CONE AND SEED VARIABILITY IN SERBIAN SPRUCE – INDICATORS OF POPULATION ENDANGERMENT

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SUMMARY

Serbian spruce (Picea omorika (Pančić) Purk., Pinaceae) is a tertiary relict and one of the rarest, most endangered, and protected conifer species in Europe. Alarmingly, many studies report a continuous decline in its population and vitality. In response to this concerning trend, the present research aims to better understand the reproductive potential of this species by analysing cone and seed characteristics, as well as intra- and inter-population variability — the critical factors for designing effective *in situ* and *ex situ* conservation strategies. To achieve this, cones were collected during the 2022/23 season (autumn/spring) from 111 trees across seven natural populations and one urban population in Bosnia and Herzegovina. Immediately after collection, cones were measured and processed, seeds were extracted, and germination tests were conducted in April-May 2023. The analysis revealed significant differences among trees and populations for all cone traits. Notably, the level of intra-population variability observed closely resembles the patterns previously identified in genetic studies of the same populations, suggesting a consistent underlying diversity structure. However, the overall germination results point to very low seed viability, 57.03% on average, with 26.85% of seeds being empty. Population-level differences were pronounced: germination rates ranged from 20.40% to 81.14%, while the proportion of empty seeds ranged from 8.10% to 59.60%. Overall, our results suggest that small and endangered populations are particularly vulnerable, producing smaller cones with a higher proportion of empty seeds and significantly lower germination success. This highlights an urgent need for conservation action — to protect the species and support natural regeneration in situ, and to establish ex situ plantations beyond its natural range.

KEY WORDS: cone morphology, endangered populations, genetic diversity, *in situ* and *ex situ* conservation, population differentiation, reproductive biology, seed viability

INTRODUCTION

The survival, or more precisely, the regeneration of a plant species involves seed production, dispersal, germination, and the successful growth of seedlings. Each species has its own unique germination requirements, which can be seen as adaptations for maximizing survival in an unstable and unpredictable environment (Fenner 1985, Iralu et al. 2019). Seed germination and seedling development are critical phases in the life cycle, shaping population dynamics and ultimately influencing

the development, structure, and survival of ecosystems (Chen and Xie 2007, Baeten et al. 2009, Grossnickle and Ivetić 2017). These phases are marked by exceptionally high mortality rates and intense natural selection (Leck et al. 2008, Grossnickle 2018). Consequently, the seedling stage is often considered a "bottleneck" in the life history of a species (Kolb and Barsch 2010, Grossnickle 2016).

A significant number of plant species worldwide are becoming endangered because processes such as seed

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dispersal, germination, and seedling development and survival are increasingly disrupted (Colin and Linda 2002, Gulias et al. 2004). The Intergovernmental Panel on Climate Change (IPCC) estimates that roughly 20-30% of vascular plants may face extinction with a global temperature rise of 2-3°C (Parry et al. 2007). Even small losses in biodiversity can result in profound changes in ecosystem services (Seppälä et al. 2009). Studying the factors behind population declines in endangered plant species, their ecological adaptability (Tumpa et al. 2022), and their mechanisms to mitigate threats has become essential. This knowledge lays the foundation for designing effective strategies to protect their genetic diversity and maintain viable population sizes, making it a priority in global biodiversity conservation efforts (Fernández and Tapias 2022, Xu et al. 2022, Xu and Zang 2023).

Seedling emergence, survival, and establishment are strongly influenced by habitat conditions (Bazzaz 1991, Mataruga et al. 2012, Tumpa et al. 2021), which makes these factors critical for understanding population dynamics and the resilience of plant species. Species with small populations and unique genetic characteristics (Mataruga et al. 2020, Aleksić et al. 2022) are especially vulnerable to the challenges posed by inbreeding depression and various demographic and ecological pressures (Hensen and Wesche 2006). Understanding these influences is crucial, as contemporary ecological factors affecting population growth in endangered plant species remain poorly understood, and there is limited knowledge on effective management strategies. Establishing scientific guidelines for conservation requires a focus on traits like seed germination and seedling development that limit population recovery, even at critically low sizes (Yates and Broadhurst 2002). One notable example of such vulnerability is Serbian spruce (Picea omorika (Pančić) Purk., Pinaceae), a species with a limited population size and unique genetic makeup, which faces significant threats due to these challenges (Pintarić 1969, Dinić 1990, Mataruga et al. 2020, Aleksić et al. 2022).

Serbian spruce is a tertiary relict found only in the mid-course of the Drina River canyon, within a narrow range from 19°09' – 44°07'N (Tisovljak site) to 19°46' – 43°20'N (Mileševa site). All sites are located at altitudes ranging from 750 to 1,550 meters above sea level on very steep, north-facing slopes (Mataruga and Milanović 2020). This species is categorized as endangered by the IUCN (Mataruga et al. 2011) and is protected by the governments of Bosnia and Herzegovina and the Republic of Serbia. The surviving populations persist in highly fragmented habitats in minimal numbers; often only a few mature trees survive, usually several dozen, and rarely up to about a thousand. The problem of natural

regeneration in these populations is recognized, with young seedlings being almost non-existent unless a catastrophic event such as a fire, avalanche, or landslide occurs. Genetic and developmental characteristics have been studied to uncover the causes of its endangerment (Aleksić and Geburek 2010, Mataruga et al. 2020), with slow natural regeneration identified as the primary factor limiting its survival (Mataruga and Milanović 2020). The species' seed germination and seedling formation traits remain unclear in terms of processes related to regeneration, stability, migration, and distribution. In addition, little attention has been given to the quality of seeds or the effects of different treatments on germination of this species (Cyjetković 2011).

To date, extensive research has been conducted on natural populations of Serbian spruce, covering aspects such as genetic diversity (Ballian et al. 2006, Nasri et al. 2008, Aleksić and Geburek 2010, Aleksić et al. 2017, Mataruga et al. 2020), morpho-anatomical variability of needles (Radovanović et al. 2014, Nikolić et al. 2015, Popović et al. 2020), and needles' chemical composition (Nikolić et al. 2009, 2025). However, studies on population variability based on cone and seed morphology remain scarce (Isajev 1987), as do investigations into seed germination dynamics. Our aim was to address these knowledge gaps by analysing population variability through cone and seed morphology, while also examining seed germination potential in the context of factors affecting population persistence. Understanding these factors is essential for developing effective conservation strategies for this critically endangered species.

