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Phenological Variability of *Pinus sylvestris* L. Provenances in the International Provenance Test in Kupres, Bosnia and Herzegovina

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ABSTRACT

Scots pine (*Pinus sylvestris* L.) is an important forest tree species growing on different soils in Bosnia and Herzegovina. Phenological traits are relevant practical indicators of the adaptability and adaptedness of forest tree species. This research aims to determine the dates of the beginning and the end and duration of phases of bud breaking and elongation in *P. sylvestris*. The results will be used in the selection of best provenances for the areas with late frosts. The materials used were *P. sylvestris* plants in the international provenance test Kupres. The test was established in 2012 with 15 provenances (three from Austria, one from Bosnia and Herzegovina, two from Germany, three from Italy, one from Poland, one from Romania, one from Slovakia, two from Norway and one from Ukraine). Six phases were observed for bud breaking and elongation. Phase 1 (start of elongation, scales partly disjoined but still covering the young shoot) was recorded on 10 May in all provenances. Phase 5 (2 needles of the same brachyblasts are clearly distinct) first occurred on 6 June on some plants of every provenances except Bugojno (Bosnia and Herzegovina), Delytayn (Ukraine), and Ca Del Lupo (Italy). Understanding phenological phenomena of Scots pine is important since the occurrence of late spring frost can damage the plants of Scots pine in the environmental conditions of Bosnia and Herzegovina. Research should be continued and include an investigation of the influence of seasonal climate and climate changes.

Keywords: Scots pine; spring phenology; selection; late frost resistance

INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is the most widely distributed pine species in the world, and it can be found all the way across Eurasia (Houston Durrant et al. 2016). It reaches 23-27 m in height on average, but can attain over 40 m and live for 400 years or more (Houston Durrant et al. 2016). It represents one of the most important conifer species in the forests of Bosnia and Herzegovina, where it inhabits large areas and as a pioneer species tolerates poor soils, drought, wind and frost (Pintarić 2002). According to the preliminary results of the Second Forest Inventory in Bosnia and Herzegovina, the area of pure stands of Scots pine, of production character, in Bosnia and Herzegovina amounts to 38,000 ha (Lojo and Balić 2011, Ballian et al. 2019).

Scots pine was investigated in Bosnia and Herzegovina on the morphological level (in clonal plantations, provenance tests, cone and seed morphology) (Daničić 2008, Ballian et al. 2009, 2019, Daničić et al. 2011, Ballian and Šito 2017, Memišević Hodžić et al. 2020a, 2020b), physiological level (Daničić 2008, 2011), phenological level (Daničić et al. 2011, 2015, Ballian and Šito 2017, Ballian et al. 2019), and molecular level using biochemical markers (Ballian and Božič 2004, Ballian et al. 2006).

Phenological traits are relevant practical indicators of the adaptability and adaptedness of forest tree species. According to some authors (Nilson and Walfridsson 1995, Eriksson and Ekberg 2001), due to changing environmental factors caused by global warming, Scots pine is migrating north. Northern and continental populations require a lower temperature sum to complete the phenophases and show a higher level of resistance. Southern and coastal provenances have a longer vegetation period and are less resistant to stressful environmental conditions (Sarvas 1962, Ballian et al. 2019).

This research aims to identify variability in the beginning, the end and the duration of phases of bud breaking and elongation in *Pinus sylvestris* per provenances, to recommend suitable provenances for afforestation in the suitable areas.

MATERIALS AND METHODS

Study Area

The materials used were *Pinus sylvestris* plants in international provenance test Kupres. The test was established in 2012 by planting two-year-old seedlings from 15 different provenances originating from ten European countries (Table 1, Figure 1).

Planting distance was 2×2 m. Thirty-six (36) seedlings in five repetitions per randomized scheme were planted for each provenance (180 plants per provenances). Provenances originated from areas with different climates: provenances from Austria, Slovakia, Poland and Romania are from a temperate continental climate and one from B&H is from a subalpine temperate continental climate. Provenances from Italy are from a continental climate and those from Germany from a moderately warm and humid climate influenced by the Atlantic Ocean. The provenance from Ukraine is from a continental climate, but from one of the coldest regions in Ukraine with an average daily maximum temperature of only 12°C and frequent rainfall and wind.

