

### INTERDISCIPLINARY DOCTORAL SCHOOL

Faculty of Silviculture and Forest Engineering

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# Phenological structure and phenotypic analysis for leaf descriptors in peripheral populations of sessile oak (*Quercus petraea* (Matt.) Liebl.) from eastern Romania

### ABSTRACT

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#### INTRODUCTION

There is a rapidly expanding body of evidence indicating that environmental changes are a growing threat to the persistence and sustainability of forests (Soularue & Kremer, 2014), with peripheral populations considered among the ecosystems most vulnerable to these phenomena (Fady et al., 2016; Ducci & Donnelly, 2017; Mátyás et al., 2018; Popescu & Şofletea, 2020). In this context, peripheral populations play an important role in preserving biodiversity (Gapare et al., 2005) and estimating species evolution (Nielsen et al., 2001).

Unlike central populations in a species' range, peripheral populations mainly exist in adverse climatic environments (Ursenbacher et al., 2016). Moreover, they are usually characterised by isolated and small population sizes (Eckert et al., 2008; Lázaro-Nogal et al., 2017), leading to reduced genetic variation (Macdonald et al., 2017) associated with an increased risk of extinction (Hardie & Hutchings, 2010; Eliades et al., 2019). However, peripheral populations, particularly those found at the lower natural range limits, are viewed as better adapted to stressful conditions, and consequently are likely to display valuable adaptations (Mátyás et al., 2009; Borovics & Mátyás, 2013; Ciocîrlan et al., 2017).

Phenology, due to its sensitivity to the variability of climatic conditions from one year to another (Campbell & Sugano, 1975; Bertin, 2008; Zhang et al., 2009; Chen & Xu, 2012) is an indicator frequently used to study the reactions of plants, and in particular tree species, to the long-term impact of global environmental changes (Menzel & Fabian, 1999; Parmesan, 2007; Richardson et al., 2013). At the same time, there are also opinions according to which not only climate change affects phenology, but also phenological variations influence the climate, mitigating or aggravating the effects of climate warming on forests depending on the relative weight of positive or negative feedback (Peñuelas et al., 2009). Therefore, assessing phenology in populations at the lower limit of a species' range can provide valuable information on the effects of climate change on forests.

In addition, phenology is considered a key determinant of the spatial distribution of trees (Chuine & Beaubien, 2001), being used to predict future changes in species' geographic ranges in response to climate change (Chuine 2010).

Due to its high phenotypic plasticity (Mátyás, 2021), sessile oak (*Quercus petraea* (Matt.) Liebl.) is considered an ideal species for assessing the effects of climate change (Czúcz et al. 2011, Borovics & Mátyás, 2013, Mátyás et al., 2018).

In Romania, sessile oak has the largest proportion of oak species, being a species of undeniable ecological and economic importance. However, knowledge regarding the phenological structure of sessile oak in peripheral populations located towards the eastern limit of the species' distribution range is lacking. Therefore, the unique characteristics of peripheral populations constantly exposed to climate pressures and soil drought is an understudied area that requires further attention.

Thus, this paper aims to analyze the phenological behavior of sessile oak, in order to characterize the phenological structure of the species in populations situated at the lower altitudinal limit, representative of its eastern range in Romania, as well as in a comparative trial of autochthonous provenances located close to the eastern limit of the species.

Also, sessile oak being a polytypic species, phenotypic analyzes were also carried out to establish the taxonomic structure at the level of foliar morphological descriptors.

The results can serve as a basis for establishing strategies for the use and conservation of the genetic resources of sessile oak, as well as for predicting future phenological changes under the threat of environmental climate change.



# 1. STATE OF THE ART REGARDING THE TAXONOMY, CHOROLOGY, ECOLOGY, PHENOTYPICAL AND PHENOLOGICAL VARIABILITY OF SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL.)

## **1.1** Sessile oak, edifying species of forest ecosystems: importance and chronological and ecological landmarks of the species

Generally, the vast area occupied by sessile oak extends between 37<sup>0</sup>-62<sup>0</sup> latitude N, respectively 10<sup>0</sup> longitude W and 50<sup>0</sup> longitude E (Vila-Lameiro & Díaz-Maroto, 2017).

In Romania, *Quercus petraea sensu lato* (including ssp. *dalechampii* and ssp. *polycarpa*) covers approximately 8.3% of the forested area (IFN, 2019). It forms its own vegetation layer, where it is detached as an edifying species of forest ecosystems (Sofletea & Curtu, 2000). It is found at altitudes that can vary between 300-800 m, but sometimes it extends to the mountain area at 900-1000 m or to the forest-steppe below 300 m. However, the rare occurrences in the plains (for example in the Hanu Conachi forest - Gafenco et al., 2023a) or those in the high mountain area (for example, the population of Mount Cozia - Sofletea & Curtu, 20 07), which reflects its high potential for adaptation in marginal or peripheral populations.

Regarding the distribution of the subspecies *dalechampii* and *polycarpa* in Romania, they grow alongside the common sessile oak (scattered or grouped), ssp. *dalechampii* being more frequent in the Central Moldavian Plateau (Chiriță, 1981), and ssp. *polycarpa* being well represented in the low hills of Oltenia, as well as in the mountains of southern Banat (Șofletea & Curtu, 2007).

Sessile oak has a typical mesothermal behavior, developing optimally at an average annual temperature of 6-9°C (Stănescu et al., 1997), and claims high values of atmospheric precipitation, generally between 600 – 800 mm annually (Stănescu et al., 1997), having from this point of view medium requirements - mesophilic species (Şofletea & Curtu, 2007; Fedorova et al., 2018). In addition, it is among the drought-tolerant species (Vander Mijnsbrugge et al. 2017), developing special adaptations in this regard (Bréda et al., 1993). The other two sessile oaks (ssp. *dalechampii* and ssp. *polycarpa*), require greater amounts of heat (subthermophile) than the common sessile oak, being also more resistant to drought than it (mesoxerophytes-mesophytes), which is why they advance more towards the silvosteppe, where they mix with the downy oak.

#### **1.2.** Taxonomic variability of sessile oak

#### 1.2.1 The taxonomic position of sessile oak within the genus Quercus L.

In the specialized literature from Romania, depending on the adopted taxonomic classification, we find 7-9 species of oaks mentioned (Georgescu & Morariu, 1948; Beldie, 1952; Stănescu et al., 1997; Șofletea & Curtu, 2007). The native species of oaks identified in the vascular flora of our country are systematized as follows: series *Sessiliflorae* Loj. (*Quercus petraea* (Matt.) Liebl. ssp. *petraea* (Liebl.) Soó, *Q. petraea* ssp. *dalechampii* (Ten.) Soó, *Q. petraea* ssp. *polycarpa* (Schur) Soó), seria *Pedunculatae* Schwz. (*Q. robur* L., *Q. pedunculiflora* K. Koch), seria *Confertae* Simk. (*Q. frainetto* Ten.), seria *Lanuginosae* Simk. (*Q. pubescens* Willd., *Q. virgiliana* Ten.) and subgenus *Cerris* (Spach.) Oerst (*Q. cerris* L.).

Among the three taxa of the *Sessiliflorae* Loj. series, only *Q. petraea* (Matt.) Liebl. is considered easily recognizable and widely accepted by most taxonomists, something that cannot be said when analyzing the taxonomic status of the other two taxa (*Q. dalechampii* Ten. and *Q. polycarpa* Schur).

Regarding *Q. dalechampii* Ten., at the European level, two contrasting approaches are distinguished within the taxonomic classifications. Thus, in some cases, it is considered a distinct species related to *Quercus petraea* (Schwarz, 1964, 1993; Filipova & Asenov, 2016), and in opposition, it is described as a distinct species related to *Quercus pubescens* (Camus, 1934-1954; Di Pietro et al., 2012).



Following the lectotypification, Di Pietro et al. (2012) fixed *Q. dalechampii* Ten. within *Q. pubescens sensu lato*. In this context, Kučera (2018) proposed the name *Quercus banatus* P. Kučera, nomen novum, for the records of *Q. dalechampii* (with glabrous stems and subsessile cups) interpreted as species taxonomically close to *Q. petraea* and reported in different countries in central and southeastern Europe.

Nor in the case of *Q. polycarpa* Schur. a consensus regarding its taxonomic position cannot be discussed. Initially, until it was discovered and described by Schur in 1851, it was considered a form of the common sessile oak, being recorded in the literature under the name *Q. sessiliflora* f. *iberica* (Steven ex M. Bieb.) Ledeb. Later, most studies confirm the morphological similarities with *Q. petraea sensu stricto*, most often being nominated as a distinct species (Matula, 2008; Bartha, 2014) or as its subspecies (Govaerts & Frodin, 1998; Şofletea & Curtu, 2007; Raab-Straube & Raus, 2013).

#### **1.2.2** Intraspecific taxonomic variability of sessile oak

In the studies on the taxonomic variability of the *Sessiliflorae* series (Beldie & Cretzoiu, 1941; Georgescu & Morariu, 1948; Beldie, 1952) the forms and varieties that its representatives present are described, being nominated for ssp. *petraea*: f. *platyphylla* (Lam.) Schwz. with the subforms: subf. *normalis* Schwz and subf. *angulata* Schwz., f. *laciniata* (Lam.) Schw. with the subforms: subf. *pinnata* (C. K. Schn) Schwz. and subf. *lobulosa* Schwz., and f. *longifolia* (Dippei) Schwz. with subform: subf. *angustifolia* (Zap) Schwz., for ssp. *dalechampii*: f. *lancifolia* (Vuk.) Schwz. And f. *pinnatifida* (Boiss.) Schwz, and for ssp. *polycarpa*: var. *typica* Beldie and var. *glabra* Beldie.

#### **1.3** Aspects regarding the phenotypic and phenological variability of sessile oak

#### 1.3.1 Phenotypic variability

In Europe, numerous studies have been carried out on the morphological differentiation of oaks and, in particular, of sessile oak.

In north-eastern France, Dupouey and Badeau (1993) compared the variability at the leaf level for *Q. petraea, Q. robur* and *Q. pubescens,* and found that the parameters with the greatest capacity to differentiate between the three species are pilosity, the number and size of intercalary veins, but also petiole length.

Later, Kremer et al. (2002) proposed a methodology for the evaluation of variability according to leaf morphology, in mixed stands of oak and sessile oak. Four categories of morphological descriptors and three methods of multivariate statistical analysis (CDA, PCA and MCA) were used to evaluate the variability at the leaf level, thus two populations, corresponding to the two species, could be clearly distinguished.

A year later, Borazan and Babaç (2003) carried out a study on the morphometric variability of leaves in oak species (*Q. robur, Q. petraea, Q. pubescens, Q. virgiliana* and *Q. cerris*), being analyzed a number of 16 morphological characters regarding the number of pairs of lobes, the width of the lobes, the width and length of the lamina or the length of the petiole.

In a work published in 2009a by Viscosi et al. leaf morphology and morphometric relationships between four species of the genus *Quercus* (*Q. petraea*, *Q. robur*, *Q. pubescens* and *Q. frainetto*) were studied.

The morphological variability of sessile oak has also been addressed in some studies at the national level. For example, in a study focused on the genetic diversity of sessile oak from natural populations (Stuparu et al., 2003), the morphological structure of the leaf was analyzed by means of six descriptors. Also, Apostol et al. (2015) aimed to identify intraspecific taxonomic units in a mixed oak stand using foliar morphological descriptors.



Bussotti and Grossoni (1997) examined with SEM (Scanning Electron Microscopy) the micromorphological characteristics on the abaxial side of the leaves of several oak species. According to this study, in sessile oak, the wax on the abaxial surface of the leaf is uniformly distributed in the form of shaped scales, of different sizes, and which do not necessarily cover the entire surface of the leaf. The stomata are elliptical in shape with the main axis longer than the secondary axis and are surrounded by wax. Regarding the trichomes, they are stellate, with 2-4 asymmetric arms flattened on the lamina. Also, samples of *Q. dalechampii* were analyzed, and in this case, a certain variability of the micromorphological characteristics was also observed (some forms are identical to *Q. petraea* while others have a completely glabrous abaxial surface).

Regarding *Q. polycarpa*, descriptions of micromorphological characteristics differ considerably among researchers. According to the description of Camus (1934-1954) the abaxial surface of the lamina is finely pubescent, according to Schwarz (1993) it is glabrous or glabrescent, while Krüssmann (1986) states that the abaxial surface is slightly pubescent when young and later glabrous.

#### **1.3.2** Phenological variability

On a large scale, phenology has been increasingly used in studies aimed at assessing or modeling climate change impacts (Hänninen, 1995; Kramer, 1995, 1996; Menzel & Fabian, 1999; Chuine, 2000; Cleland et al., 2007; Vitasse et al., 2011; Kolář et al., 2016).

Nizinski and Saugier (1988) were among the first researchers to develop an empirical model for forecasting bud burst in sessile oak. In relation to other models, the one proposed by the two previously mentioned authors proved to be simple (for the calculation of the production of the leafing phenophase it takes into account only two variables: the average daily temperature and the photoperiod) and with high precision (± 2 days, compared to other models where the differences were up to 3-4 days).

For the impact analysis of climate change in relation to phenology in various stands, Kramer (1994, 1996), Kramer et al. (2000) used more complex, mechanistic models. Thus, for the species of the *Quercus* genus (*Q. petraea* and *Q. robur*), the effect of the temperature increase on the phenological dynamics proved to be a small one, because the date on which the leaves fall (October 16) has advanced more than the one on which the leafing takes place (May 6).