MATERIALS AND METHODS

Studied species

Serbian spruce is a medium-sized evergreen conifer, reaching heights of 20 to 40 meters. It is distinguished by its remarkably slender, spindle-shaped form, and narrow pyramidal crow (Vidaković 1982). The bark is thin, reddish-brown, and scaly, gradually fissuring with age. Its needles measure 7-20 mm in length and approximately 2 mm in width (Nikolić et al. 2024), dark green on the upper side, with two distinct white stomatal bands on the underside. The needle tips range from rounded to pointed, and they are elliptical, triangular or rhomboidal in cross-section (Radovanović et al. 2014, Nikolić et al. 2015), attached singly to small, persistent peg-like structures (pulvini or sterigmata) on the branches. Male cones are small and reddish, while female cones are positioned higher in the crown and exhibit a similar reddish hue (Krüssmann 1972). Flowering occurs from April to June, with wind pollination ensuring reproductive success. The seed cones are elongated, measuring 2-6 cm in length and 1–2 cm in width (Idžojtić 2019). Initially deep purple to bluish-brown, they mature to dark brown with a subtle bluish tint in autumn of the first year. Seed dispersal relies on wind, facilitated by light, ovate seeds with an 8 mm long wing. Serbian spruce thrives in diverse habitats and is highly tolerant of air pollution, contributing to its widespread cultivation in botanical gardens and arboretums worldwide. However, despite these adaptations, it is gradually disappearing from its natural habitats due to poor natural regeneration, making conservation efforts crucial for its survival.

Study area

In 2022, an abundant yield of Serbian spruce cones was observed in a significant number of natural populations. The cones were collected in the autumn of 2022 by climbing trees, ensuring the identification of each maternal (test) tree – half-sib lines. All cones were

collected from a total of 111 trees across seven natural and one urban population (Figure 1, Table 1). For each tree, coordinates were recorded, basic dendrometric data were taken (diameter (DBH) and height (TH)), and the trees were marked in the field. Out of the 26 described populations in Bosnia and Herzegovina (Mataruga and Milanović 2020), seven spatially isolated natural populations were included (Table 1). The most endangered and habitat-specific populations with cone yield were chosen following previous recommendations (Mataruga and Milanović 2020). Habitat conditions in all sampled populations are similar. The soil is very shallow and of the colluvium type. Detailed climatic data for the study sites are provided by Mataruga and Milanović (2024). In addition to cones collected from natural populations, cones were also collected from mature trees in the urban area of the city of Banja Luka in early spring 2023.

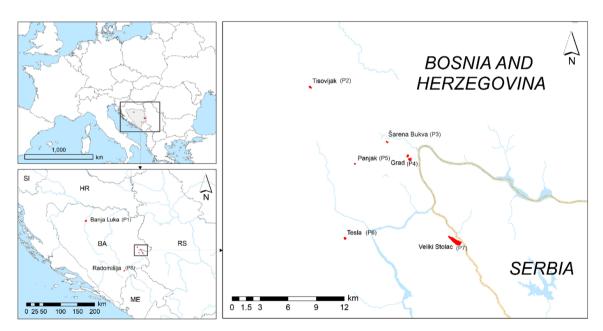


Figure 1 Spatial distribution of Serbian spruce populations where cone samples were collected.

Table 1 Basic characteristics of studied populations: population ID, population name, longitude (E), latitude (N), altitude (m), area (ha), estimated number of mature trees (EN), offspring (OF), number of trees from which seeds were collected (NT), multivariate diversity index (MDI).

Population ID	Population name	Longitude (E)	Latitude (N)	Altitude (m)	Area (ha)	Estimated number of mature trees (EN)	Offspring (OF)	Number of trees from which seeds were collected (NT)	Multivariate diversity index (MDI)
P1	Banja Luka	17.198876	44.764852	160-190	-	-	None	24	1.497
P2	Tisovljak	19.091117	44.072907	980-1080	2.6	100	Rare	17	1.313
P3	Šarena Bukva	19.192886	44.019238	1000-1090	1.0	150	Rare	10	1.515
P4	Grad	19.219963	44.005645	1100-1220	4.4	300	Rare	11	1.540
P5	Panjak	19.149446	43.998802	1270-1330	0.6	30	None	9	0.617
P6	Tesla	19.134806	43.927498	970-1110	2.6	50	Sporadic	11	1.366
P7	Veliki Stolac	19.282496	43.922926	1050-1580	43.8	1000	Sporadic	21	1.076
P8	Radomišlja	18.606057	43.459431	850-1395	44.1	2000	Sporadic	8	1.324

Morphometric analysis and germination tests

Immediately after collection, the total mass of all freshly collected cones from each tree was measured (TMFC). In addition, mass (CM), length (CL), and width (CW) at the widest part were recorded for 12 randomly selected cones from each tree. The cones were then placed in a BCC drying chamber at ±48°C for 72 hours (Rietz and Torgeson 1937, Aldhous 1972). After drying, the seeds were extracted from the cones, and both the dry mass of the cones (TMDC) and the dry mass of the seeds with (SMW) and without wings (SMNW) were measured.

The cone moisture content (CMC) was calculated using the formula:

$$CMC (\%) = \frac{(TMFC - TMDC) \cdot 100}{TMFC}$$

The extraction factor or mass of seeds obtained from the cones is presented in percentages (SMC) using the formula:

$$SMC = \frac{SMW \cdot 100}{TMDC}$$

After manual seed deburring, impurities were removed using the BCC gravity separator. To determine the absolute mass (mass of 1000 seeds) and the number of seeds per kilogram, four sets of 100 seeds were weighted for each tree. Absolute mass and the number of seeds per kilogram were analysed using samples from five randomly selected trees from each originating natural population, totalling 35 test trees. The seeds were then stored in a refrigerator at $4\pm1^{\circ}$ C.