Provenance test is located on 1140 m of altitude, on terrain characterized by sinkholes and numerous hills

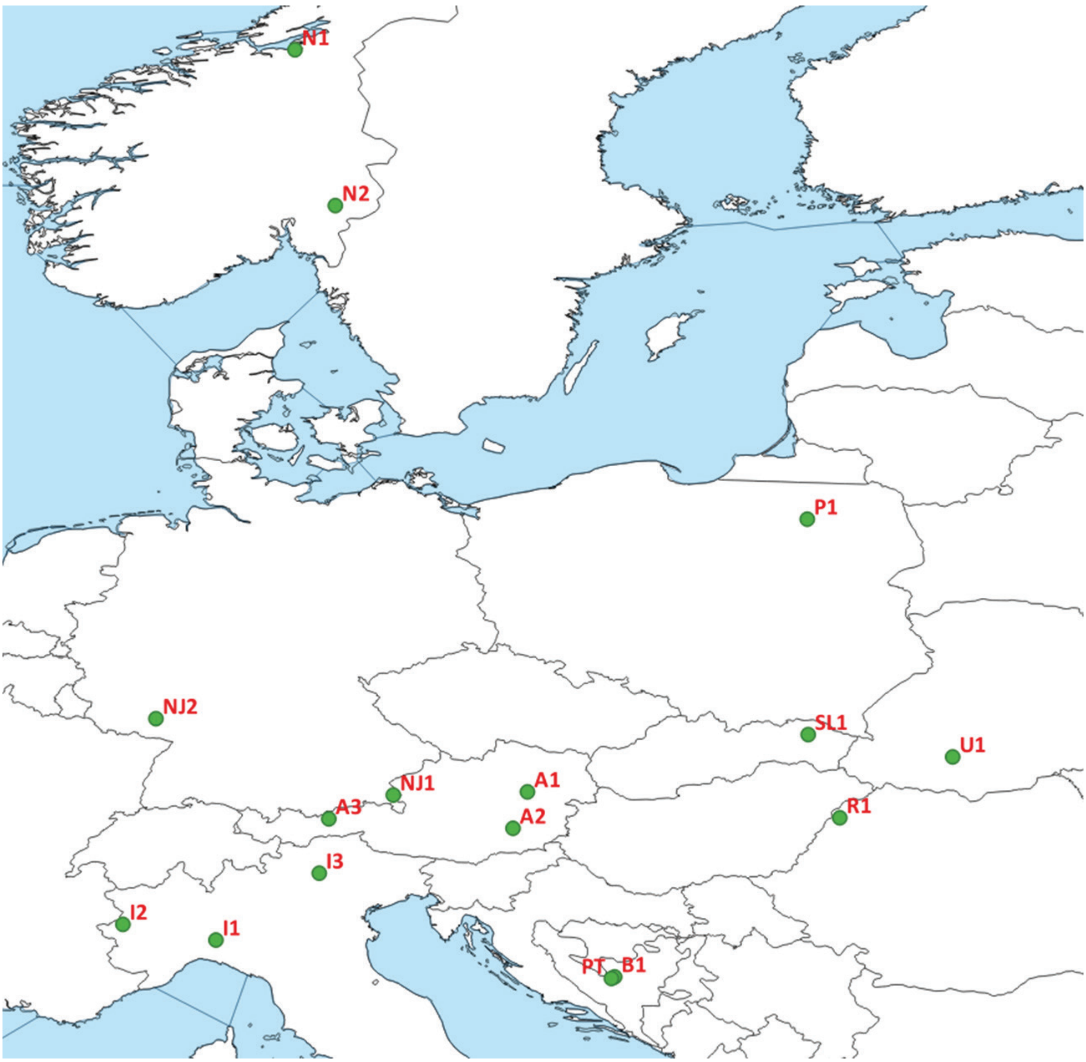

Figure 1. Scots pine provenances included in the provenance test.

Table 1. List of investigated provenances.

No	Provenance label	Country	Locality	Latitude	Longitude	Altitude (m)
1.	A1	Austria	Kobersdorf/Lackenbach	47° 53' 12''	15° 31' 39''	551
2.	A2	Austria	Panholz	47° 07' 14''	15° 17' 14''	424
3.	A3	Austria	Lans/Tirol	47° 13' 49''	11° 26' 12''	1017
4.	B1	Bosnia and Herzegovina	Bugojno	44° 03' 00''	17° 27' 00''	623
5.	NJ2	Germany	Trippstadt	49° 21' 35''	7° 46' 29''	445
6.	I1	Italy	Ca Del Lupo	44° 45' 25''	9° 05' 07''	532
7.	I2	Italy	Fenestrelle (TO)	45° 01' 47''	7° 03' 38''	1182
8.	I3	Italy	Valda (TN)	46° 13' 00''	11° 16' 00''	959
9.	P1	Poland	Ruciane – Nida	53° 37' 00''	21° 29' 00''	149
10.	R1	Romania	Sacueni	47° 21' 09''	22° 05' 29''	104
11.	SL1	Slovakia	Hanušovce	49° 01' 35''	21° 30' 01''	249
12.	NJ1	Germany	Teisendorf	47° 51' 00''	12° 49' 00''	505
13.	N1	Norway	Malvik	63° 22' 22''	10° 45' 03''	201
14.	N2	Norway	Arnes	60° 07' 20''	11° 27' 55''	174
15.	U1	Ukraine	Delytayn	48° 32' 41''	24° 30' 10''	743
Provenance test (PT)		Bosnia and Herzegovina	Kupres	43° 58' 18''	17° 15' 48''	1140