Delpierre et al. (2009) attempted to model the spatial variability of leaf senescence in sessile oak, pedunculate oak and beech, using data provided by the RENECOFOR network (France) from 1997-2006.

From research carried out in comparative trials of sessile oak (Liepe, 1993; Deans & Harvey, 1995; Ducousso et al., 1996), it has been observed that the phenology of leafing varies with latitude (southern populations leaf earlier than northern ones). Similar clinal variations have also been reported along altitudinal gradients (Alberto et al., 2011, 2013).

In sessile oak, variations with latitude were also found regarding the phenophase of leaf senescence (Deans & Harvey, 1995; Jensen & Hansen, 2008). As a result of variations in photoperiod length, senescence occurs earlier in high-latitude populations than in low-latitude populations. However, Vitasse et al. (2009), observed that the gradient variation in senescence with altitude is much more likely to be induced by temperature than by photoperiod.

In Romania, the first observations on the main forest species phenophases (including sessile oak) began in 1946, within the Forestry Research Institute (Bălănică & Tomescu, 1953) and continued until 1965.

Based on the observations made between 1956 and 1965, it was found that the vegetation season for sessile oak, regardless of the geographical gradients, lasts on average 180 days (200 days at 100 m altitude and 160 days at 650 m altitude). Also, the onset of bud burst occurred on average 10 days earlier in the south than in the north (the difference representing approximately 5 days for 1<sup>o</sup> latitude) (Tomescu, 1967).



Regarding the autumn coloring of the leaves, it is shown that this phase takes place after a period of time in which there was a sudden and very strong drop in average daily temperatures, compared to the beginning of autumn (Tomescu, 1957). In addition, due to the climatic conditions (high temperatures, severe drought) in late summer and autumn, in the southern regions leaf coloration is earlier than in the north.

At the end of the last century, within the ICP Forest network, systematic phenological research on the most important forest species in our country was resumed (Badea, 1998), mainly aiming at assessing climate changes, as well as establishing correlations between phenophases and different measured parameters (health status, tree growth, litter accumulations, etc.) (Beuker et al., 2016).

Later, Teodosiu et al. (2005) carried out phenological observations on sessile oak, in two points belonging to the FENOFOR network.

Lately, phenological research on some oak species were carried out in the Fundeanu mixed forest (Chesnoiu et al., 2009; Chesnoiu, 2017). Also, Crăciunesc (2013) studied the implications of spring phenology on the phenomenon of natural hybridization between four species of oaks belonging to the subgenus *Lepidobalanus* (*Q. petraea*, *Q. robur*, *Q. pubescens*, *Q. frainetto*).

Phenological studies also indicate that individual trees can be classified according to their phenological rank into distinct phenological categories: early, intermediate, and late trees (Chesnoiu et al., 2009; Delpierre et al. 2017, Denéchère et al. 2021). Lechowicz (1984) attributed differences in the timing of bud burst between cohabiting trees to variations in their wood anatomy. More recently, other studies have provided evidence that wood anatomy influences species differences in bud burst phenology (Panchen et al., 2014, Savage et al., 2022).

Furthermore, combined studies using both wood anatomical traits and phenological data have a high potential to be useful for explaining environmental signals recorded at the tree level in different stages of wood formation (Pérez-de-Lis et al., 2016).

Typically, the timings of leaf and cambium phenology do not occur concurrently and may be influenced differently by environmental cues (Delpierre et al., 2016).

Recent studies revealed that functional characteristics such as wood density might serve as determinants for the development of species phenophases (Sun & Frelich, 2011; Galvão et al., 2021; Wang et al., 2021). Leaf phenology and wood density are plant functional traits that are useful in assessing species responses to climate change and estimating biomass production.



#### 2. AIM AND OBJECTIVES

#### 2.1 Research aim

Sessile oak is regarded as one of the key species for forestry in the future (Kuster et al., 2014), being considered a drought and heat-tolerant species (Nölte et al., 2020) due to specific morphological adaptations in the structure of leaves and wood (Fonti et al., 2013; Kuster et al., 2013), as well as in root growth (Arend et al., 2012).

Even if the common sessile oak is a hill species with typical mesothermal behaviour, there are situations when this species enters the forest-steppe area, forming pure or mixed stands with the other two subthermophilic sessile oak trees (Paşcovschi & Doniţă, 1967; Sanda et al., 2004). The climate in these marginal populations differs sharply from the hill area (higher average annual temperatures, lower average annual rainfall, and intense evapotranspiration), leading to numerous consequences for mesophilic species such as sessile oak at the limit of its tolerance range.

In this context, the unique characteristics of peripheral populations constantly exposed to climate pressures and soil drought represent an understudied area that requires further consideration. Therefore, the present research aimed to evaluate the phenological and taxonomic structure in populations of the lower altitudinal limit representative for the eastern range of the species in Romania.

The research carried out primarily aimed at assessing the phenological structures of low-altitude populations to better understand their adaptive and evolutionary potential under conditions expected to occur due to global climate change. In addition, the data obtained from the comparative analysis of populations will provide a foundation for establishing strategies for the use and conservation of sessile oak genetic resources.

Secondly, it aimed to highlight some possible correspondences between the phenological categories and certain characteristics of the annual growth rings.

At the same time, the analysis of the phenological behavior and some wood traits in a comparative trial of sessile oak provenances installed in the research area, in which provenances from different areas of the species' range in our country are tested, will contribute to the selection and promotion in cultures of the most suitable provenances, so as to ensure the stability of forest ecosystems.

#### 2.2 Research objectives

To fulfill the purpose of the research, the following objectives were established:

i. Assessment of spring and autumn phenology in order to establish correlations between phenophases and local climatic conditions.

ii. Analysis of the influence of the phenological category on different wood traits

iii. Analysis of phenotypic variability in a comparative trial of sessile oak provenances installed in the research area

iv. Assessment of phenotypic variability at the level of foliar macro and micromorphological descriptors and comparison of the results using multivariate statistical analyses.

v. Identification by phenotypic and phenological criteria of valuable populations or provenances with high potential for adaptation to local climatic conditions.



#### **3. RESEARCH METHODOLOGY**

#### **3.1 Research location**

The research included four populations of sessile oak (Table 3.1.1 și Figure 3.1.1), three of which (SAT, OLT, FUN) are representative for their peripheral/marginal position (at low altitude for sessile oak in the research area, along a north–south transect), respectively, in close proximity to the eastern limit of the general sessile oak distribution in Romania. In choosing the three peripheral populations, it was also considered that the extreme points of the transect were located at approximately equal distances from its middle. The straight line between the extremes of the transect is approximately 120 km long.

In addition, for the comparative analysis, were chosen a reference population (HEL) from the inner part of the species' range (non-peripheral), in the ecologically optimal area (being included in the category of mesophyll sessile oak forests (Gancz et al., 2008)), and a sessile oak comparative trial (FAN), where 33 provenances from the sessile oak area in Romania are tested (Table 3.1.2). The comparative trial was installed in 1978.

		Geo	graphical pos	sition	Climatic c	Martana		
Code	Label Latitude		Longitude	Altitude	Annual average	Annual rainfall	iviar conne	
		(N)	(E)	(m)	temperature (°C)	(mm)	anulty muex	
Peripheral populations								
FUN	Fundeanu	45°58′	27° 41'	210	9.6	470	24.0	
OLT	Oltenești	46°34′	27° 53' 305 9.2		9.2	530	27.6	
SAT	Satu Nou	46°58′	27° 49'	300	9.2	535	27.9	
			Reference	population	(non-peripheral)			
HEL	Heltiu 46°14′ 26°55′ 490 9.2 655		34.1					
				Comparativ	e trial			
FAN	Fântânele	46°39′	26° 43′	260	9.6	590	30.1	

Table 3.1.1 Geographic location and climatic conditions of the sampled populations and of the sessile oak comparative trial



Figure 3.1.1 Location of sampled sessile oak populations and of the comparative trial (adapted from Forest Map by Ecosystem Units, Gancz et al. 2008). The codes can be found in Table 3.1.1.



Table 3.1.2 Location and geographical coordinates of the tested provenances in the FAN comparative trial.

		Ecological regi	on and	l subregion of	Prov	enance loca	ation	
		pro	ovenan	ce	1100			
Provenance	Cada*	Pagion	Cada*	Subragion	Forest district	Latitude	Longitude	Altitude
	coue	Region	Coue	Subregion	Compartment	(N)	(E)	(m)
					Botosani			
1	G	Moldavian	G26C	Jijia Plain	IV Pojenita-Tulbureni	47°45′	26°40′	130
_	•	Plateau	0100	0.9.00 1.00.00	46 B			
				Suceava Tableland	Dolhasca			
2**	G	Noldavian	G150	and Siret and Iasi	V Dolhasca	47°07	26° 37′	325
		Plateau		Hills	20 A			
		Moldavian		Barlad Tableland	Roman			
3	G	Plateau	G350	and Bacau Hills	V Vulpășești	46°52′	26°55′	300
		Thateau			76 B			
		Moldavian		Suceava Tableland	Vaslui			
4	G	Plateau	G150	and Siret and Iasi	ll Lipovăț	48°38′	27°48′	240
				Hills	5 A			
-	~	Moldavian	C150	Suceava Tableland	Zeletin	460201	27005/	240
5	G	Plateau	G150	and Siret and lasi	II Rachitoasa Vest	46°20'	27°05'	340
				пші	Saccut			
6**	G	Moldavian	G250	Barlad Tableland	JV Gaiceana	16°10'	27°05′	300
0	U	Plateau	0350	and Bacau Hills	52A	40 10	27 05	500
				Curved	Panciu			
7	В	Curvature	B250	Carpathians-	l Varnita	45°52′	23°03′	370
		Carpathians		Exterior range	42 B			
		Constant		Curved	Râmanicu Sărat			
8	В	Carnathians	B250	Carpathians-	III Buda	45°23′	23°03′	410
		Carpatnians		Exterior range	12 A			
		Southern		Meridional	Târgoviște			
9	С	C Carnathians	C250	Carpathians –	VII Gura Ocniței	44°55′	25°25′	280
		carpatinans		Southern range	131 A			
	_	Southern		Meridional	Meridional Mihăiești			
10	С	Carpathians	C250	Carpathians –	II Hârticești	44°08′	08′ 25°08′	550
		•		Southern range	48 A			
11**	C	Southern	C2E0	Vieridional	Babeni	A 40FF/	240141	200
11	C	Carpathians	C250	Carpathians –		44 55	24 14	290
				Southern tange	10 A, 20 B			
12***	Unk	nown informa	tion					
		Southern		Meridional	Tismana			
13	С	Carpathians	C250	Carpathians –	IV Tismana	45°05′	22°50′	310
				Southern range	138 A			
a a 4 4	-	Curvature		Curved	Väleni			100
14**	В	Carpathians	B250	Carpathians-		45°10′	26°02′	490
				Exterior range	45 A			
15**	F	Apuseni	F250	Western Apuseni	VII Cerbu	16°10'	าว∘วว/	300
15	-	Mountains	2250	Mountains	201 C 202 B	40 40		. 500
					Făget			
16**	D	Banat	D250	Banat Mountains:	V Vlădeana	45°45′	22°05′	410
		Mountains		Tarcu-Poiana Rusca	90 B, 91 B			
				Transition	Reghin			
17	F	Plataau	F250	Tahlaland	III Săcele	46°45′	24°42′	440
		Plateau		Tablelallu	40 A			



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		Ecological regi	on anc	l subregion of	Prove	enance loca	ation	
Provenance	Code*	Region	Code*	ce Subregion	Forest district Production unit Compartment	Latitude (N)	Longitude (E)	Altitude (m)
18	D	Banat Mountains	D150	Mehedinți-Cerna- Semenic	Orșova IV Mracovia Lespezi 3 A	44°41′	22°26′	180
19	В	Curvature Carpathians	B250	Curved Carpathians- Exterior range	Vidra I Bolotești 38	45°36′	27°10′	370
20	В	Curvature Carpathians	B250	Curved Carpathians- Exterior range	Buzău V Barbu 81 F	45°10′	26°55′	250
21	В	Curvature Carpathians	B250	Curved Carpathians- Exterior range	Panciu I Varnița 42 B	45°52′	27°06′	370
22	G	Moldavian Plateau	G350	Barlad Tableland and Bacau Hills	Fântânele I Trebeș 70	46°40′	26°40′	450
23	В	Curvature Carpathians	B250	Curved Carpathians- Exterior range	Râmnicu Sărat III Buda 12 E	45°32′	27°03′	410
24**	G	Moldavian Plateau	G26C	Jijia Plain	Botoşani IV Poieniţa-Turbuleni 46 B	47°45′	26°40′	130
25	E	Apuseni Mountains	E150	Apuseni Mountains-Zarand and Metaliferi Mountains	Lipova III Păltiniș 17 B	46°06′	21°44′	300
26***	D	Banat Mountains	D150	Mehedinți-Cerna- Semenic	Oravița Foratic 58 C	45°03′	21°44′	280
27	D	Banat Mountains	D150	Mehedinți-Cerna- Semenic	Bocșa Montană IV Smida 49 E	45°25′	21°45′	330
28**	F	Transylvanian Plateau	F250	Transylvania Tableland	Blaj II Valea Lunga 19 G	46°12′	23°54′	420
29	В	Curvature Carpathians	B250	Curved Carpathians- Exterior range	Râmnicu Sărat III Buda 12 E	45°25′	27°03′	410
30	с	Southern Carpathians	C250	Meridional Carpathians – Southern range	Voinești II Gemenea 4 A	45°04′	25°16′	610
31**	F	Transylvanian Plateau	F150	Transylvania Plain	Lechința V Matei 16	47°02′	24°20′	425
32**	E	Apuseni Mountains	E350	Eastern Apuseni Mountains	Cluj IX Mara 38	46°45′	23°34′	600
33	E	Apuseni Mountains	E350	Eastern Apuseni Mountains	Zalău VII Cehu Silvaniei 50	47°15′	22°48′	250

\*Ecological regions and subregions of provenance established in Romania (Pârnuță et al., 2010) \*\* Provenances evaluated phenologically and from the point of view of wood traits \*\*\* Provenances excluded from the phenotypic analysis



#### 3.2 Research material and method

#### 3.2.1 Research material and method for the phenological analysis

In order to determine the phenological structure in the sampled sessile oak populations, it was monitored for four consecutive years (2017-2020) to establish the dates at which certain phenophases occur and their duration.