Between 11 April 11 to 2 May 2023, the seeds were placed on a germination table, known as the "Copenhagen table" or "Jacobsen table" (Germinationtable GR10), which maintains a constant temperature and precisely controlled moisture levels, ensuring uniform treatment across all seed samples. Four sets of 100 seeds were placed on filter paper in continuous contact with water at a temperature of 21°C. A total of 101 trees out of 111 were tested, as some trees from which the cones were collected did not yield enough seeds for testing. The number of normally germinated seeds was recorded on days 7, 14, and 21 from the start of the test. On the final day, the number of normally germinated seeds, full but ungerminated seeds, and empty seeds were documented. Seeds were considered normally germinated when the root had broken through the seed coat and grown to at least half the seed's length.

Four parameters were calculated: germination percentage (GP), germination energy (GE), the number of empty seeds (ES), and the number of full but ungerminated seeds (FS) as follows:

- GP = GP_21 (%) = (total number of germinated seeds on the 21st day \times 100) / total number of seeds set for germination
- GE = GP_7 (%) = (number of seeds germinated on the 7th day \times 100) / total number of seeds set for germination
- ES (%) = (total number of empty seeds × 100) / total number of seeds set for germination
- FS (%) = (number of full but ungerminated seeds \times 100) / total number of seeds set for germination

Statistical analysis

Descriptive statistics were performed using Statistica 7.0 (StatSoft Inc. 2004). Analysis of variance (ANOVA) and Duncan's test were employed to determine the significance of differences, with significance levels set at p < 0.01. The Statistica program was also used for correlation analysis to assess the interdependence of seed, cone and tree traits. Principal Component Analysis (PCA) was conducted to reveal the interactions between the analysed variables and to reduce them to a smaller number of factors. A biplot was constructed using two principal components to illustrate analysed individuals and traits. To quantify variability within populations, the multivariate diversity index (MDI) was calculated for each studied population (Poljak et. 2024, Vidaković et al. 2024).

RESULTS

Basic characteristics of the studied trees

The eight studied populations—P1 (Banja Luka), P2 (Tisovljak), P3 (Šarena Bukva), P4 (Grad), P5 (Panjak), P6 (Tesla), P7 (Veliki Stolac) and P8 (Radomišlje)—yielded a total of 111 sampled trees (Table 2), with per-population sample sizes ranging from eight trees in Radomišlje (P8) to 24 in Banja Luka (P1). Across all sites, mean tree height was 16.54 m, with population means ranging from 10.67 m in Banja Luka (P1) to 24.15 m in Tisovljak (P2); individual heights ranged from 7.00 to 32.60 m. Diameter at breast height (DBH) averaged 21.75 cm, population means varied from 18.03 cm in Šarena Bukva (P3) to 26.40 cm in Tisovljak (P2), and single-tree DBH values lay between 12.10 and 41.72 cm. These results highlight substantial variability among the eight populations.

Mass of cones and seeds, cone moisture content and the percentage of seeds per cone

On average, 840.34 grams of cones (747.03 grams in dry weight) were collected from a single tree, yielding 28.34 grams of seeds with wings and 14.06 grams without wings (Table 2). The smallest quantity of cones was collected from the tree Veliki Stolac_16 (P7), amounting to 68.18 grams in fresh weight (59.18 grams in dry weight), while

the largest collection came from the tree Radomišlja_3 (P8), totalling 1,986.54 grams in fresh weight (1,741.54 grams in dry weight). The highest seed yield was recorded from the tree Tisovljak_1 (P2), producing 82.08 grams of seeds with wings (48.22 grams without wings), followed closely by the tree Radomišlja_3 (P8) which yielded 69.32 grams of seeds with wings (50.21 grams without wings).

The average moisture content of cones (CMC) was 11.12%, ranging from 7.31% in the Banja Luka population (P1) to 14.07% in the Radomišlje population (P8). Since cones from Radomišlje (P8) were collected in late autumn and those from Banja Luka (P1) in early spring, it is evident that the time of cone collection, and the prevailing weather conditions are a primary determinant of cone moisture content.

The percentage of seeds per cone (SMC) ranged from 2.53% (P6 – Tesla) and 2.73% (P5 – Panjak) to 5.26% in the Veliki Stolac (P7) population. In contrast to cone moisture content, seed yield from cones appears to be more dependent on the specific population. Notably, Tesla and Panjak—both characterized by a limited number of trees and considered highly endangered—exhibited the lowest seed yield values.

On average, the smallest and lightest seeds were collected from the Tesla (P6) and Panjak (P5) populations, with more than 600,000 seeds per kilogram. In contrast, the largest seeds were found in the Veliki Stolac (P7) population (378,000 seeds per kilogram), while the Grad (P4), Šarena Bukva (P3), Radomišlja (P8), Tisovljak (P2) and Banja Luka (P1) populations had slightly over 400,000 to 430,000 seeds per kilogram (Table 2). The highest seed production per tree was recorded in the Radomišlja (P8) population with approximately 21,000 seeds. This number was calculated by multiplying the absolute mass of a single seed by the total seed mass obtained from the tree $(21,877 = 435,714 \times 0.05021)$. It is important to note that a significant number of trees did not yield any seeds after cone extraction, as their cones remained closed even after several days of drying. This was observed in multiple trees from the Tesla (P6) and Panjak (P5) populations, such as Tesla_1, 7, 8, 9, 10; Panjak_1, 5, 6, 9. In addition, a higher number of seeds per kilogram does not necessarily indicate smaller seeds, as large proportion of empty seeds was present. Although seed size was not directly measured, populations with a higher seed count per kilogram generally had smaller seeds, based on observations during seed processing.

Table 2 Basic characteristics of the studied trees, mass of collected cones and seeds, cone moisture content, percentage of seeds per cone and seed count per kilogram.

