključne reči: trnjina, adaptivnost, funkcionalnost, antioksidativni kapacitet plodova, usluge ekosistema, toplotni talasi, ekotoni.

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