Initially, in each of the four research populations (FUN, OLT, SAT and HEL) a number of 50 mature trees from the dominant layer were identified and selected, located at a minimum distance of 30 m (typically 50 m) from each other. Trees within each plot were numbered and mapped using a Trimble Juno SC GPS.

Subsequently, for the FUN population, the morphological analyzes on the leaves (supplemented by those of the twigs) showed that a number of 16 individuals could not be classified as *Quercus petraea* species, thus they were eliminated from the study, sessile oak being the species of interest. Also, in the HEL population, following an extreme weather event that occurred in 2017, at the end of May, a number of seven trees from those included in the study were uprooted and felled. Since the event occurred after the observations on the leafing phenophase were carried out, the phenological studies continued only on the 43 specimens that remained unaffected.

In the comparative trial Fântânele, 10 provenances from different ecological subregions were selected (the list of provenances is shown in Table 3.1.2), and for each provenance, five specimens were randomly selected.

Phenological observations were made on the spring phenophases (bud burst, abv. BB, leaf development, abv. LD and flowering, abv. FL) and on senescence in autumn (leaf senescence, abv. LS). In general, the spring phenology observations were carried out between the last decade of March - the first decade of May, respectively the last decade of September - the second decade of November, for the autumn phenophase, depending on the average daily temperatures of the respective year. Phenological observations were performed in all sampled populations and in the comparative trial (FAN) at an interval of 2–4 days by examining the entire crown of each individual tree, always from the same direction (Preushler, 1999), with  $20 \times 50$  binoculars.

The assessment of the dynamics of the phenophases followed the protocol proposed for oaks within the Tree4Future international project (Pâques, 2016), being suitable for tall trees.

Thus, the determination of the leafing stages was carried out according to the following evaluation scale (Figure 3.2.1.1): stage 0 – more than 50% of the crown buds are dormant, stage I – swelling and cracking in more than 50% of the buds, stage II – more than 50% of the buds have leaves, and stage III – more than 50% of the crown has fully formed, but incompletely developed leaves.



Stage 0







Stage III

Figure 3.2.1.1 The main stages of leaf flushing (Foto: Ioana Maria Gafenco)



For the flowering phenophase, only the maturation of male flowers was monitored, because it is easier to evaluate than for female flowers (Chesnoiu et al., 2009), the latter having very small dimensions (a few mm), and at the same time, it is known that the maturation of male and female flowers is intercorrelated (Bacilieri et al., 1995).

Thus, the following stages were assigned for the phenophase of male flowering (Figure 3.2.1.2): stage 0, for the case when more than 50% of the catkins are green or yellowish-green, without pollen release, and stage I, for the situation when more than 50% of the catkins are yellow-brown and pollen release occurs.



Stage 0

Stage I

Stage III

Figure 3.2.1.2 The main stages of male flowering (Foto: Ioana Maria Gafenco)

For the phenophase of the autumn coloring of the leaves (senescence), the following three stages were used (Figure 3.2.1.3): stage I – more than 50% of the tree crown has dark green leaves with small yellowish spots; stage II – more than 50% of the tree crown has light green leaves with numerous yellow spots and stage III – more than 50% of the tree canopy has yellow leaves.



Stage I

Stage II

Figure 3.2.1.3 The main stages of leaf senescence (Foto: Ioana Maria Gafenco)

The length of the growing season was defined as the number of days between the date of bud burst (stage I of the leafing phenophase) and that of senescence (stage III of the autumn coloring phenophase).

In order to make comparisons between phenological stages, the start date of the monitored phenophases stages was recorded using the DOY (Day Of Year) system. In this way, January 1 is considered the first day of the year, and the DOY of each phenological stage is considered to be the number of days from the beginning of the year until that stage takes place.

Also, in order to enable the correlation of phenophases stages with climate, a HOBO Pro v2 sensor was installed at each site, with the help of which the temperature and the relative humidity of the air were recorded at hourly intervals throughout the duration of the research. Thus, the average daily values were calculated based on the hourly values provided by the sensor.

Phenological data were analysed using the STATISTICA v.8.0 statistical software (StatSoft Inc., Tulsa, OK, USA).



As the data were found not to be normally distributed, the Kruskal-Wallis test was used to explore whether there were significant differences between them.

In addition, for the comparative trial, the relationship between the phenological data and the geographical parameters, including the ecophysiological latitude, of the place of origin of each provenance was assessed using the Spearman rank correlation coefficient ( $r_s$ ). Ecophysiological latitude (Le) is latitude (Lat) corrected for altitude (Alt), where a difference in altitude of 100 m equals one degree of latitude, and was determined with the formula Le = Lat + Alt/100 (Wiersma, 1962) (Equation 1).

#### **3.2.2** Research material and method for the wood traits analysis

For a better understanding of how the phenological behavior of trees could influence certain wood properties, it was aimed to highlight possible correspondences between phenological categories and basic wood density, annual ring width, sapwood traits (sapwood width and number of sapwood rings) in sessile oak in the four sampled populations, and in addition, with the phenology of the 2021 annual ring in the comparative trial FAN (Table 3.1.1, Table 3.2.2.1).

In the FAN comparative trial, of the 10 phenologically evaluated provenances (Table 3.1.2), three fall into the category of peripheral populations from the eastern-northeastern limit of sessile oak (Dolhasca, Sascut and Botoșani), and the other seven are non-peripheral, originating from areas with a climate considered favorable for sessile oak (Băbeni, Văleni, Beiuș, Făget, Blaj, Lechința and Cluj).

Population	Stand status	Stand density (trees∙ha⁻¹)	No of trees for phenological observations	Trees age (ani)	Number of sampled trees
FUN	Deningly and a second stice	291	34	80-95	10
OLT	Peripheral population,	283	50	70-75	10
SAT	flatural regeneration	289	50	75-110	10
HEL	Non-peripheral population, natural regeneration	294	43	75-110	10
FAN*	Non-peripheral, plantation	340	50	30-40	50

Table 3.2.2.1 Characteristics of the sampled sessile oak populations and FAN comparative trial and sample size

\*Sessile oak comparative trial

To achieve this objective, individuals with different phenological behavior indicated by the time of bud burst were compared. Thus, having the timeline of the bud burst already established (Gafenco et al., 2022), the trees were differentiated according to their phenological rank. Consequently, each tree was placed in a phenological category (early, intermediate, late).

In the peripheral populations and in the non-peripheral population, wood traits were analyzed in a number of 10 trees (5 early trees and 5 late trees) (Table 3.2.2.1).

The number of trees for wood trait analysis was previously determined using the sample size formula for continuous data (Bartlett et al., 2001).

On the other hand, in the comparative trial, wood traits were analyzed for all 50 phenologically evaluated trees (5 trees per provenance)(early, intermediate and late trees) (Table 3.2.2.1).

In 2021, at the beginning of April, growth cores (at 1.3 m height) were extracted with a Pressler increment borer from the trees selected for the analysis of wood traits. The samples were conditioned to a moisture content of 10% until future analysis.



In the laboratory, for each core, the sapwood (SAW) was delimited from the heartwood and the heartwood was divided into juvenile heartwood (JHW) and mature heartwood (MHW) depending on the dynamics of the annual ring width. The delimitation between JHW and MHW was done depending on the variation of the width of the annual ring from pith to bark, and in parallel the variation of the proportion of latewood content was examined. Juvenile wood samples included wide inner rings with large variations in width from year to year (Zobel & Sprague, 1998). However, in the comparative trial, as the trees were younger (Table 3.2.2.1), the sequence of mature wood was not long enough to separate the cores into three sections, so they were only separated (SAW) into sapwood and heartwood (HW).

Prior to basic density determination, for each resulting section, was counted the number of annual rings in the section and the number of sapwood rings (NSR); also were measured the section length (L) and the proportion of sapwood (SAW). Since in sessile oak annual rings are easily distinguishable due to the alternation of earlywood and latewood, the number of annual rings was obtained by direct observation through a Zeiss Discovery V.1.2 stereomicroscope.

Moreover, for trees from the comparative trial, the stage of annual ring formation was also evaluated, microscopically, at the time of sample collection (2 April 2021). The following stages were distinguished: 1 - the 2021 ring had not formed, 2 - the 2021 ring contained only a single row of pores in the earlywood zone, 3 - the 2021 ring contained only two rows of pores in the earlywood zone and 4 - the 2021 ring contained both earlywood and part of the latewood.

Subsequently, following the maximum moisture content method (Smith, 1954; Dumitriu-Tătăranu et al., 1983), all sections were saturated with water by boiling them for 12 hours in order to determine the basic wood density (BWD). During this process, the water was replaced several times in order to eliminate the content of dissolved extractives that could lead to an overestimated value of basic density (Zobel & Van Buijtenen, 1989). In the next phase, the sections were weighed twice: (1) immediately after boiling, and (2) in the anhydrous state, after drying them at 104 °C on a thermobalance until they attained a constant weight.

Finally, the basic density (in g·cm-3) of each section was calculated using Equation (2) (Smith, 1954; Dumitriu-Tătăranu et al., 1983):

$$BWD = \frac{1}{\frac{m_m - m_0}{m_0} + \frac{1}{G_{so}}},$$
 (2)

where BWD is basic wood density,  $m_m$  is the mass of saturated wood sections,  $m_0$  is the mass of anhydrous wood sections and  $G_{so}$  is the average density of wood substance in the anhydrous state; a constant usually accepted as having a value of 1.53 g·cm-3, since the density of cell-wall substances is practically identical for all wood species (Subhasis et al., 2019).

Additionally, the average ring width (ARW) was calculated by dividing the length of sections by the number of rings in each section (Equation 3):

Statistical analysis was conducted using STATISTICA v.8.0 software (StatSoft Inc., Tulsa, OK, USA) and the confidence level was predefined at 95%.

Firstly, the goodness of fit to normality was assessed using the Kolmogorov-Smirnov and Shapiro–Wilk test (Sheskin, 2004), and the homogeneity of variances was assessed using Levene's test (Zar, 2010). If the statistical assumptions were not satisfied (normality of observations and homogeneity of variances), we performed the Kruskal–Wallis non-parametric test. In the case of a normally distributed random variable, variance analysis was practiced.

Because tree age and radial growth are key factors in the development of wood structure(Zobel & van Buijtenen, 2012), to remove their interaction in the relationship of tree phenology with the studied wood properties, covariance analysis was called (Dytham, 2011).



After controlling for tree age and ring width, the partial correlation was used to estimate the relationship between wood and phenology variables.

Further, we used principal component analysis (PCA) to study the variation among wood traits and phenological categories. In addition, before performing PCA we transformed all variables (we applied a square-root transformation or log10 transformation) that were non-Gaussian distributed and we checked if the data set was appropriate for this type of analysis by implementing the Kaiser–Meyer–Olkin Measure of Sampling Adequacy (KMO) and Barlett's Test of Sphericity (BTS). The data are considered adequate if the KMO value is between 0.5–1.0 and the p-value is less than 0.05 for BTS.

Furthermore, cluster analysis was applied to find similarities between populations or provenances by using different wood traits. For this, Ward's method and Euclidean distance were used as an amalgamation rule and distance measure, respectively.

# **3.2.3** Research material and method for the phenotypic analysis in the comparative trial Fântânele

During the autumn of 2022, we performed the following phenotypic assessments: survival (% of an existing number of trees from the initial one), diameter at breast height (cm), total height (m), pruned height (m), stem straightness (index), trunk shape (index), branch insertion angle (index) and branch diameter (index).

Indexes were visually assessed with the following scores:

- stem straightness: 1= straight stem, 2= stem with curvature in one plane, 3= curvature in two planes, 4= sinuous steam;

- trunk shape: 1= not forked, 2= trunks with one fork, 3= trunks with two forks;

- the branch insertion angle:  $1 = angle < 60^{\circ}$ ,  $2 = angle between 60 and <math>90^{\circ}$ ,  $3 = angle > 90^{\circ}$ ;

- branch diameter: 1=branches with a diameter less than 5 cm, 2=branches with a diameter of 5-8 cm, 3=branches with diameters higher than 8 cm.

In order to identify certain common features, provenances were grouped according to the region of provenances to which they belong (Table 3.1.2).

For all traits, we assessed the goodness of fit for normality and homogeneity of variances using Shapiro– Wilk test and Levene's test respectively. If the previous statistical assumptions were satisfied, we used analysis of variance (ANOVA) to estimate the difference between the assessed traits.