		Number of trees	Descriptive parameters	Trees from which seeds were collected		Mass of cones (g)		Mass of seeds (g)		Cone	Percentage	Number
Population ID	Population			Height (m)	Diameter at breast height (cm)	After collection	After drying	With wings	Without wings	moisture content (%)	of seeds per cone (%)	of seeds per kilogram
			Average	10.67	18.31	927.42	858.58	28.78	8.77	7.31	3.33	436,820
P1	Banja Luka	24	Min	7.00	13.06	228.54	216.54	5.86	2.76	3.94	2.26	360,358
			Max	12.50	24.52	1,670.54	1,546.54	68.72	22.74	9.47	4.56	620,320
	Tisovljak		Average	24.15	26.40	1113.71	981.18	46.21	22.14	11.72	4.69	433,034
P2		17	Min	17.40	16.88	394.18	339.18	11.22	6.60	5.42	2.73	322,581
			Max	30.20	39.81	1,933.18	1,560.18	82.08	48.22	19.29	6.61	555,556
	×		Average	12.93	18.03	887.88	775.38	27.52	12.52	13.16	3.82	406,722
P3	Šarena Bukva	10	Min	10.00	12.10	305.18	267.18	9.30	3.86	8.24	2.60	294,118
			Max	16.70	21.97	1,894.18	1,738.18	45.22	24.04	22.27	5.84	588,235
		11	Average	12.53	20.76	752.54	658.45	31.69	19.77	12.40	4.88	407,576
P4	Grad		Min	9.70	15.92	278.18	239.18	10.26	6.34	9.74	3.45	333,333
			Max	17.30	27.71	1,822.18	1,608.18	72.12	45.02	15.13	6.29	500,000
	Panjak	9	Average	15.96	21.76	467.51	419.62	6.65	3.51	9.94	2.73	600,446
P5			Min	11.30	15.92	179.18	165.18	0.00	0.00	7.81	1.33	437,500
			Max	25.80	40.45	843.18	767.18	19.46	13.80	14.56	5.62	714,286
			Average	19.24	24.61	814.36	724.00	11.93	4.62	10.60	2.53	621,947
P6	Tesla	11	Min	14.90	15.92	151.18	137.18	0.00	0.00	9.26	1.47	384,615
			Max	32.60	41.72	1,723.18	1,512.18	44.11	13.84	12.42	3.62	833,333
	.,		Average	19.30	22.40	596.99	519.51	27.83	17.11	13.00	5.26	378,716
P7	Veliki Stolac	21	Min	14.20	13.38	68.18	59.18	2.65	1.22	7.05	3.05	277,778
	otolac		Max	26.20	29.94	950.18	845.18	52.76	32.48	18.58	6.63	568,182
			Average	21.50	25.00	1,153.42	998.42	33.75	23.61	14.07	3.38	435,714
P8	Radomišlje	e 8	Min	19.90	20.06	300.54	276.54	8.96	5.10	7.79	1.70	250,000
			Max	23.10	29.94	1,986.54	1,741.54	69.32	50.21	28.37	5.13	714,286
Total	Total sample		Average	16.54	21.75	840.34	747.03	28.34	14.06	11.12	4.09	465,122

Morphometric traits of cones

The results show considerable variability in cone traits among populations, with significant differences in mass (CM), length (CL), and width (CW). The Grad (P4) population had the highest average cone mass (5.27 g), while Panjak (P5) and Tesla (P6) had the smallest (2.61 g and 2.86 g, respectively), whereas Banja Luka (P1) exhibited

a relatively high cone mass (4.89 g). In terms of length, Banja Luka (P1) stood out with the longest cones (46.90 mm), while Panjak (P5) and Tesla (P6) had the shortest cones, measuring around 30 mm. Regarding cone width, Radomišlja (P8) recorded the largest values (17.80 mm), closely followed by P1 – Banja Luka (17.13 mm), whereas Panjak (P5) had the smallest width (13.75 mm). Statistical analysis confirmed highly significant differences

Table 3 Mean values and standard deviation for cone traits. Values are represented as mean \pm standard deviation. Duncan test results are shown as homogenous groups – same letters above the mean. The last row presents the *F* value from the analysis of variance and the *p*-value of probability.

Population ID	Population	Cone mass (g)	Cone length (mm)	Cone width (mm)
P1	Banja Luka	$4.89c \pm 1.83$	46.90e±7.19	17.13e±2.15
P2	Tisovljak	$4.38b \pm 2.68$	$37.97b \pm 4.62$	$15.98c \pm 1.33$
P3	Šarena Bukva	$4.60c \pm 2.01$	$38.50b \pm 6.02$	$16.15c \pm 2.07$
P4	Grad	$5.27d \pm 1.54$	39.96c±5.98	$16.58d \pm 1.41$
P5	Panjak	2.61a±0.98	29.78a±5.56	13.75a±1.60
P6	Tesla	2.86a±1.21	30.72a±6.10	$14.25b \pm 1.42$
P7	Veliki Stolac	4.72c±1.32	$38.45b \pm 4.27$	$16.10c \pm 1.46$
P8	Radomišlja	4.82c±1.20	$43.38d \pm 5.01$	17.80f±1.48
	All populations	4.37±1.68	39.26±7.81	16.10±2.01
	$F_{\text{pop}}, p_{\text{pop}}$:	58.12, <i>p</i> < 0.001	169.01, <i>p</i> <0.001	83.97, <i>p</i> < 0.001

among populations (p < 0.001 for all traits), indicating strong population-level variability, likely influenced by environmental or genetic factors.

Germination energy and germination rate

The germination analysis revealed significant variation in rates and energy levels across different Serbian spruce populations. The Tesla (P6) and Panjak (P5) populations exhibited lower germination rates and energy levels, whereas the Veliki Stolac (P7) population demonstrated the highest values in both parameters. Notably, the Banja Luka (P1) population showed reduced germination rates compared to the others. Analysis of variance, supported by Duncan's test, confirmed significant differences

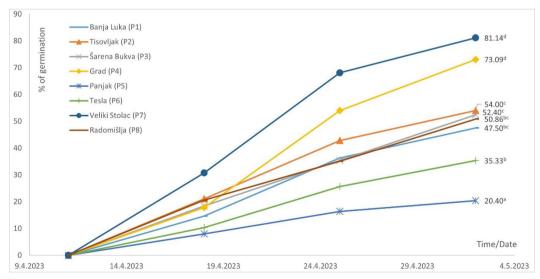


Figure 2 Seed germination dynamics across tested populations (%). Duncan's test results indicate homogenous groups, represented by letters above the germinations values.

among the populations, classifying them into four distinct homogeneous groups (as shown in Figure 2).