and valleys. The soil type is calcocambisol or brown soil, and geological substrate are limestones and dolomites. According to the classification of climate types in B&H (Milosavljević 1973), this area belongs to the mountain climate, with short summers and long, cold, and very snowy winters. The average annual temperature is 6.2°C. The minimum temperature was measured in January (-26.8°C) and the maximum in September (34.9°C). The annual average of registered frosts is 155 days. The average annual precipitation is 1221 mm, and its distribution varies slightly by season.

Field Observations

Six phases were observed for bud breaking and elongation (Ducci et al. 2012): 0 = dormant bud with joined scales covered by resin; 1 = start of elongation, scales partly disjoined but still covering the young shoot; 2 = significant elongation of terminal bud, scales still present but the green young shoot is visible; 3 = brachyblasts are well visible but still in their envelope; 4 = needles joined in two start to appear; 5 = the two needles of the same brachyblasts are clearly distinct (Figure 2).

Field observations started in March and were conducted every week regularly until the first plant with phase 1 was observed. After that date, the observations were made every third-day, until all plants were in phase 5.

Table 2 provides an overview of the basic climatological data for the nearest meteorological station (Bugojno) for the first half of 2022 (Federalni hidrometeorološki zavod, 2023), which influenced the development of the phenological phases of Scots pine in the researched provenance test.

Score 1 Score 2 Score 3 Score 4 Score 5

Figure 2. Scoring system adopted for bud breaking and elongation in *Pinus sylvestris* (according to Ducci et al. 2012).

Table 2. Climatological data for meteorological station Bugojno for the first half of 2022.

Month	Average air temperature (°C)	Maximum daily temperature (°C)	Date of max temperature	Minimum daily temperature (°C)	Date of min temperature	Sum of precipitation (mm)	Max height of snow cover (cm)
I	-1.1	16.0	05	-18.5	25/04	25.9	10
II	3.9	16.4	10 and 19	-8.2	02	42.5	11
III	3.9	21.3	24	-10.3	12	12.7	15
IV	9.3	25.3	15	-4.0	19	79.8	9
V	16.6	30.8	27	1.6	19	87.8	0
VI	21.8	36.0	29	7.7	15	55.8	0

Statistical Analysis

The observed data were processed in Microsoft Excel to determine the first and last occurrence of phases per provenances, the duration of individual phenophases, and SPSS 20.0 for ANOVA analysis was used to determine the duration of phases and cluster analysis.

RESULTS

The first and last occurrence of phases per provenance are shown in Table 3.

Phase 1 (start of elongation, scales partly disjoined but still covering the young shoot) was recorded on 10 May in all provenances. Phase 5 (the two needles of the same brachyblasts are clearly distinct) first occurred on 5 June in some plants of every provenances except Bugojno (Bosnia and Herzegovina), Delytayn (Ukraine), and Ca Del Lupo (Italy).

Average duration of phases are shown in Table 4.

In Table 4, it is visible that the durations of phases were different per provenance. Phase 1 had the shortest duration, and in the case of some provenances it was not even registered, which means it happened between two observations.

The results of variance analysis for duration of phases are shown in Table 5.

Variance analysis (Table 5) showed statistically significant differences among provenances for all phases (phases 0 and 5 were not included because of incomplete data, i.e. phase 0 started before we began with observations and phase 5 continued after we stopped.)

The results of cluster analysis for duration of phases are shown in Figure 3.

Cluster analysis (between groups linkage, Squared Euclidean distance) showed separation of A1 and I1 in separate group, and in the duration of phenophases (Table 4) it can be seen that these two provenances included phase 1, while in other provenances it was finished between two observations, and other phases started later (Table 3).

Cluster analysis for the duration of phenophases showed that the nearest provenances were the provenance from Ukraine and the provenance from Germany, which are geographically very distant, followed by the provenance from Austria and the provenance from Germany (which are geographically close to each other).

Table 3. First and last occurrence of phases per provenances.