Considering the adopted experimental design, the total variance was separated into the variance due to replications, provenances, groups, and the variance of error (residual) (Nanson 2004). Consequently, provenance component significance was tested using the following statistical model (Equation 4):

$$Y_{ijk} = m + \alpha_i + \beta j + \gamma_k + \varepsilon_{ijk}, \qquad (4)$$

where *m* is the overall mean value,  $\alpha_i$  is the overall mean values *i*<sup>th</sup> provenance,  $\beta_j$  is the effect of the *j*<sup>th</sup> replication;  $\gamma_k$  is the effect of the *k*<sup>th</sup> group and  $\varepsilon_{ijk}$  is the random error associated with the *ijk* trees.

Furthermore, if ANOVA indicated a significant *F*-value, Duncan's test for a 5% transgression probability was applied to compare the means.

The relationship between the analyzed traits and the geographical position (latitude, longitude, altitude, and ecophysiological latitude) of the seed stands origin was evaluated using the Pearson correlation coefficient (r). The ecophysiological latitude (Le) was determined using equation (1) (presented in chapter 3.2.1).

All statistics were performed using STATISTICA v.8.0 software (StatSoft Inc., Tulsa, OK, USA).



#### 3.2.4 Research material and method for foliar morphological analyses

From the sampled trees for phenological assessments in the four natural populations and in the comparative trial FAN (Table 3.1.1 and Table 3.1.2), vegetative material was also collected for leaf morphological analyses.

The methodology for collecting biological samples followed the recommendations of previous morphological studies conducted on oaks (Kremer et al., 2002; Borozan & Babaç, 2003; Curtu et al., 2011; Apostol et al., 2017). Thus, leaves were taken from the inner part of the upper crown, avoiding those exposed to direct sunlight. From all sampled specimens, between 6 and 12 fully developed leaves were collected from the same branch (Bacilieri et al., 1995). These leaves were dried and herborized for further processing.

For assessing the intraspecific taxonomic variability of the three subspecies of sessile oak based on the phenotypic characteristics of their leaves, the description of intraspecific taxonomic units presented in the Romanian literature was used (Beldie & Cretzoiu, 1941; Georgescu & Morariu, 1948; Beldie, 1952; Şofletea & Curtu, 2007).

For studying the intraspecific taxonomic variability, both macromorphological and micromorphological descriptors were used, their selection being based on studies conducted at both international (Bussotti & Grossini, 1997; Bruschi et al., 2000, 2003; Kremer et al., 2002; Viscosi et al., 2009b; Fortini et al., 2015; Di Pietro et al., 2016; Yücedağ et al., 2021) and national levels (Curtu et al., 2011; Şofletea et al., 2011; Enescu et al., 2013; Apostol et al., 2017) for various species of the *Quercus* genus.

Therefore, for the analysis of macromorphological variability, a set of 18 phenotypic descriptors were used, including seven dimensional variables, two counted variables, four observed variables, and five transformed variables (Table 3.2.4.1)

	Foliar macromorphological descriptors	Code						
	Leaf area (cm <sup>2</sup> )	LA						
	Leaf perimeter (cm)							
Dimonsional	Lamina length (cm)	LL						
variables	Petiole length (cm)	PL						
(measured)	Lamina width (cm) - measured between the median rib and the tip of the lobe	LW						
(measured)	in the area of maximum width							
	Sinus width (cm) - below the lobe furthest from the midrib	SW						
	Length of lamina at largest width (cm)	WP						
Counted	Number of lobes	NL						
variables	Number of intercalary veins	NV						
	Basal shape of the lamina	BSL						
Observed	Abaxial laminar pubescence	AB PU						
variables	Adaxial laminar pubescence	AD PU						
	Petiole pubescence	PE PU						
	Lamina shape or obversity: WP/LL·100	OB						
Transformed	Petiole ratio: PL/(LL+PL) ·100	PR						
variables	Lobe depth ratio: (LW-SW)/LW·100	LDR						
(calculated)	Percentage venation: NV/NL·100	PV						
	Lobe width ratio: LW/LL·100	LWR						

Table 3.2.4.1 List of foliar macromorphological descriptors analyzed



To determine the dimensional characters, the leaves were scanned, and the seven dimensional descriptors were evaluated using the WinFOLIA PRO 2020 software (Figure 3.2.4.1), specialized for foliar morphological analyses.



Figure 3.2.4.1 Graphic representation of the evaluated dimensional characters (LL – lamina length, PL – petiole length, LW – lobe width, SW – sinus width, WP – length of lamina at largest width)

Regarding the observed variables, for assessing the leaf base shape, the numerical scale (1 - cuneate to 9 - auriculate) proposed by Kremer et al. (2002) was used (Figure 3.2.4.2). For evaluating the degree of pubescence on the petiole and on both sides of the lamina, the scale (1 - no hair to 6 - very pubescent) developed by Kissling (1977) was employed (Figure 3.2.4.3), and it was performed using a stereomicroscope (40X) (Figure 3.2.4.4).



2 1: Fárá pározítate 4 4 5

Figure 3.2.4.2 Assessment of the base shape of lamina (Kremer et al., 2002)

Figure 3.2.4.3 Assessment of pubescence (Kissling, 1997)



Figure 3.2.4.4 General aspects of the abaxial, adaxial surfaces, and petiole of the sessile oak leaf (Foto: Ioana Maria Gafenco)



In addition to macromorphological analyses, micromorphological analyses were also conducted. In this case, a number of nine descriptors were used for the analysis of micromorphological variability (Table 3.2.4.2).

Foliar micromorphological descriptors	Code
Stomatal density (număr · mm <sup>-2</sup> )	StoD
Stomata rim length (μm)	SRL
Stomata rim width (μm)	SRW
Freedom of stomatal rim (µm)	FSR
Stomatal area index: SD·SRL	SAI
Number of stellate trichomes	NST
Number of glandular trichomes	NGT
Length of rays of stellate trichomes	LRS
Number of rays of stellate trichomes	NR

Table 3.2.4.2 List of foliar micromorphological descriptors analyzed

For the determination of micromorphological descriptors, the leaves previously analyzed from a macromorphological perspective were used. Specifically, six leaves per tree were used to prepare microscopic slides, which were examined using an Olympus CX43 microscope equipped with a PROMICAM LITE 5 camera. The number and dimensions of stomata were obtained using the QuickPHOTO MICRO 3.2 image acquisition and processing software.

The microscopic slides were prepared by applying a thin layer of nail polish on the abaxial (lower) surface of the leaves, in the interveinal area where the lamina width was maximum. The determinations were performed on a standardized area (98278  $\mu$ m<sup>2</sup>  $\approx$  0.1 mm<sup>2</sup>), and the average values of stomata were obtained based on measurements taken for 10 randomly selected stomata.

In addition, besides the micromorphological descriptors of stomata, certain characteristics of trichomes (hairs) were also determined (Table 3.2.4.2).

Characteristically, on the abaxial surface of the sessile oak leaf, glandular trichomes and non-glandular stellate and stellate-bifurcate trichomes were observed (Figure 3.2.4.5).



Figure 3.2.4.5 Glandular trichome (a) and non-glandular stellate trichomes (b) and stellate-bifurcate trichomes (c). In the first image, the red arrow indicates the distal cell of the glandular trichome. (Foto: Ioana Maria Gafenco).

All the obtained data were imported into the XLFolia software, where the average value for each descriptor was calculated at the level of each tree. Subsequent statistical analyses were performed using the STATISTICA 8.0 program.



For characterizing the intra- and inter-populational variability at the level of macromorphological and micromorphological leaf descriptors, the main statistical indicators (mean - abv. med, variance - abv. var, standard deviation - abv. SD, and coefficient of variation - abv. CV%) were determined.

Subsequently, for the entire dataset, normality was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests (Sheskin, 2004), and homogeneity of variances was tested using the Levene test (Zar, 2010). In cases where the statistical assumptions were not met (normality of observations and homogeneity of variances), the variables were transformed using the square root or logarithm.

To evaluate the differences between the means of the three subspecies, analysis of variance (ANOVA) was used. Following ANOVA, the significance of differences between the mean values of the variables was tested using Welch's t-test, regardless of whether the variances of the groups were equal or not.

Among the methods frequently used in taxonomic classifications, principal component analysis (PCA), discriminant analysis (DA), and cluster analysis were applied.



#### 4. RESULTS AND DISCUSSIONS

## **4.1** Phenology of sessile oak in peripheral populations from eastern Romania and in the comparative trial Fântânele

#### Bud burst and leaf development

Amongst the peripheral populations FUN, OLT, and SAT, sessile oak showed an extremely small difference in the average date for bud burst (Table 4.1.1.). Moreover, there was only a few days difference between them and the reference HEL population, with a maximum average advance of 5 days for the FUN population. Unlike the peripheral populations, HEL showed the lowest inter-annual variability and the smallest range (9 days) for the start of the bud burst.

Table 4.1.1 Inter-individual variability of bud burst (BB) and leaf development (LD) observed for sessile oak in the analyzed populations and in the FAN comparative trial for the period 2017–2020

	Bud burst (DOY) - Stage I					Lea	Leaf development (DOY) - Stage III				BB-LD Dynamic		
Population	Min	Max	Mean ± SE	IV	SD	Min	Max	Mean ± SE	IV	SD	No. days	T <sub>med</sub> (°C)	∑ T (°C)
					Periph	neral p	opulat	ions					-
FUN	88	104	96 ± 0.38	16	4.4	101	114	$109 \pm 0.27$	13	3.2	13.2	11.1	230.4
OLT	90	107	98 ± 0.27	17	3.7	104	120	$111 \pm 0.26$	16	3.8	13.2	10.9	217.8
SAT	90	104	98 ± 0.23	14	3.1	106	120	$112 \pm 0.26$	14	3.5	14.0	10.5	217.1
Mean of peripheral populations	89	105	98 ± 0.16	16	3.7	104	118	111 ± 0.16	14	3.5	13.5	10.8	221.8
				Referen	се рор	oulatio	n (non	-peripheral)					
HEL	97	106	$101 \pm 0.20$	9	2.4	106	120	116 ± 0.25	14	3.3	15.4	10.4	214.5
					Cor	npara	tive tri	al					
FAN	95	112	$103 \pm 0.32$	17	4.6	109	124	117 ± 0.29	15	3.9	15.4	10.4	236.9

Min – minimum DOY; Max – maximum DOY; SE – standard error; IV – range of variation; SD – standard deviation;  $T_{med}$  – average temperature( $^{\circ}C$ );  $\Sigma T$  ( $^{\circ}C$ ) – sum of average daily temperatures.

The bud burst starts in the peripheral populations before the reference population, except for 2018, when it starts almost at the same time (Figure 4.1.1).

The later onset of bud burst in the peripheral populations in 2018 compared to the other years was associated with lower temperatures between leaf senescence in the previous year and the start of bud burst in the current year. Consequently, the later start of vegetation growth in 2018 resulted in a shorter time for leaf phenophase (i.e., the number of days from bud burst (stage I) until leaves reached the full size (stage III)) in the three peripheral populations.

In the FAN comparative trial, the bud burst date range was the largest at 17 days, the earliest bud burst dates recorded in 2017 (DOY 95) and the latest in 2020 (DOY 112) (Figure 4.1.1).

The number of days required for leaves to complete expansion (from stage I to stage III) was between 13 to 16 days on average across all populations, with the process at its fastest in the peripheral populations.





Figure 4.1.1 Boxplot diagram of sessile oak bud burst phenophase in the studied populations and in the FAN comparative trial. The bottoms and tops of boxes denote the 25 and 75% quartiles; the bold lines denote the median. The whiskers represent the minimum and the maximum DOY values. The green crosses indicate the mean value across all trees in each population or in the FAN comparative trial.

The average bud burst date in the peripheral sessile oak populations (DOY 98, 8 April) was similar to that reported by Tomescu (1957) in the Dobrina forest in Romania (DOY 99, 9 April), located at 200 m altitude near our OLT population. However, the duration of the time series observations and the locations were not the same and, therefore, these comparisons should be treated with caution. Still, the results show that similar bud burst dates 70 years ago and nowadays could be encountered.

Moreover, compared to extra-Carpathian sessile oak phenological studies, the average bud burst in our peripheral populations occurred almost two weeks earlier than the average bud burst indicated for sessile oak in the OPTIMix experimental plots in France (DOY 111; Perot et. al, 2021) and 3 to 6 days later than the average bud burst obtained for sessile oak in low-elevation populations (<387 m above sea level) in the Gave Valley, France (DOY 92–95; Dantec et al., 2014). However, this difference is only illustrative because both previous studies considered different evaluation scales for leaf unfolding.

However, these differences highlight the adaptive phenological features of sessile oak populations located near the eastern limit of the species' general area.

It was observed that the average bud burst in the reference HEL population was very close to that of the peripheral populations (+3 days), and the range of bud burst variation was almost twice as small as the peripheral populations. However, most individuals completed phenophase close to the average date, suggesting a greater reduction in behavioral diversity in the reference population for bud burst. For instance, de Sauvage et al. (2022) studied nine populations of sessile oak along an altitudinal gradient in the central Pyrenees in France and found smaller phenological variability for populations situated at higher elevations.



There were extremely small differences in the thermal sum (sum of daily average temperatures) between 1 January and bud burst across peripheral populations, decreasing from the southern to the northern regions of the transect (Table 4.1.2). In the HEL population, the thermal sum was slightly higher than in the peripheral populations (11.3% for SAT, 3.6% for OLT, and 1.8% for FUN) over this period and about 4.3% smaller than the FAN comparative trial.