The lower germination rates observed in the Tesla (P6) and Panjak (P5) populations can be attributed to a higher percentage of empty seeds (ES). However, differences in

the number of full but ungerminated seeds (FS) were relatively small across all populations, averaging 16.12% (Figure 3). Additionally, these populations contain fewer mature trees compared to others (see Table 1). Duncan's test confirmed significant differences, which align with the observed germination rate groupings.

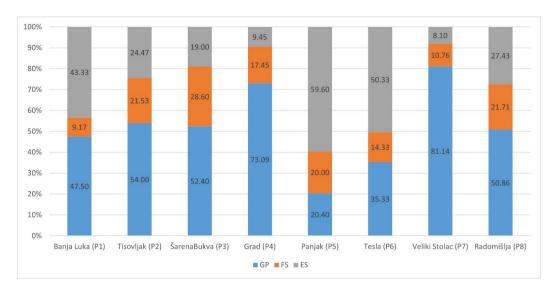


Figure 3 The percentage of germinated seeds (GP), full but ungerminated seeds (FS), and empty seeds (ES).

Correlation analysis

Table 4 shows the results of the correlation analysis. First, germination percentages at 7, 14 and 21 days (GP_7, GP_14, GP_21) are all strongly positively correlated (r = 0.701-0.922, p < 0.01), while the proportion of empty seeds (ES) is strongly negatively correlated with those same germination metrics (r = -0.610 to -0.863, p < 0.01). The percent of full but ungerminated seeds (FS) shows a low negative correlation with GP_7 (r = -0.224, p < 0.05) and slightly stronger negative correlation with later germination GP_14 (r = -0.366, p < 0.01), GP_21(r = -0.333, p < 0.01). Notably, seed germination was found to be independent of cone length and width.

Total mass of fresh (TMFC) and dry (TMDC) cones are essentially redundant (r = 0.996, p < 0.01), as are seed mass with wings (SMW) and without wings (SMNW) (r = 0.904, p < 0.01). These seed weight traits correlate positively with total mass of fresh (TMFC vs. SMW, r = 0.793, p < 0.01; TMFC vs. SMNW, r = 0.645, p < 0.01) and dry cones (TMDC vs. SMW, r = 0.791, p < 0.01; TMDC vs. SMNW, r = 0.630, p < 0.01), as well as with the percent of seeds per cone (SMC) ($r \approx 0.60$, p < 0.01), and moderately with cone size parameters – mass (CM), length (CL), and width (CW) (r = 0.386-0.473, p < 0.01). Cone length (CL) and width (CW) are likewise highly positively correlated (r = 0.834, p < 0.01), and both scale closely with cone mass (CM) (r = 0.842 and r = 0.843, respectively, p < 0.01). Additionally, these three cone traits (CL, CW, CM) were moderately correlated with total mass of fresh (TMFC) and dry (TMDC) cones (r = 0.40-0.47, p < 0.40-0.470.01).

Cone moisture content (CMC) exhibited only a few statistically significant and weak correlations, namely, with the percent of full but ungerminated seeds (FS; r = 0.243, p < 0.05) and the percent of empty seeds (ES; r = -0.295, p < 0.01). CMC also showed a positive correlation with total cone mass (CM; r = 0.228, p < 0.05) and tree height (TH; r = 0.294, p < 0.01), while remaining uncorrelated with germination rates (GP_7, GP_14, GP_21), seed weights (SMW, SMNW), and most cone size attributes (TMFC, TMDC, CL, CW).

Tree size metrics—height (TH) and diameter at breast height (DBH)—are themselves very strongly correlated (r = 0.796, p < 0.01), but generally display only weak associations with seed and cone traits. Tree height (TH) showed low but statistically significant correlations with seed mass with wings (SMW; r = 0.241, p < 0.05), seed mass without wings (SMNW; r = 0.296, p < 0.01), cone moisture content (CMC; r = 0.294, p < 0.01), the percentage of seeds per cone (SMC; r = 0.201, p < 0.05), cone mass (CM; r = -0.199, p < 0.05), cone length (CL; r =-0.304, p < 0.01), and early germination at 7 days (GP_7; r = 0.217, p < 0.05). Similarly, DBH exhibited weak but statistically significant correlations with cone fresh weight (TMFC; r = 0.293, p < 0.01), dry weight (TMDC; r = 0.272, p < 0.01), seed mass with wings (SMW; r =0.230, p < 0.05), and seed mass without wings (SMNW; r = 0.212, p < 0.05). No meaningful correlations were observed between DBH and germination rates (GP_7-GP_21), cone moisture content (CMC), seed output (SMC), or cone size dimensions (CM, CL, CW). Overall, all associations for TH and DBH fell within the low correlation range ($|\mathbf{r}| \approx 0.20-0.30$).

Table 4 Pearson's correlation coefficients between seed, cone and tree traits. Analysed traits: GP_7 - % of germinated seeds on day 7; GP_14 - % of germinated seeds on day 14; GP_21 - % of germinated seeds on day 21; FS - % of full but ungerminated seeds; ES - % of empty seeds; TMFC - total fresh cone mass per tree; TMDC - total dry cone mass per tree; SMW - seed mass with wings; SMNW - seed mass without wings, CMC - cone moisture content; SMC - % of seeds per cone; CM - cone mass; CL - cone length; CW - cone width; TH - tree height; DBH - diameter at breast height. Significant correlation values are marked in red.