Provenance	Phase 0	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
A1	until 12.5.	10.5.-21.5.	15.5.-24.5.	22.5.-5.6.	30.5.-11.6.	from 5.6.
A2	until 12.5.	10.5.-12.5.	12.5.-24.5.	22.5.-4.6.	30.5.-11.6.	from 5.6.
A3	until 12.5.	10.5.-12.5.	12.5.-24.5.	22.5.-2.6.	27.5.-9.6.	from 5.6.
B1	until 12.5.	10.5.-12.5.	12.5.-24.5.	25.5.-29.9.	30.5.-11.6.	from 8.6.
U1	until 12.5.	10.5.-12.5.	12.5.-24.5.	22.5.-3.6.	30.5.-11.6.	from 8.6.
SL1	until 14.5.	10.5.-14.5.	12.5.-21.5.	22.5.-26.5.	27.5.-7.6.	from 5.6.
R1	until 12.5.	10.5.-12.5.	12.5.-21.5.	22.5.-2.6.	27.5.-11.6.	from 5.6.
N1	until 12.5.	10.5.-12.5.	12.5.-21.5.	18.5.-30.5.	27.5.-7.6.	from 5.6.
N3	until 12.5.	10.5.-12.5.	12.5.-21.5.	22.5.-26.5.	27.5.-4.6.	from 5.6.
NJ1	until 12.5.	10.5.-12.5.	12.5.-21.5.	22.5.-29.5.	30.5-9.6.	from 5.6.
NJ2	until 12.5.	10.5.-12.5.	12.5.-24.5.	22.5.-4.6.	30.5.-11.6.	from 5.6.
P1	until 12.5.	10.5.-14.5.	12.5.-21.5.	22.5.-4.6.	30.5.-11.6.	from 5.6.
I1	until 12.5.	10.5.-17.5.	15.5.-26.5.	25.5.-4.6.	3.6.-11.6.	from 8.6.
I2	until 12.5.	10.5.-12.5.	12.5.-26.5.	25.5.-4.6.	30.5.-11.6.	from 5.6.
I3	until 12.5.	10.5.-12.5.	12.5.-24.5.	22.5.-4.6.	27.5.-9.6.	from 5.6.

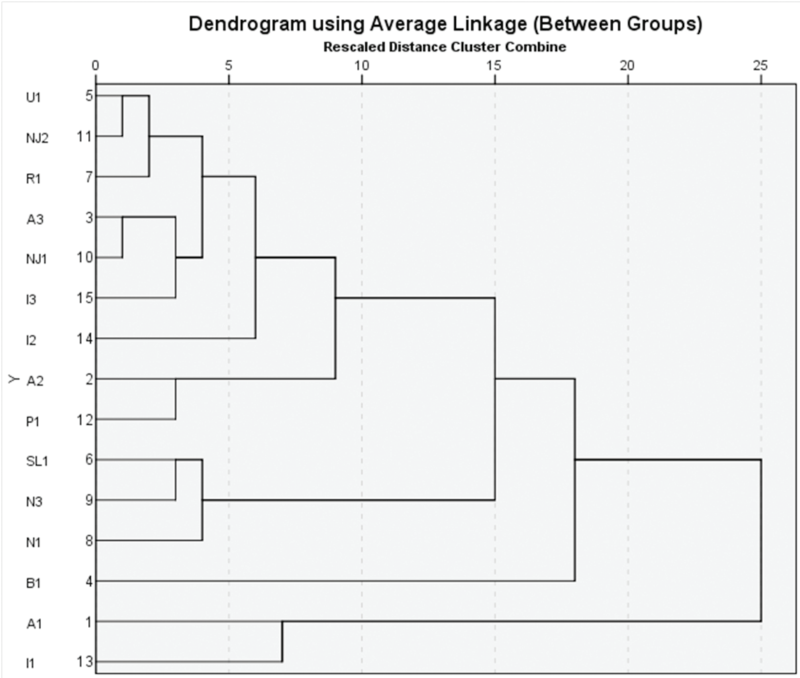

Figure 3. Grouping of provenances according to cluster analysis for duration of phases.

Table 4. Average duration of phases (in days) per provenances.

Provenance	Average duration of phases (days)			
	Phase 1	Phase 2	Phase 3	Phase 4
A1	4.4	7.4	10.2	4.6
A2	0.0	11.2	10.8	4.6
A3	0.0	11.2	7.2	7.8
B1	0.0	13.0	5.0	10.6
U1	0.0	11.2	9.2	7.4
SL1	0.6	9.4	5.0	10.6
R1	0.0	10.0	9.0	8.4
N1	0.0	8.4	7.2	9.0
N3	0.0	10.0	5.0	9.0
NJ1	0.0	10.0	8.0	8.2
NJ2	0.0	11.2	9.6	6.6
P1	0.6	9.4	10.0	5.8
I1	4.2	9.2	9.8	6.2
I2	0.0	13.4	8.2	6.6
I3	0.7	12.4	6.2	8.0
Total	0.6	10.5	8.0	7.6

Table 5. Variance analysis for duration of phases.