Conversely, the thermal sum between leaf senescence in the previous year and bud burst in the current year increased from the most southerly peripheral population to the most northerly. However, the differences were very small. The thermal sums of the HEL and FAN populations were similar and higher than those of the peripheral populations.

Table 4.1.2 Average daily temperature summed from January  $1^{st}$  to bud burst (A) and from LS in the previous year until BB in the current year (B).

Population ∑ T (°C) ± SD	FUN	OLT	SAT	HEL	FAN*
(A) January 1 <sup>st</sup> - BB DOY	191.5 ± 5.5	188.0 ± 5.6	173.0 ± 5.6	195.1 ± 5.5	203.8 ± 5.6
(B) LS DOY - BB DOY	438.4 ± 5.0	445.3 ± 5.3	447.0 ± 5.1	500.2 ± 4.7	500.8 ± 5.0

SD –standard deviation; ΣT (°C)— sum of average daily temperatures. \*Comparative trial

The local provenances 2 Dolhasca, 6 Sascut, and 24 Botosani—all within the G region (The Moldavian Plateau) where the comparative trial was installed—were among the fastest to reach bud burst dynamic stage I in all studied years. While the differences in the average bud burst date across provenances are not considerable, the slowest onset of bud burst was found for the provenances 32 Cluj and 15 Beius, both in the E region (Apuseni Mountains).

Furthermore, a significant positive correlation was found between the bud burst and provenance altitude in the data of the FAN comparative trial for all four years of the study. Moreover, the bud burst was significantly and indirectly correlated with the longitude of the sites, reflecting the positive correlation between site longitude and the rate of leafing. Therefore, our sessile oak data indicate a delay in bud burst from east to west of 0.5 to 1.4 days for each degree of longitude.

Numerous studies performed in comparative trials of sessile oa (Liepe, 1993; Deans & Harvey, 1995; Ducousso et al., 1996) have shown that the bud burst phenology varies with latitude, with southern leafing earlier than northern populations.

However, these findings contrast with those of Jensen (2000), who found in comparative trials of sessile oak and pedunculate oak that northern provenances budded earlier than southern provenances. Moreover, some studies have also reported similar clinal variations along altitudinal gradients (Alberto et al., 2011, 2013; Firmat et al. 2017).

### > Flowering

In the spring of 2017, after a period with mean air temperatures above 10°C, the air temperature decreased and persisted for several days (DOY 109–115). The sudden drop in temperature was accompanied by heavy snow, catching the male inflorescences in full maturation process, and the minimum temperatures dropped below 0°C at night. In both the four populations and the comparative trial, minimum temperatures varied between -2.0°C and -0.9°C.

Therefore, the unfavourable climatic conditions in 2017 during pollen release likely harmed the flowering process, interrupting microsporogenesis and the elongation of the catkins, leading to their death(Garca-Mozo et al., 2001).

The reference HEL population had the highest proportion of frost-injured individuals among the studied populations (47.5%) because its average flowering DOY was closer to the average frost date. The differences between populations in the damage intensity can be attributed to the stage of development



at the time of frost exposure (Liepe, 1993; Chaar & Colin, 1999). In the peripheral populations, the percentage of individuals that suffered from low temperatures was smaller, 24.0% in SAT, 12.0% in OLT, and 11.8% in FUN.

In the FAN comparative trial, there were injuries only to individuals in provenances 11 Babeni, 14 Valeni, and 24 Botosani, the latter having the largest proportion of injured trees. In this case, flower injuries were also associated with the developmental stage at the time of frost exposure. Consequently, the three provenances (11 Babeni, 14 Valeni, and 24 Botosani) that had trees in a more advanced flowering stage suffered flower damage in 2017, potentially explaining the increased frost sensitivity. However, these results should be treated with caution, since there was only one episode of spring frost during this study, and further studies are required to confirm this observation.

The flowering phenophase overlapped with the leaf-development phenophase for all populations, except for the FUN population, where the lowest average DOY values were recorded (Table 4.1.3).

Deputation		F	lowering (DO	Temper	Na dava ð				
Population	Min	Max	Mean ± SE	IV	SD	T <sub>med</sub>	∑T > 5 °C	No.days "	
	Peripheral populations								
FUN	101	114	109 ± 0.31	13	3.7	12.4	80.0	7.0	
OLT	104	117	$110 \pm 0.22$	13	3.1	11.8	96.6	8.3	
SAT	106	120	111 ± 0.23	14	3.4	11.4	94.3	8.5	
Mean of periphe- ral populations	104	117	110 ± 0.15	13	3.4	11.9	90.3	7.9	
		Ref	erence popula	tion (non-pe	eripheral)				
HEL	106	120	113 ± 0.28	14	3.1	11.1	90.5	8.0	
			Сотра	rative trial					
FAN	109	124	117 + 0.24	15	3 /	10.1	107.9	95	

Table 4.1.3 Inter-individual variability of flowering for sessile oak in the studied populations and in the FAN comparative trial.

Min – minimum DOY; Max – maximum DOY; SE – standard error; IV – range of variation; SD – standard deviation; Tmed – average temperature(<sup>o</sup>C); ΣT (<sup>o</sup>C) – sum of average daily temperatures. <sup>a</sup> The difference between DOY BB and DOY flowering.

The average daily recorded temperatures of the OLT and SAT populations were highly similar. The FUN population, located at the southernmost point in terms of altitude and latitude, had the highest temperature, leading to faster flowering compared to the other populations.

Within the comparative trial, flowering time was positively correlated with latitudinal and longitudinal gradients, except in 2017, when it was only correlated with longitude. Moreover, flowering time and altitude were significantly positively correlated in 2019 (p < 0.05, Spearman's rank correlation test), but not in the other years. However, no significant correlation was observed between flowering time and ecophysiological latitude.

In addition, there were geographic patterns with latitude and longitude and, to a lesser extent, altitude in flowering time in the FAN comparative trial. The flowering date was negatively correlated with latitude and longitude, indicating that flowering occurs earlier in northern and eastern provenances than in southern and western provenances.

In sessile oak, the role of geographic gradients in flowering onset has not been studied as extensively as bud burst. For example, Grundström et al. (2019) found an association between the start of the oak pollen season and latitude, where southern populations flowered earlier than northern populations.

In the four consecutive years studied, no meaningful differences in flowering period length (7–10 days) were observed among sites. This interval falls within limits observed in previous studies in mixed oak stands that reported a flowering period of 6 (Chesnoiu et al., 2009) to 12 days (Crăciunesc, 2013; Chesnoiu, 2017).



#### > Leaf senescence and growing season length

Sessile oak showed greater inter-individual variation in the date of leaf senescence (DOY 281–313, 32 days) than in bud burst (DOY 88–112, 24 days) for both the four populations and the FAN comparative trial (Table 4.1.1 and 4.1.4). The average date of leaf senescence did not vary greatly among peripheral populations (Table 4.1.4). Moreover, the average value of senescence of the peripheral population differs from the reference population by almost a week (Table 4.1.4).

Table 4.1.4 Inter-individual variability of senescence (LS) and growing season mean values observed for sessile oak in the analyzed populations and in the FAN comparative trial for the period 2017–2020

	LS (DOY)				Bioactive season			
Population	N 45-5	Max	Mean ± SE	IV	No. days <sup>a</sup>	Climate Conditions		SD
	IVIIN					T <sub>med</sub> (°C)	∑ T (°C)	
Peripheral populations								
FUN	287	325	304 ± 0.74	38	208	17.8	3806.7	8.7
OLT	287	322	301 ± 0.62	35	203	17.5	3576.3	8.8
SAT	287	322	300 ± 0.69	35	202	17.4	3500.4	9.2
Mean of peripheral	207	225	202 / 0.44	20	204	17.0	2627.0	0.5
populations	287	325	302 ± 0.41	30	204	17.0	3627.8	9.5
Reference population (non-peripheral)								
HEL	281	315	296 ± 0.80	34	195	17.1	3271.2	10.5
Comparative trial								
FAN	281	315	297 ± 0.60	34	194	17.2	3347.1	8.2

Min – minimum DOY; Max – maximum DOY; SE – standard error; IV – range of variation; Tmed – average temperature(<sup>e</sup>C); ΣT (<sup>e</sup>C) – sum of average daily temperatures; SD – standard deviation.

<sup>a</sup> The difference between DOY BB and DOY leaves senescence.

For the year 2018, which had a later bud burst start date compared to the rest of the years, the peripheral populations had an earlier onset of autumn leaf senescence (Figure 4.1.2).

During the first two seasons (2017-2018), the peripheral populations had later leaf colouring compared to the HEL population by more than 14 days in 2017 and almost a week in 2018 (Figure 4.1.2). Conversely, in the last two seasons (2019-2020), the autumn phenophase in the peripheral populations occurred either approximately at the same time as the reference population (in 2019, recording a delay of 4 days for the FUN population and one day for the OLT and SAT populations), or simultaneously (in 2020). Compared to the HEL population, the FAN comparative trial also had a similar inter-individual variation range, but with a later average DOY value of leaf senescence.

The average leaf senescence date observed in the peripheral populations (DOY 302) is later than the average senescence DOY values reported in previous studies performed over half a century ago at the national level in sessile oak forest stands. For example, Tomescu (1957) reported a DOY of 289 for sessile oak in the Dobrina forest, and Tomescu (1967) reported a DOY of 273 for sessile oak in the Poieni forest located 360 m from SAT population, suggesting a delay in leaf senescence of 2.0–4.3 days per decade. As mentioned previously, these differences should be treated with caution, since the phenological observation protocol is not the same, and the influence of inter-observer variability is very high (Liu et al., 2021). Similarly, we observed a small delay in the average date of leaf senescence in the reference HEL population (DOY 296) and the FAN comparative trial (DOY 297) compared with nearby sites such as the Bradatel forest (DOY 273) and Casa cu Nuci forest (DOY 289; Tomescu (1967)).

However, the average leaf senescence date for the peripheral populations was very close to that obtained by Chesnoiu (2017) in the Fundeanu forest (DOY 299). Moreover, the differences in the leaf colouring average DOY in the peripheral populations were small compared to some European studies on sessile oak that reported DOY of 299 (Delpierre et al., 2009) and 303 (Vitasse et al., 2011).





Figure 4.1.2. Boxplot diagram of sessile oak leaf senescence phenophase in the studied populations and in the FAN comparative trial. The bottoms and tops of boxes denote the 25 and 75% quartiles; the bold lines denote the median. The whiskers represent the minimum and the maximum DOY values. The green crosses indicate the mean value across all trees in each population or in the FAN comparative trial.

Unlike bud burst, leaf senescence was significantly correlated with the geographic gradients of the place of origin only in 2017.

For sessile oak, in some previous studies, variations with latitude were also found regarding the phenophase of leaf coloration (Deans & Harvey, 1995; Jensen & Hansen, 2008). As a result of variations in photoperiod length, senescence occurs earlier in populations at higher latitudes than in populations at lower latitudes. However, Vitasse et al. (2009), observed that the gradient variation in senescence with altitude is much more likely to be induced by temperature than by photoperiod. However, the results obtained by Torres-Ruiz et al. (2019) in sessile oak do not support a clinal tendency of senescence with temperature.

While leaf senescence showed small variations across years for the FAN comparative trial, some differences were observed between provenances. For instance, the provenance 32 Cluj showed late bud burst and early senescence across the three autumn seasons studied, likely reflecting its marginal location within the experimental trial.

In general, the year-to-year variation in leaf senescence was small in the comparative trial. However, there were some changes at the provenance level, especially provenance 32-Cluj, which had the earliest leaf coloration in each of the four consecutive years.

The recorded temperatures during the bioactive season were higher in the peripheral populations, with only slight differences between them. The onset of senescence occurred earlier and finished faster in the HEL reference population and in the FAN comparative trial because these are located in the sub-Carpathian area, resulting in a shorter bioactive season in these areas.



Numerous studies have indicated that leaf senescence will be progressively delayed over time under future climate-change scenarios (Menzel et al., 2001; Menzel et al., 2008; Vitasse et al., 2011). The models explored by Nölte et al. (2020) for sessile oak in Southwest Germany predicted a delay of 6–11 days per decade for the period 2070–2100 compared to 1985–2015. In contrast, it was shown that, due to global warming, the bud burst phenology of oaks starts earlier by 1–3 days·°C<sup>-1</sup> (Thomas & Gausling, 2000; Fu et al., 2014) up to 5–7 days·°C-1 Morin et al., 2010; Vitasse et al., 2010).

These changes in the occurrence of phenological phases are also reflected in the length of the vegetation season (Menzel & Fabian, 1999; Vitasse et al., 2009b, 2010; Crabbe et al., 2016), as can be seen in comparisons between our results and those reported previously for bioactive season lengths. Based on observations between 1956–1965, Tomescu (1967) found that the vegetation period in sessile oak lasted, on average, 180 days regardless of geographical gradients (200 days at 100 m altitude and 160 at 650 m). Therefore, we can observe a lengthening of the growing season by cca. 30 days over the last few decades.

#### > Correlations

For sessile oak, in the years of phenological observations, significant correlations were found between spring phenophases, more precisely between bud burst and leaf development, respectively flowering. However, between bud burst and leaf senescence situations where there were significant correlations were not frequent, suggesting that the onset of budding does not influence the onset of senescence

Furthermore, in most cases, no significant correlations were observed between bud burst in the current year and senescence in the previous year, which contradicts previous results obtained for sessile oak by Marchand et al. (2020).