Trait	GP_7	GP_14	GP_21	FS	ES	TMFC	TMDC	SMW	SMNW	СМС	SMC	СМ	CL	CW	TH
GP_14	0.774**														
GP_21	0.701**	0.922**													
FS	-0.224*	-0.366**	-0.333**												
ES	-0.610**	-0.765**	-0.863**	-0.188											
TMFC	-0.080	-0.186	-0.139	0.039	0.123										
TMDC	-0.086	-0.184	-0.145	0.018	0.141	0.996**									
SMW	0.144	0.085	0.124	0.079	-0.171	0.793**	0.791**								
SMNW	0.251*	0.212*	0.266**	0.121	-0.341**	0.645**	0.630**	0.904**							
CMC	0.154	0.066	0.160	0.243*	-0.295**	-0.008	-0.085	0.000	0.135						
SMC	0.456**	0.523**	0.523**	0.050	-0.569**	0.039	0.032	0.567**	0.598**	0.135					
CM	-0.061	0.081	0.204*	-0.141	-0.135	0.399**	0.386**	0.467**	0.440**	0.228*	0.333**				
CL	-0.184	-0.104	-0.038	-0.183	0.135	0.466**	0.473**	0.484**	0.354**	-0.035	0.294**	0.842**			
CW	-0.157	-0.039	0.056	-0.036	-0.039	0.403**	0.404**	0.455**	0.403**	0.041	0.306**	0.843**	0.834**		
TH	0.217*	0.085	0.026	0.165	-0.114	0.162	0.137	0.241*	0.296**	0.294**	0.201*	-0.199*	-0.304**	-0.143	
DBH	-0.017	-0.093	-0.124	0.132	0.058	0.293**	0.272**	0.230*	0.212*	0.180	0.052	-0.114	-0.133	-0.020	0.796**

**Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level

Principal component analysis (PCA)

This analysis provides a comprehensive overview of the grouping and dispersion patterns among populations based on 16 studied seed, cone, and tree traits (Figure 4). The first five principal components have eigenvalues

greater than 1. The first principal component (PC1) explains 26.90% of the total variation. In the positive direction of PC1, populations exhibit higher germination rates and a greater percentage of seeds per cone. Trees from the Veliki Stolac (P7), Tisovljak (P2), and Grad (P4) populations are notably positioned on this side of

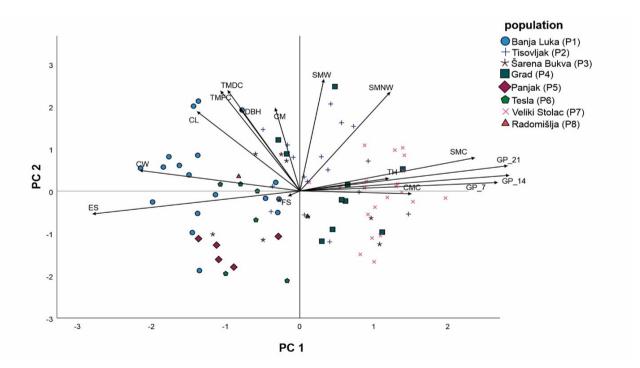


Figure 4 Principal component analysis (PCA) of the studied populations (P1–P8) based on analysed seed, cone, and tree traits. Analysed traits: GP_7 - % of germinated seeds on day 7; GP_14 - % of germinated seeds on day 14; GP_21 - % of germinated seeds on day 21; FS - % of full but ungerminated seeds; ES - % of empty seeds; TMFC - total fresh cone mass per tree; TMDC - total dry cone mass per tree; SMW - seed mass with wings; SMNW - seed mass without wings, CMC - cone moisture content; SMC - % of seeds per cone; CM - cone mass; CL - cone length; CW - cone width; TH - tree height; DBH - diameter at breast height.

Table 5 Pearson's correlation coefficients between the studied traits and scores of the first six principal components, along with eigenvalues, explained variance (%) and cumulative variance (%) for each component in the PCA (all components with eigenvalues greater than 1 and first bellow 1 are included. Studied traits: acronyms as defined in Table 4 and Figure 4.

Trait			PC – principa	PC – principal component						
IIdil	PC1	PC2	PC3	PC4	PC5	PC6				
GP_7	0.797	0.067	-0.159	-0.305	-0.029	-0.099				
GP_14	0.844	0.124	-0.357	-0.271	0.023	-0.044				
GP_21	0.837	0.201	-0.407	-0.140	-0.001	-0.083				
FS	-0.048	-0.042	0.376	0.716	-0.382	0.261				
ES	-0.836	-0.184	0.221	-0.233	0.202	-0.052				
TMFC	-0.292	0.804	0.333	-0.143	-0.166	-0.299				
TMDC	-0.319	0.799	0.309	-0.188	-0.190	-0.263				
SMW	0.096	0.893	0.305	-0.079	-0.223	0.053				
SMNW	0.364	0.791	0.266	0.079	-0.207	0.076				
CMC	0.451	-0.023	0.131	0.587	0.292	-0.463				
SMC	0.705	0.265	-0.011	0.064	-0.055	0.487				
CM	-0.101	0.663	-0.497	0.340	0.339	-0.125				
CL	-0.415	0.635	-0.532	0.080	0.208	0.064				
CW	-0.647	0.164	-0.441	-0.272	-0.065	0.283				
TH	0.358	0.100	0.741	-0.119	0.424	0.153				
DBH	-0.243	0.659	-0.448	0.234	0.277	0.249				
Eigenvalues	4.57	4.24	2.86	1.46	1.11	0.94				
Variance (%)	26.9	24.94	16.85	8.57	6.56	5.54				
Cumulative variance (%)	26.9	51.84	68.69	77.27	83.82	89.36				

the diagram. Conversely, the negative direction of PC1 is associated with a higher proportion of empty seeds. This component also shows grouping in the positive direction of cone-related traits. Panjak (P5) and Tesla (P6) populations are distinctly located in the lower-left quadrant of the PCA plot, reflecting smaller cones and lower germination energy and rates. This pattern corresponds with the negative axis of PC2, which accounts for 24.94% of the variation, yielding a cumulative variance of 51.84% (Table 5). PC2 predominantly represents traits related to seed mass. Additionally, the Banja Luka (P1) population stands out in the upper-left quadrant, indicating distinct cone traits compared to the other populations.