Phase	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	158.587	14	11.185	11.882	0.000
	Within Groups	64.400	60	1.073		
	Total	220.987	74			
2	Between Groups	195.547	14	13.968	6.021	0.000
	Within Groups	139.200	60	2.320		
	Total	334.747	74			
3	Between Groups	282.347	14	20.168	2.753	0.003
	Within Groups	439.600	60	7.327		
	Total	721.947	74			
4	Between Groups	241.680	14	17.263	3.443	0.000
	Within Groups	300.800	60	5.013		
	Total	542.480	74			

DISCUSSION

This research included observation of phenological phases of Scots pine provenances in the international provenance test in Kupres, Bosnia and Herzegovina. Provenances originated from different parts of Europe, latitudes, longitudes, altitudes, ecological niches and climatic conditions. Observing phenological development of these provenances planted ex-situ in the same conditions could give important results for using Scots pine in afforestation, since late spring frosts can damage the plants of Scots pine in the environmental conditions of Bosnia and Herzegovina.

Ducci et al. (2012) stated that phenological characteristics are relevant practical indicators of the adaptability and adaptedness of forest trees. They are directly related to the growth and to the tree architecture. There is a correlation between them and the frost damage, whose incidence is expected to increase with the climate change. A series of assessments on the same individuals over more years leads to conclusions about the climate development.

In this research, phase 1 first occurred at the beginning of May in most of the provenances, and in the mid-May in other provenances (A1, P1, I1). The dates are in compliance with the results by Salminen and Jalkanen (2015) in Finland, but unlike our results, they recorded buds beginning to extend at the beginning of May in the southernmost stand and in mid-May in the northernmost stands.

In 2017 (Ballian et al. 2019), the latest date when all plants were in phase 0 was 20 April, while in this research it was 10 May. The meteorological data for the nearest meteorological station (Bugojno), shown in Table 2, indicated snow in April 2022, which could cause the later start of bud breaking. Contrary, in this research, all plants were in phase 5 on 5 June, while in 2017 (Ballian et al. 2019) it occurred on 21 June.

In 2012, Ballian and Šito (2017) found that 4 April was the beginning of the growth season (the buds begin to develop) in the international provenance test of Scots pine in Žepče (600 m of altitude), and all plants were in phase 6 on 6 June.

Salminen and Jalkanen (2015) observed bud break phases in periods 2001-2003 and 2008-2010 in two locations in Lapland, Finland. On average, buds began to extend at the beginning of May in the southernmost stand and in mid-May in the northernmost stands, and the variation between years was in the range of 3 weeks (Salminen and Jalkanen 2015).

Although the results of this research showed statistically significant differences in the duration of certain phenophases among provenances, the differences in the first appearance of certain phenophases occur in an interval of no more than seven days. In this sense, we cannot recommend using certain provenances in areas where late frost occurs based on the results of this research, especially considering that the year of observations (2022) was a year with meteorological conditions that deviated from the average. Therefore, it is necessary to carry out research in several consecutive years to be able to give recommendations for the use of certain provenances in areas where late frost occurs.

CONCLUSIONS

Phase 1 (start of growth season) first occurred on 10 May in all provenances. Phase 5 first occurred on 5 June in some plants of every provenances except Bugojno (Bosnia and Herzegovina), Delytayn (Ukraine), and Ca Del Lupo (Italy). Variance analysis has shown statistically significant differences in the duration of phenophases 2 (significant elongation of terminal bud, scales still present but the green young shoot is visible), 3 (brachyblasts are well-visible but still in their envelope) and 4 (needles joined by two start to appear). The first and last appearances of phases are in very short interval for all provenances, but differences between the first and last plant in one provenance (the duration of a phase) are great. Research should be continued and should include an investigation of the influence of seasonal climate and climate changes.

Author Contributions

MMH, DB conceived and designed the research, TM carried out the field measurements, MMH processed the data and performed the statistical analysis, DB supervised the research, MMH, TM and DB wrote the manuscript. The main part of these results was presented at the international IUFRO Conference - Abies&Pinus 2022, “Fir and pine management in a changing environment: Risks and opportunities”, held on 19-22 September 2022 in Sarajevo, Bosnia and Herzegovina.

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Conflicts of Interest

The authors declare no conflict of interest.

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