In agreement with previous studies (Cole & Sheldon, 2017; Delpierre et al., 2017), the bud burst timing was repeatable from year to year. However, the magnitude of repetition ( $r_s < 0.6$ ) was medium to low, suggesting that not all sampled trees start to bud burst at the same time in different years (Bacilieri et al., 1995). Nevertheless, microenvironmental variation can help explain the individual consistency between years in bud burst development (Morin et al., 2010).

Similarly, the repeatable order of flowering in consecutive springs was observed despite inter-year differences in the time of pollen release. However, the low-to-medium Spearman's correlation coefficients we obtained indicate that only a small proportion of the sampled trees maintain their order when pollen is released, consistent with the observations of Craciunesc (2012) and Chesnoiu (2017).

Furthermore, were obtained positive correlations of bud burst with leaf development and flowering. Therefore, the timing of bud burst is a good indicator for the onset of leaf development and flowering.

In comparison, the timing of bud burst and leaf senescence were correlated, albeit to a lesser extent, indicating that bud burst is a poor indicator for the onset of leaf senescence.

#### **4.2** Analysis of the influence of the phenological category on wood traits

#### 4.2.1 The influence of the phenological category on the basic wood density

For all trees, the mean BWD of sessile oak was 0.579 g·cm<sup>-3</sup> ( $\pm$ 0.04 standard deviation), with a coefficient of variation of 6.6% (Table 4.2.1.1) and values in the range 0.470–0.694 g·cm<sup>-3</sup>.

Complianting	BWD (g⋅cm <sup>-3</sup> )			
Sampling location	Mean±SD	CV(%)		
Peripheral populations (FUN, OLT, SAT)	0.572±0.06	10.1		
Reference population (HEL)	0.584±0.04	6.3		
Comparative trial (FAN)	0.589±0.05	8.1		
Total	0.579±0.04	6.6		

Table 4.2.1.1 Basic wood density of sessile oak according to sampling location



At tree level, BWD was a standard Gaussian random variable (d from Kolmogorov-Smirnov test = 0.102, Lilliefors p < 0.05). The differences between the trees of the whole sample were statistically significant (t = 146.93, p < 0.001). These differences were not directly due to the age of the trees, but to the width of the annual wood rings (Table 4.2.1.2), trees with vigorous growth having higher BWD (Spearman *R* correlation = 0.266, p = 0.01), this effect being masked by the age of the trees (partial correlation between BWD and ARW after controlling for tree age was -0.001, p = 0.93).

Demondent	Predictors		Fixed Effects: Tree phenological category		
Variable	Trace age	Ding width	Covariates		
Variable	Tree age	King width	Tree age	Ring width	
BWD	0,39	0,03*	0,01**	0,01**	
SW	0,98	0,006**	0,34	0,32	
NSR	< 0,001***	< 0,001***	0,57	0,36	
2021 tree ring phenology	0,38	0,03*	0,02**	0,03**	

Table 4.2.1.2 ANCOVA of wood	examined traits	( <i>p</i> from F test)
	chaining a traits	

Level of significance: \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

The effect of phenology differences between trees on wood density was influenced by both tree age and by their radial growth (Table 4.2.1.2). When was considered the entire sample, early flushing trees had the BWD with 31 kg·m<sup>-3</sup> higher than late-flushing trees. Basic wood density varied with the status of the sampled tree populations (peripheral/non-peripheral, natural/plantation): F = 3.18, p = 0.04. It turned out that at the same age of the trees, the trees in the peripheral populations have the wood in the base section 10 kg·m<sup>-3</sup> lighter (Figure 4.2.1.1).



Figure 4.2.1.1 The variation of BWD according to the tree phenological category (1- early flushing, 2- intermediate flushing, 3- late flushing) in the comparative trial (**a**) and in the natural regenerated populations (**b**).

At the comparative trial level, which was installed in a non-peripheral station, the peripheral provenances (Dolhasca, Sascut and Botosani) had slightly higher BWD than non-peripheral provenances (Babeni, Valeni, Beius, Faget, Blaj, Lechinta and Cluj), but the differences were not statistically assured (F = 0.47, p = 0.50).

The wood density in the comparative trial, where the trees were younger, exceeded the density in the older stands (Figure 4.2.1.1). The result could be explained by the higher growth rate of young trees, translated into wider rings, which are associated with heavier wood, due to the high latewood content Bergès et al., 2000, 2008; Vavrčík & Gryc, 2012; Pretzsch et al., 2018).

When the heartwood was separated from the sapwood, the differences between early flushing and late flushing trees regarding wood density were accentuated (Figure 4.2.1.2). In the comparative trial, the SAW basic density was consistently lower than HW basic density (Kruskal–Wallis test: H = 18.95, p < 0.001), but the differences were higher in late flushing trees (Figure 4.2.1.2a). Also, in the natural regenerated populations, BWD differences were observed between juvenile and mature heartwood, the latter being less dense (Figure 4.2.1.2b).





Figure 4.2.1.2 Sapwood and heartwood BWD (mean ± standard deviation) according to the phenological category (1- early flushing, 2- intermediate flushing, 3- late flushing) in the comparative trial (**a**) and in the natural regenerated populations (**b**).

Within the comparative trial, the sapwood and heartwood basic density of early and intermediate flushing trees was greater than that of late flushing trees (Figure 4.2.1.2a).

Although it has generally been stated that density gradually increases from pith to juvenile wood, and then to mature wood (Knapic et al., 2007), we attained greater values for BWD in the JHW section. The higher density in the juvenile wood of the oaks is the consequence of the higher proportion of latewood in the annual rings (Figure 4.2.1.2). Furthermore, in support of these findings, Pásztory et al. (2014), as well as Diaz-Maroto & Tahir (2018), observed a higher oven-dry density in the juvenile heartwood of sessile oak.

The difference in BWD between early flushing and late flushing trees (Figure 4.2.1.1, Figure 4.2.1.2) is, in fact, the result of differences in growth: a faster start of radial growth is assumed in early flushes and, implicitly, a longer duration of growth, leading to wider rings, which will have a higher density (Wang et al., 2021).

Geographical variations of basic density are highlighted both in the natural stands sampled and by the provenances from the comparative trial (Figure 4.2.1.1). Although the differences were not substantial, peripheral populations had the lowest average values for BWD, which confirms that wood density in ring-porous hardwoods is higher under optimal ecological conditions (Bergès et al., 2008).

#### 4.2.2 The influence of the phenological category on the structural traits of wood

Differences between sampling sites were statistically significant (W = 22.60, p = 0.04) and it was primarily due to the age of the trees, especially those from the comparative trial (H = 50.88, p < 0.001). At the same tree age, peripheral populations had narrower rings at breast height than non-peripheral population, as well as a greater amplitude of ARW, but the differences were not statistically significant (p from Kruskal Wallis multiple comparison array = 0.13).

In regard to the variation of the ARW according to phenological category (Figure 4.2.2.1), was observed that the widest average annual rings were found for intermediate-flushing trees, and the narrowest for late flushing trees, the differences being statistically significant (Kruskal–Wallis test: H = 8.369, p < 0.05) independent of the age of the trees.

Comparing different wood parts, the sapwood had narrower average rings than heartwood, and both wood parts had narrower average rings in late flushing trees (Figure 4.2.2.1).



Figure 4.2.2.1 The variation of ARW (mean  $\pm$  standard deviation) according to phenological category (1 - early flushing, 2 - intermediate flushing, 3 - late flushing) in trees from the comparative trial, 30-40 years old (**a**) and in natural regenerated populations, 70-80 years old trees (**b**).

There were no differences between site plots with respect to SAW (H = 19.42, p = 0.11). Yet, there were statistically significant (t = 27.21, p < 0.1) differences between trees with respect to SAW, and were due to differences in radial growth expressed by ARW (Table 4.2.1.2).

Differences between trees with respect to NSR were statistically significant (t = 25.55, p < 0.01), as well as differences between sites (H = 42.83, p < 0.001). The average NSR of all trees from the comparative trial (trees 30-40 years old) was  $9 \pm 2.5$  (Figure 4.2.2.2a), with a range of variation between 5 and 16 rings, and of the natural regenerated populations (70-80 years old trees),  $14 \pm 4.4$  (Figure 4.2.2.2b), ranging from 6 to 27 rings. The NSR was dependent both on the age of the trees and their growth rate (Table 4.2.1.2). Apparently, NSR increased with tree age (as revealed from the simple correlation), but after controlling for ring width effect, NSR on the contrary decreased with age (partial correlation = -0.279, p = 0.01).



Figure 4.2.2.2 The variation of NSR according to phenological category (1 - early flushing, 2 -intermediate flushing, 3 - late flushing) in the comparative trial (**a**) and in the natural regenerated populations (**b**).

Additionally, was tested the relationship between the NSR and the geographical coordinates of the ten provenances' origins, and we found that the NSR was significantly and indirectly correlated with the longitude of the provenances' origins (*R* Spearman rank order correlation = -0.230, p < 0.05). Therefore, although the correlation was weak, our sessile oak provenances showed a decreasing tendency of the NSR from west to east, i.e., the peripheral provenances had fewer NSR. When we compared the natural regenerated populations, the peripheral populations presented an obviously higher NSR compared to the non-peripheral one (Figure 4.2.2.2b).

Possible differences between trees with different phenology in relation to SAW and NSR (NSR decreased from late, to intermediate, to early flushing trees, Figure 4.2.2.2a) were due to radial growth (Table 4.2.1.2). For example, in comparative trial, trees NSR decreased with ARW (Spearman Rank Order Correlations = -0.354, p = 0.02).

In the comparative trial (30-40 years old trees), tree phenology was also analysed in relation to the 2021 annual ring formation. The non-parametric test (Kruskal–Wallis test: H = 13.151, p < 0.05) revealed



significant differences between the phenological stages of 2021 ring formation according to the trees' phenological category.

Unexpectedly, in 49% of the early-flushing trees (22% of the total number of trees from the comparative trial), a ring had not formed by 2 April 2021, while in some late-flushing trees, the ring was advanced in formation (Figure 4.2.2.3). Furthermore, in most intermediate flushing trees (61%, or 23% of the total number of trees from the comparative trial), the formation of the 2021 ring was advanced (Figure 4.2.2.3).

Also, was verified with the non-parametric Kruskal–Wallis test, the differences between the provenances of the comparative trial regarding the average width of the annual rings were checked and it turned out that they are not statistically significant (H = 4.92, p = 0.84). However, this fact does not mean that there are no differences between the trees, which is why the ring width was confronted with the 2021 ring phenology, resulting in an incompatibility between the two variables (Spearman rank correlation test R = -0.01, p = 0.95). In addition, from the graphic representation of the average width of the annual ring classified according to the phenology of the 2021 ring (Figure 4.2.2.4), it can be noted that among the trees in which the formation of the 2021 ring had not started at the time of collection, there are also trees with wide rings, which highlights that there is still no connection between the width of the rings and the phenology of their formation.



Figure 4.2.2.3 Phenology of the 2021 annual ring according to the phenological category for the trees in the comparative trial: 1 - the 2021 ring has not formed, 2 - the 2021 ring contains only one row of pores in the early wood zone, 3 - the 2021 ring contains only two rows of pores in the early wood zone and 4 - the 2021 ring contains both earlywood, as well as part of the latewood.

Figure 4.2.2.4 Histogram of the average annual ring width classified according to the phenology of the 2021 ring (1 - the 2021 ring is not formed, 2 - the 2021 ring contains only one row of pores in the earlywood zone, 3 - the 2021 ring contains only two rows of pores in the earlywood zone and 4 - the 2021 ring contains both earlywood, as well as part of the late wood).

Unlike the BWD, sapwood width does not reflect phenological gaps between trees. However, in the comparative trial where trees experience homogeneous environmental conditions, the NSR indicates a delay in the transition to heartwood formation in late flushing trees by an average of two years compared to early flushing trees (Figure 4.2.2.2).

Besides the geographical location of the sampling sites, the different NSR values observed between the natural populations and the comparative trial are related to tree age, with younger trees having fewer sapwood rings (Jevšenak et al., 2019). As with tree phenology, a delay in the transition to heartwood can be assumed in peripheral populations, which had a consistently higher NSR than in non-peripheral populations (Figure 4.2.2.2b).



The structure of the annual rings is a complex of growth-related variables, which make the properties of the wood dependent on the width of the annual rings (Rao et al, 1997; Gričar et al., 2013; Sousa et al., 2021). In *Q. petraea*, as a ring-porous heartwood species, ring width is positively correlated with the proportion of latewood (Bergès et al., 2000; Guilley et al., 2004), in the sense that, along with an increase in the annual ring width, there is also an increase of the proportion of latewood, and implicitly of wood density (Vavrčík & Gryc, 2012).

The fact that wood formation in intermediate flushing trees preceded in most cases wood formation in early flushing trees appears to be unrelated to the intercepted phenological process. A possible explanation for this situation is that the precocity and lateness of leaf phenology could be related to the anthesis process which is related to flower formation and not to the formation of the annual ring. Another possible explanation is related to the provenance origins of the trees with intermediate flushing trees. As shown in a previous study, the bud burst was negatively correlated with the longitude of the provenances and positively with the provenance altitude origins (Gafenco et al., 2022). Thus, as observed in other provenance studies (Chmura & Rozkowski, 2002), the effect of longitude on bud burst may be considered a possible mechanism for adapting to local conditions which allows provenances to avoid exposing leaves to late frosts.

In a previous study on pedunculate oak, Puchałka et. al (2017) did not find a correlation between the phenology of leaf flushing and earlywood-vessel formation. Others studies (Gričar et al., 2022) support the findings that the two events are not associated. However, there are also studies that report that leaf phenology is likely to be connected to wood formation (Michelot et al., 2012; Guada et al., 2019).