Multivariate diversity index

Based on all measured parameters, the MDI coefficients indicate the highest diversity in the P4–Grad (1.540) and P3–Šarena Bukva (1.515) populations, which are geographically close. In contrast, the P5–Panjak population exhibits the lowest diversity (0.617). The remaining studied populations show MDI values ranging from 1.076 and 1.497 (Table 1).

DISCUSSION

The dimensions of Serbian spruce cones vary significantly among the studied populations, highlighting potential ecological and genetic influences on their

morphology. According to Vidaković (1982) and Idžojtić (2019), Serbian spruce cones typically range from 20 to 60 mm in length and 10 to 20 mm in width, while Krüssmann (1972) reports a length range of 30 to 60 mm and a width of 10 mm. The cones measured in Bosnia and Herzegovina, spanning eight populations of Serbian spruce, had an average length of 39.3 mm and a width of 16.1 mm. These measurements fall within the ranges previously documented in dendrological literature.

In our study, average cone length across natural populations ranged from 29.8 to 43.4 mm, whereas the urban population exhibited the longest cones, averaging 46.9 mm. Similarly, cone width in natural populations varied from 13.7 to 17.8 mm, while the urban population had an average width of 17.1 mm. These differences may be attributed to specific growth conditions in urban environments, including resource availability, microclimatic factors, and potential anthropogenic influences. Another possible explanation is that trees in the urban population in northern Bosnia and Herzegovina may originate from a different population rather than those analysed in this study. This possibility suggests that genetic differences among populations could play a role in the observed cone size variations. Further research, including genetic analyses, would be valuable in clarifying the origins and adaptability of urban populations compared to their naturally occurring counterparts.

As already stated in the introduction, previous research has primarily focused on genetic variability (Ballian et al. 2006, Nasri et al. 2008, Aleksić and Geburek 2010, Aleksić et al. 2017, Mataruga et al. 2020) and phenotypic analysis (Radovanović et al. 2014, Nikolić et al. 2015, Popović et al. 2020, Nikolić et al. 2025) based on needles, while studies examining cone morphology have been relatively scarce. One notable investigation was conducted by Isajev (1987), who analysed the variability of Serbian spruce trees across three planted forests in Serbia. His morphometric analysis of 1,000 cones harvested in 1981 and 1983 revealed significant variability between trees in cone length (20–70 mm), width (10-23 mm), and the number of seeds per cone (20-113 grains). The values for cone length and width in our study align with these findings. In addition, his study highlighted that cone size varied within 5% on the same tree across different harvest years, indicating strong genetic control of this trait (Isajev 1987). The obtained results showed that the Serbian spruce seeds from all three planted forests are characterized by very good quality (average germination rate was 90%).

In forest trees, reproductive dynamics and success are intricately linked to seed production, which varies across demographic stages and depends heavily on favourable environmental conditions (Moran and Clark 2012, Younginger et al. 2017). Fragmentation and climate change significantly affect seed quality and dispersal, both of which are crucial for species survival (McConkey et al. 2012). Empirical research highlights the complex relationship between seed size and germination time, with studies showing positive correlations between seed size and germination percentage and energy (Parker et al. 2006, Jones and Reekie 2006, Upadhaya et al. 2007, Souza and Fagundes 2014, Kaliniewicz et al. 2018), negative correlations (Simons and Johnston 2000, Gomez 2004, Tiscar and Lucas 2010, Hojjat 2011), and instances where no correlation exists (Larson 1963, Vaughton and Ramsey 1998, Tumpa et al. 2021). Serbian spruce populations with smaller/lighter seeds, such as those in the Tesla (P6) and Panjak (P5) populations with over 600,000 seeds per kilogram, exhibited poor germination rates, posing a threat to their already endangered status. This further supports the positive correlation observed between seed size and germination efficiency in Serbian spruce, as larger seeds likely store more nutrients that facilitate rapid germination and early seedling growth (Domic et al. 2020). Understanding these relationships is essential for conservation efforts aimed at preserving Serbian spruce populations. Improving seed quality and ensuring favourable germination conditions are critical strategies for mitigating the impact of environmental stressors on this endangered species.

Germination rates of Serbian spruce seeds in our study ranged from 20.4% to 81.4%, with an overall average of 51.8% across all investigated populations, aligning with findings for Norway spruce reported by Sarvas (1986) and Himanen et al. (2016). In contrast, Pintarić (1956, 1957, 1969) documented exceptionally high germination rates under optimal conditions, with one-year-old seeds reaching 97-98% and nearly 100% after 10 days. Furthermore, Pintarić (1970) demonstrated that proper storage preserves seed viability, as seeds aged up to three years still germinated at around 96%. However, the unspecified seed origin in Pintaric's studies limits the broader applicability of his findings. More recently, Cvjetković et al. (2013) observed significant differences in germination based on seed provenance, with seeds from planted forests exhibiting the highest germination rate at 84%, whereas those from natural populations germinated at 58.57% and 65.15%, showing extended dormancy periods. Refrigerator storage at ±4°C for six months improved germination by 20-28%, resembling the effects of stratification. The study conducted by Cvjetković et al. (2013) indicated considerable variation in seed germination depending on whether they were collected in natural or planted forests. However, this pattern was not observed in our study, where the Banja Luka (P1) population exhibited a germination rate of 47.5%, slightly below the average for all analysed populations. These results highlight the variability in germination success across different Serbian spruce populations and suggest that environmental factors and seed provenance may play a critical role in determining germination rates. Further highlighting the variability of Serbian spruce seed germination, Ostojić and Dinić (2009) examined germination under natural conditions, revealing poor seed germination and seedling failure in the first year. Comparative experiments confirmed that Serbian spruce seedling establishment is heavily influenced by abiotic and biotic factors, including early spring and autumn frosts, dense forest canopy, thick layers of litter, herbaceous competition in open areas, and soil overheating in dry summer periods. Dinić (1988, 1989) previously reported similar findings, attributing poor seed germination and seedling survival to the sharp decline in tree numbers at the study location—from 40 trees recorded in early 1950s to only four remaining today. Later comparative experiments further validated these observations, confirming the sensitivity of Serbian spruce seeds and seedlings to multiple environmental pressures (Dinić 1990).