However, since it is only one year (2021), the results regarding the phenology of the annual ring cannot be extrapolated and require further investigation.

#### 4.2.3 Principal component analysis and cluster analysis

The adequacy of the data for PCA was confirmed (KMO = 0.517, and BTS returned a significance value lower than 0.000). The PCA results showed that the first two factors described 72.33% of the total variance, i.e., 46.48% and 25.85%, respectively (Figure 4.2.3.1).



Figure 4.2.3.1 Principal component analysis (a) and scatter plot of PCA scores (b). Notes: BWD – basic wood density, T – tree age at breast height; D – tree diameter at breast height; SAW – sapwood width; ARW – average ring width; NSR – number of sapwood rings.



From the hierarchical cluster analysis, we could distinguish two distinct groups based on the five wood traits selected (BWD, tree age, ARW, SAW and NSR) (Figure 4.2.3.2): one represented by the comparative trial and one by the natural populations. Furthermore, their differences were highlighted by the long Euclidean distance.

The dendrogram separated the natural regenerated populations from the comparative trial, and within the comparative trial were differentiated two large groups of provenances, which, however, were not associated according to their geographical position within the Romanian area of sessile oak (Figure 4.2.3.2). For instance, the Sascut peripheral provenance fell into a separate subcluster along with the non-peripheral provenance Lechința. Furthermore, in the natural populations, there were some similarities between the peripheral populations and the non-peripheral population: these similarities stemmed from the trees' age.



Figure 4.2.3.2 Cluster dendrogram based on four wood traits (BWD, tree age, ARW, SAW, NSR). The horizontal axis represents the Euclidean distance; the vertical axis represents the comparative trial provenances and the four natural regenerated populations (the non-peripheral provenances are represented with blue; the peripheral provenances are represented with red; the non-peripheral natural population with green and the peripheral natural populations with orange).

Regarding the PCA (Figure 4.2.3.1) it could be observed that among the identified phenological categories, intermediates flushing had a more reliable association with wood traits. Also, the cluster analysis (Figure 4.2.3.2) allowed us to separate the natural regenerated populations from the comparative trial when considering all investigated wood traits. The lack of a clear separation between provenances inside the comparative trial might be related to the limited traits included in the analysis (Bessa et al., 2022).

#### 4.3 Phenotypic analysis in the comparative trial Fântânele

#### Provenance survival

Analysis of variance showed highly significant differences between all three analyzed factors in terms of survival (p < 0.001) (Table 4.3.1).

Survival after 44 years from planting was very low, ranging from 6.7% (provenance 5 Zeletin) to 22.0% (provenance 25 Lipova), with an average of 13.1% (Figure 4.3.1).



Table 4.3.1 Analysis of variance for the phenotypic traits studied in the FAN comparative tri	Table 4.3.1 Ana	vsis of variance	for the phenoty	pic traits studied in	n the FAN comparative tri
---	-----------------	------------------	-----------------	-----------------------	---------------------------

Trait	Factors	DF	SS	MS	F	р
Survival	Replications	2	3472.83	1736.41	50.55	0.000
	Provenances	30	6663.33	222.11	6.47	0.000
	Groups	5	1643.80	328.76	7.02	0.000
	Error	304	10614.98	34.35		
	Replications	2	57.30	28.70	0.85	0.429
Breast	Provenances	30	1366.00	45.50	1.35	0.112
height diameter	Groups	5	412.40	7104	2.42	0.036
	Error	304	10445.50	33.80		
	Replications	2	32.90	16.40	2.14	0.119
Trees'	Provenances	30	443.60	14.80	1.93	0.003
height	Groups	5	144.80	29.0	3.62	0.003
	Error	304	2372.70	7.70		
	Replications	2	2.04	1.02	0.23	0.798
Drunod boight	Provenances	30	180.78	6.03	1.33	0.120
Pruned neight	Groups	5	59.60	11.92	2.62	0.024
	Error	304	1398.75	4.53		
	Replications	2	0.12	0.06	1.12	0.326
Volume	Provenances	30	2.84	0.09	1.65	0.019
per tree	Groups	5	0.99	0.20	3.38	0.005
	Error	304	17.71	0.06		
	Replications	2	0.523	0.26	0.73	0.483
Stom straightness	Provenances	30	18.09	0.60	1.68	0.017
Stem straightness	Groups	5	3.25	0.65	1.73	0.128
	Error	304	110.90	0.35		
	Replications	2	0.84	0.42	1.04	0.354
Trunk chono	Provenances	30	23.23	0.77	1.92	0.003
Trunk snape	Groups	5	7.10	1.42	3.36	0.006
	Error	304	124.88	0.40		
	Replications	2	0.03	0.02	0.14	0.873
Duou ch in contion on alc	Provenances	30	5.63	0.19	1.70	0.014
Branch insertion angle	Groups	5	0.88	0.18	1.52	0.184
	Error	304	34.07	0.11		
	Replications	2	1.05	0.52	1.05	0.351
Branchac' diamater	Provenances	30	51.44	1.72	3.43	0.000
Branches diameter	Groups	5	12.43	2.49	4.29	0.000
	Error	304	154.41	0.50		

DF = degrees of freedom, SS = sum of squares, MS = mean squares, F value, p < 0.05 is significant, p < 0.01 is distinctly significant, p < 0.01 is highly significant (bold underline).



Figure 4.3.1 The survival variation of the sessile oak provenances in the Fantanele comparative trial. The whiskers represent the standard deviation. The provenance code and groups are the same as in Table 3.1.2.


Some non-local provenances (belonging to B, C, D, E, and F groups) proved to be better adapted compared to the local ones (belonging to group G – where the comparative trial was established) (Figure 4.3.1). Therefore, provenances that grow under similar environmental conditions to those at their source site do not necessarily display higher adaptation capacity. Although it has been previously demonstrated that non-local provenances have often reduced survival rates compared to local ones (Grotehusmann & Schönfelder 2011, Piqué et al. 2014), this cannot be considered a general rule since the influence of other factors (which were not included in the study; e.g., soil type, slope) cannot be neglected.

Moreover, in a recent study, it has been reported that bud burst phenology for the provenances tested in the FAN comparative trial followed an east-to-west longitudinal tendency (Gafenco et al. 2022). Thus, the non-local provenances may have registered higher survival rates than the local provenances most likely because of the slight delay in bud burst timing, therefore avoiding exposure to late frost. Therefore, the slightly advanced beginning of bud burst associated with low survival rates for some of the local provenances could also reflect the effect of climate disturbances in recent decades (Gafenco et al., 2023b).

## Diameter at breast height

After the ANOVA analysis, significant differences were detected only within groups (p<0.05) (Table 4.3.1). The average diameter at breast height value was 21.8 cm, with values between 17.4 cm (6 Sascut) and 26.2 mm (33 Zalau) (Figure 4.3.2).

If we compare our results to those obtained by Stuparu (2009) in the same comparative trial 25 years after plantation we observe that the provenances 6 Sascut and 33 Zalau kept their position in the ranking as the provenance with the smallest, respectively largest growth diameter. However, within the ranking, there were numerous position changes between the provenances, such as provenance 19 Vidra which was in the second position moved to the ninth position, and provenance 22 Fantanaele which was above the average diameter value per experiment now is on position 28.



Figure 4.3.2 The diameter at breast height variation of the sessile oak provenances in the FAN comparative trial. The whiskers represent the standard deviation. The provenance code is the same as in Table 3.1.2.

Although the trial is located inside the sessile oak optimum for the average annual temperature, the local provenances group (group G) had overall the smallest average diameter at breast height (Figure 4.3.3). However, in the test site, the average annual rainfall falls within the ecologically suboptimal conditions of sessile oak. This may be an explanation for the fact that six of the eight local provenances (group G) have poor performance regarding the diameter at breast height. The provenances originating from Apuseni Mountains (group E) had the best diameter growth and were 11% greater than the overall mean.





Figure 4.3.3 The diameter at breast height variation among groups. The whiskers represent the standard deviation.

#### Total height

The average value of total height was 19.7 m, with a range of variation between 17.7 m in provenance 4 Vaslui and 22.7 m in provenance 29 Ramnicu Sarat.

From the results reported by Stuparu (2009) for FAN comparative trial at 25 years from the plantation, we observed that provenance 4 Vaslui was among the provenances with the best height growths (6<sup>th</sup> position on the ranking), whereas 29 Ramnicu Sarat was among the provenances with the lowest height growths (30<sup>th</sup> position on the ranking). Also, the previous evaluation of Stuparu (2009) showed that the same sessile oak provenances exhibited significant differences in growth performance among three different sites (Fântânele, Mihăești and Râmnicu Sărat). Therefore, results obtained from a single comparative trial cannot be used to establish which provenances are superior and which are inferior than for the specific conditions of the respective test site. The advantage of tests in multisite comparative trials resides, among other things, in providing information regarding the adaptive plasticity of the provenances tested.

As in the case of diameter at breast height, the analysis of the performance of groups revealed that the local provenances group (group G) was the last in rank (Figure 4.3.4). Within this group, the top two provenances were 2 Dolhasca and 1 Botosani, both located around the overall mean. These local provenances also had the highest diameter at breast height. For the other groups, the rank position for the average total height was similar to that of diameter at breast height.



Figure 4.3.4 The total height variation among groups. The whiskers represent the standard deviation.

Distinctly significant differences in height performance were found only between provenances and groups (p < 0.01) (Table 4.3.1). By testing the significance of the differences between the provenances using the Duncan test for a 5% transgression probability, six homogeneous classes of variation were differentiated (Figure 4.3.5).

The best homogenous group comprises half of the total number of provenances, belonging to all six regions of provenance, but mainly originating from Curvature Carpathians (group B) and Southern Carpathians (group C). The best-ranking provenances for total height were 29 Ramnicu Sarat, 33 Zalau and 32 Cluj, while the worst-ranking included the local provenances 24 Botosani, 6 Sascut and 4 Vaslui.



Drovenence	Mean	Hom	mogeneous groups for the transgression								
Provenance	(m)			probabil	ity of 5%						
29	22.7	****									
33	22.3	****	****								
32	22.0	****	****	****							
27	21.3	****	****	****	****						
9	20.8	****	****	****	****	****					
25	20.8	****	****	****	****	****					
30	20.5	****	****	****	****	****	****				
13	20.4	****	****	****	****	****	****				
21	20.4	****	****	****	****	****	****				
20	20.3	****	****	****	****	****	****				
31	20.2	****	****	****	****	****	****				
19	20.0	****	****	****	****	****	****				
23	20.0	****	****	****	****	****	****				
1	19.9	****	****	****	****	****	****				
2	19.8	****	****	****	****	****	****				
17	19.8	****	****	****	****	****	****				
28	19.7		****	****	****	****	****				
8	19.5		****	****	****	****	****				
11	19.4		****	****	****	****	****				
5	19.3		****	****	****	****	****				
10	19.3		****	****	****	****	****				
14	19.3		****	****	****	****	****				
15	19.1			****	****	****	****				
16	19.0			****	****	****	****				
22	19.0			****	****	****	****				
3	18.9				****	****	****				
18	18.8				****	****	****				
7	18.5				****	****	****				
24	18.4				****	****	****				
6	18.0					****	****				
1	17.7						****				

Figure 4.3.5 Duncan test for the average total height (local provenances are highlighted)

## Pruned height

For pruned height, ANOVA revealed a distinctly significant influence of the group factor (p<0.01), while replication and provenance had an insignificant influence (p>0.05) (Table 4.3.1). On average, the pruned height was 9.7 m, varying between 8.4 m in provenance 28 Blaj and 11.0 m in provenance 6 Sascut.

Among the groups, the local provenance group (group G) had the highest pruned height average (Figure 4.3.6).



Figure 4.3.6 The pruned height variation among groups. The whiskers represent the standard deviation.



#### Volume per tree

Among the studied provenances, the difference between the best growing provenance (33 Zalau) and the slowly growing provenance (6 Sascut) (Figure 4.3.7) reached 35%. For oak provenances at 35 years, Kleinschmit (1993) found differences in volume production up to 100%.

Drevenence	Mean	Homogeneous groups for the transgression									
Provenance	(m³)		pro	bability of	5%						
33	0.622	****									
29	0.619	****	****								
32	0.542	****	****	****							
13	0.507	****	****	****	****						
9	0.498	****	****	****	****						
14	0.482	****	****	****	****	****					
20	0.465	****	****	****	****	****					
30	0.437	****	****	****	****	****					
19	0.421	****	****	****	****	****					
31	0.406	****	****	****	****	****					
18	0.393	****	****	****	****	****					
28	0.379	****	****	****	****	****					
11	0.373	****	****	****	****	****					
2	0.366		****	****	****	****					
1	0.365		****	****	****	****					
21	0.365		****	****	****	****					
3	0.352			****	****	****					
25	0.351			****	****	****					
16	0.345			****	****	****					
27	0.343			****	****	****					
8	0.340			****	****	****					
17	0.330			****	****	****					
15	0.329			****	****	****					
5	0.327			****	****	****					
24	0.317			****	****	****					
10	0.298			****	****	****					
23	0.298			****	****	****					
22	0.287			****	****	****					
4	0.243				****	****					
7	0.223					****					
6	0.219					****					

Figure 4.3.7 Duncan test for the average volume per tree (local provenances are highlighted)

Furthermore, through the analysis of variance were found statistically significant differences between provenances (p<0.05), distinctly significant differences between groups (p<0.01) and non-significant between replications (p>0.05) (Table 4.3.1).