The failure of Serbian spruce natural regeneration is primarily linked to frequent fires (Aleksić et al. 2022), limited availability of suitable habitats, and strong

competition with other species (Tucić and Stojković 2001, Ostojić 2005). Additionally, recent studies suggest that poor genetic connectivity, even at small spatial scales, may further hinder seed dispersal (Aleksić and Geburek 2010, Mataruga et al. 2020). Despite these challenges, genetic diversity monitoring between two generations of Serbian spruce did not reveal negative effects on the genetic structure of the younger generation, even though only 25% of adult trees from the same population contributed to its formation, with 66% of pollen coming from unknown sources (Aleksić et al. 2022). These findings emphasize the strong link between the number of mature trees and seed quality, which is crucial for the species' survival in critical habitats. Moreover, the weak germination of seeds from endangered populations with fewer adult trees further supports the existence of mechanisms that help prevent inbreeding in this species (Mataruga and Milanović 2020).

In addition to confirming a positive correlation between seed mass and germination rate, we also established a statistically significant positive correlation between cone size (length and width) and mass, as well as between cone dimensions and seed mass. Similar findings have been reported by other authors investigating variability across different species of gymnosperms (Gómez 2004, Moya et al. 2007, Castanha et al. 2013, Memišević Hodžić et al. 2020, Poljak et al. 2020) and angiosperms (Poljak et al. 2021, Vidaković and Poljak 2024, Vidaković et al. 2025). According to Poljak et al. (2025), these results align with the general expectation that an increase in one cone/ fruit dimension is often accompanied by proportional increases in other related dimensions, reflecting the interconnected nature of cone/fruit development. For instance, larger fruits tend to have a greater width and mass due to integrated growth processes (Vidaković et al. 2025).

Previous analyses of the genetic structure of Serbian spruce using SSR markers across 14 natural populations in Bosnia and Herzegovina (Mataruga et al. 2020) included all populations except Tesla (P6) and Banja Luka (P1), which are examined in the present study. When comparing the results of Bayesian clustering from that research with the outcomes of the principal component analysis (PCA) in this study, certain similarities in the population grouping patterns can be observed. However, clustering did not always reflect genetic origin. For example, samples from the Grad (P4) and Veliki Stolac (P7) populations were grouped together based on morphological and germination traits, yet SSR markers placed them in distinct genetic clusters. In contrast, genetically close populations like Šarena Bukva (P3) and Panjak (P5) were consistently grouped together across both

phenotypic traits and molecular data. These findings suggest that, in addition to genetic variability, environmental conditions play a major role in shaping cone and seed morphology and affecting germination success. In addition to the differentiation of the urban population Banja Luka (P1) from the natural ones (P2–P8), a general trend was observed in the PCA biplot: samples from smaller natural populations tended to cluster within the section characterized by reproductive constraints, such as reduced germination rates and a higher proportion of empty seeds.

Genetic and phenotypic variations in Serbian spruce populations generally follow similar patterns, though not always in direct proportion. Previous research (Mataruga et al. 2020) identified Panjak (P5), Tisovljak (P2), and Grad (P4) as populations with the lowest contribution to total allelic diversity, while Veliki Stolac (P7), Šarena Bukva (P3), and Radomišlja (P8) exhibited the highest genetic variation. In our analysis, Šarena Bukva (P3) and Grad (P4) demonstrated the highest levels of phenotypic variability, based on multivariate diversity index (MDI) values of 1.515 and 1.540, respectively. Interestingly, despite Grad's (P4) lower genetic contribution, its high phenotypic diversity suggests environmental or developmental factors may be influencing morphological traits beyond what is reflected by neutral markers. Veliki Stolac (P7), although genetically diverse and reproductively vigorous, recorded a relatively modest MDI (1.076), possibly due to environmental homogeneity. The Panjak (P5) population—characterized by low genetic diversity, geographic isolation, and poor seed quality—also exhibited the lowest morphological variability (MDI = 0.617), reinforcing its designation as the most vulnerable unit. Overall, Serbian spruce populations display intrapopulation morphological variation comparable to that of other woody taxa, including Salix eleagnos Scop. (MDI = 1.154–3.480; Poljak et al. 2024), *Juniperus oxy*cedrus (MDI = 1.506-1.772, Vidaković et al. 2024) and *J. deltoides* (MDI = 1.110–1.385, Vidaković et al. 2024), indicating a notable reservoir of phenotypic diversity despite the species' narrow distribution and endangered status.

CONCLUSIONS

Significant differences were observed in cone and seed traits, as well as in seed germination characteristics, among the examined Serbian spruce populations (seven natural and one urban). Germination rates were notably low and considerably lower compared to earlier findings. This decline is likely a consequence of climatic conditions during the cone harvest year and the fact that, for the first time, cones were collected from endangered populations

characterized by very limited number of mature trees. The biological quality of the seeds—reflected in their smaller size and low absolute mass—combined with the harsh environmental conditions in which these populations persist, led to a substantial loss of viable seeds. These constraints severely hinder natural regeneration and the sustainability of populations. This may also help explain why Serbian spruce tends to regenerate poorly in its native habitats.

The number of trees and their spatial distribution are crucial factors influencing cone and seed traits, as well as seed quality and germination rates at the population level. Threatened populations with fewer and more spatially isolated trees exhibit the poorest seed germination rates, suggesting the presence of a natural mechanism of blocking self-fertilization (inbreeding) in this species. This reproductive strategy, while beneficial for maintaining genetic integrity, may also reduce regeneration potential in fragmented and declining populations.

To ensure the long-term survival of Serbian spruce populations and support their recovery and expansion, conservation priorities should focus on protecting and restoring their native habitats and plant communities. At the same time, translocation and *ex-situ* conservation efforts could be targeted toward areas with more favourable environmental conditions, such as better-preserved vegetation, higher humidity, flatter terrain, and well-drained, loose soil. These combined measures would enhance the effectiveness of regeneration efforts and contribute to the conservation of the genetic resources of this valuable and critically endangered species.

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