The ranking according to the Duncan test showed that the provenances were divided into five homogenous groups (Figure 4.3.7). The best homogeneous group (with the highest volume per tree average values) included 13 provenances covering all groups except group G. The ranking of provenances for volume per tree does not correspond to that of the total height, but there were some consistencies. Specifically, in both rankings, the first three ranked provenances were the same (except that provenance 33 Zalau out-performed provenance 29 Ramnicu Sarat), and the same two local provenances (4 Vaslui and 6 Sascut) were amongst the lowest ranked.



#### Stem straightness

For this trait the average value was 1.68, indicating good steam straightness at the experimental level. The amplitude of variation varied between 1.31 and 2.25, with the provenance 30 Voinesti having the lowest average index (most straight stems), respectively 29 Ramnicu Sarat having the higher average index. ANOVA revealed only significant differences between provenances (p<0.05) (Table 4.3.1), which were confirmed by the Duncan test (Figure 4.3.8).

Brovonanco	Mean	Homogeneous groups for the transgression									
Frovenance	(index)		probabil	ity of 5%							
30	1.31	****									
7	1.33	****									
15	1.33	***									
1	1.36	***	****								
21	1.40	****	****								
10	1.42	****	****								
13	1.43	****	****								
3	1.43	****	****								
23	1.44	****	****								
24	1.50	****	****	****							
16	1.58	****	****	****							
11	1.62	****	****	****	****						
27	1.62	****	****	****	****						
8	1.62	****	****	****	****						
17	1.63	****	****	****	****						
28	1.65	****	****	****	****						
19	1.67	****	****	****	****						
9	1.75	****	****	****	****						
20	1.75	****	****	****	****						
18	1.78	****	****	****	****						
14	1.80	****	****	****	****						
5	1.83	****	****	****	****						
31	1.85	****	****	****	****						
6	1.86	****	****	****	****						
22	1.88	****	****	****	****						
4	1.90	****	****	****	****						
2	1.91	****	****	****	****						
25	1.95	****	****	****	****						
32	2.00		****	****	****						
33	2.14			****	****						
29	2.25				****						

Figure 4.3.8 Duncan test for the average stem straightness index (local provenances are highlighted).

The provenances were arranged in four overlapping homogenous groups, with most provenances being included in the most favourable group. Only provenances 32 Cluj, 33 Zalau and 29 Ramnicu Sarat were outside this group.

Despite statistically assured differences between provenances for most of the considered traits (except diameter at breast height and pruned height), the provenances stem form ranking was not consistent with that of total height (Figure 4.3.5) and volume per tree (Figure 4.3.7). A similar finding was reported by Fober (1998) in an experimental trial of Polish sessile oak and pedunculate oak provenances.



#### Trunk shape

In this comparative trial, the average value was 1.54, suggesting good trunk shape. Trees from provenance 24 Botosani had an ideal trunk shape (the average index was 1.00), while trees from provenance 29 Ramnicu Sarat had the worst trunk shape (the average index was 2.13).

Regarding this trait, distinctly significant differences were found for the provenance and group factors (p<0.01) (Table 4.3.1). According to Duncan's test, the mean trunk shape of all provenances was arranged into five homogenous groups (Figure 4.3.9).

Duction	Mean	Homogeneous groups for the transgression									
Provenance	(index)		pro	bability of	5%						
24	1.00	****									
7	1.11	****	****								
3	1.14	****	****								
23	1.22	****	****	****							
8	1.25	****	****	****							
2	1.27	****	****	****							
1	1.27	****	****	****							
6	1.29	****	****	****							
33	1.29	****	****	****							
5	1.33	****	****	****	****						
28	1.35	****	****	****	****						
11	1.38	****	****	****	****						
4	1.40	****	****	****	****						
21	1.40	****	****	****	****						
9	1.50	****	****	****	****	****					
22	1.50	****	****	****	****	****					
27	1.50	****	****	****	****	****					
25	1.55	****	****	****	****	****					
30	1.62	****	****	****	****	****					
10	1.67	****	****	****	****	****					
14	1.70		****	****	****	****					
16	1.75		****	****	****	****					
31	1.77		****	****	****	****					
15	1.78		****	****	****	****					
32	1.86			****	****	****					
17	1.88			****	****	****					
19	1.89			****	****	****					
20	1.92			****	****	****					
13	2.00				****	****					
18	2.00				****	****					
29	2.13					****					

Figure 4.3.9 Duncan test for the average trunk shape index (local provenances are highlighted).

All local provenances were characterized by very good trunk shapes being included in the most favourable homogenous group. Furthermore, among groups, group G (local provenances group) had the best average index for trunk shape, while the rest of the provenance groups recorded slightly higher average values with very small differences between them (Figure 4.3.10).







Figure 4.3.10 The trunk shape index variation among groups. The whiskers represent the standard deviation.

#### Branch insertion angle

The ANOVA results showed a statistically significant difference between the investigated provenances for the branch insertion angle (with p<0.05) (Table 4.3.1). Additionally, the Duncan test confirmed that there is a significant difference between the means of the provenances, resulting in three homogenous groups (Figure 4.3.11).

Drovonanco	Mean	Homogeneous	groups for the t	ransgression
Provenance	(index)	p	robability of 5%	
24	1.50	****		
9	1.42	****	* * * *	
18	1.33	****	****	****
20	1.33	****	****	****
21	1.30	****	****	****
14	1.30	****	****	****
13	1.29	****	****	****
22	1.25	****	****	****
7	1.22	****	****	****
2	1.18	****	****	****
1	1.18	****	****	****
3	1.14		****	****
8	1.13		****	****
17	1.13		****	****
28	1.12		****	****
15	1.11		****	****
19	1.11		****	****
4	1.10		* * * *	***
16	1.08		* * * *	***
10	1.08		****	****
11	1.08		* * * *	***
6	1.07		* * * *	***
25	1.05			***
33	1.00			***
32	1.00			****
31	1.00			****
27	1.00			****
5	1.00			****
23	1.00			****
30	1.00			****
29	1.00			****

Figure 4.3.11 Duncan test for the average trunk shape index (local provenances are highlighted).

Previous studies that investigated the branching angle in oak provenances (Hodžić & Ballian, 2020) pointed out that the most favourable branching angle range for better-quality individuals is between 67.5° and 90°. As such, in our case, trees scored with 2 have relatively good insertion angles, which means that an average index value of 2 is considered favourable. The highest percentage of trees with a branching angle of 60-90° were found for provenance 24 Botosani.



## Branches' diameter

The analysis of variance for this trait highlighted highly significant differences between provenances and group factors (p<0.001) (Table 4.3.1). Duncan's test (Figure 4.3.12) confirmed the existence of high variability between the provenances, these being separated into eight homogenous groups.

Provenance	Mean	Homogeneous groups for the transgression									
	(index)				probabil	ity of 5%					
30	2.8	****									
33	2.7	****	****	ale ale ale ale							
29	2.5	****	****	****	* * * *						
28	2.4	****	****	****	****	****					
32	2.3	****	****	****	****	****	****				
27	2.5	****	****	****	****	****	****				
31	2.2	****	****	****	****	****	****				
11	2.1		****	****	****	****	****	****			
14	2.0		****	****	****	****	****	****	****		
9	1.9			****	****	****	****	****	****		
19	1.9			****	****	****	****	****	****		
8	1.9			****	****	****	****	****	****		
24	1.9			****	****	****	****	****	****		
3	1.9			****	****	****	****	****	****		
2	1.8			****	****	****	****	****	****		
6	1.8			****	****	****	****	****	****		
18	1.8			****	****	****	****	****	****		
7	1.8			****	****	****	****	****	****		
13	1.7				****	****	****	****	****		
20	1.7				****	****	****	****	****		
5	1.7				****	****	****	****	****		
15	1.7				****	****	****	****	****		
10	1.6				****	****	****	****	****		
23	1.6					****	****	****	****		
16	1.5						****	****	****		
21	1.5						****	****	****		
22	1.4							****	****		
4	1.3								****		
1	1.3								****		
17	1.3								****		

Figure 4.3.12 Duncan test for the average branch diameter index (local provenances are highlighted).

Provenance 30 Voinesti obtained the highest average branch diameter index value, while provenances 17 Reghin, 1 Botosani and 4 Vaslui showed the lowest values of the branch diameter index.

For broadleaves species, and in particular for sessile oak, the branch's diameter size (measured close to the junction of the branch with the stem) is a major determinant of wood quality (Struck & Dohrenbusch 2000). As such, branches with smaller diameters are preferable because the self-pruning process occurs more rapidly (Kint et al. 2010). However, Harmer (1992) pointed out that the growth potential of trees should be considered when estimating the influence of branch diameter on wood quality, as vigorous trees may carry heavy stem branches that reduce the wood value, while less vigorous ones might have small branches but perform poorly. For provenances tested in the FAN comparative trial, it is observed that provenances with higher indices for branch diameter also have better growth performance.



## Correlation analysis between traits and geographical gradients of origin

The analysis of the experimental data allowed the highlighting of some significant statistical links between the studied characters, on the one hand, and the geographical gradients of the place of origin of the provenances, on the other hand, by determining and establishing the level of significance of the phenotypic correlations (Table 4.3.2).

Caracterul	S	D	Ht	He	v	Rt	Ft	Ur	Dr	Lat	Long	Le	Alt
S	-	-0.156**	-0.001	0.034	-0.136**	-0.079	-0.103	-0.034	-0.064	-0.160**	-0.248***	-0.154**	-0.033
D	-	-	0.662***	-0.084	0.967***	-0.045	0.278***	0.180***	0.577***	-0.055	-0.012	-0.013	0.032
Ht	-	-	-	0.174***	0.718***	-0.106	0.122*	0.060	0.459***	-0.065	-0.105	0.043	0.102
He	-	-	-	-	-0.055	-0.199***	-0.252***	0.008	-0.175**	-0.012	0.091	-0.052	-0.047
V	-	-	-	-	-	0.425***	0.271***	0.145**	0.573***	-0.065	-0.027	0.013	0.069
Rt	-	-	-	-	-	-	0.284***	0.049	0.131*	0.085	-0.004	0.053	-0.015
Ft	-	-	-	-	-	-	-	-0.008	0.232***	-0.135*	-0.069	0.006	0.120*
Ur	-	-	-	-	-	-	-	-	0.044	-0.028	0.103	-0.172**	-0.164**
Dr	-	-	-	-	-	-	-	-	-	-0.103	-0.179***	0.075	0.169**

Table 4.3.2 Phenotypic correlations between traits in the FAN comparative trial

S – survival; D – breast height diameter; H – total height: He – pruned height; V – volume per tree; Rt – stem straightness; Ft – trunk shape; Ur – Branch insertion angle; Dr – branch diameter; Lat – latitude; Long – Longitude; Le – ecophysiological latitude; Alt – altitude.

The level of significance: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001.

Correlation between survival and the geographical gradients of seed stand origins showed that survival had no significant correlation with altitude, but there were found small influences of the other geographical gradients. Specifically, the correlations between survival and latitude and ecophysiological latitude were distinctly significant and negative ( $r=-0.160^{**}$ , respectively  $r=-0.154^{**}$ ) and highly significant and negative ( $r=-0.248^{***}$ ). Thus, in the Fantanele comparative trial western and southern provenances (from lower latitudes and longitudes) are better adapted.

The diameter at breast height was highly significant and positively correlated with tree height, volume per tree, trunk shape, branch insertion angle, and branch diameter. Therefore, the provenances with larger diameters at breast height had superior heights, and relatively good branch architecture (higher index values) but with inferior trunk shapes (higher trunk shape indices).

Total height was directly and highly significantly correlated with pruned height, volume per tree and branches diameter and significant with trunk shape, which indicates that the provenances that register the highest heights have the stem pruned over a greater length, as well as trunks with lower qualities and greater branch thicknesses (indices with higher values).

The volume per tree was positively and highly significantly correlated with all form traits, which denotes that the trees with higher volumes had good branch architecture and inferior stem and trunk shapes.

Steam straightness and trunk shape were directly and highly correlated, and both were positively correlated with branch diameter, meaning that straight stems and trunks had thicker branches. In addition, trunk shape was indirectly and significantly correlated with latitude ( $r = -0.135^*$ ) and positively and significantly with altitude ( $r = 0.120^*$ ), meaning that southern and lower altitude provenances had straighter trunks.

Among the geographical gradients of seed stand origins, the altitude had a small influence on the branches' architecture. In this sense, provenances from inferior altitudes had sharper insertion angles and thinner branches. Also, a weak and negative correlation was observed between longitude and branch diameter, meaning that western provenances had thicker branches.



#### 4.4 Phenotypic variability of the leaves

#### 4.4.1 Intrapopulational and interpopulational morphological variability of sessile oak

The assessment of intrapopulation morphological variability for the four populations of the oak species and for the FAN comparative trial was conducted by identifying infraspecific taxonomic units (subspecies, forms, subforms, varieties) based on leaf characteristics, according to their description presented in the specialized literature.

Among the 227 trees evaluated, 164 individuals showed phenotypic characteristics specific to the ssp. *petraea*, 54 specific to the ssp. dalechampii and 9 specific to the ssp. *polycarpa* (Table 4.4.1.1). In the case of ssp. *petraea* and ssp. *dalechampii*, all the infraspecific units described in the literature were found, instead, for ssp. *polycarpa* only individuals corresponding to var. *typica* (Figure 4.4.1.1).

Table 4.4.1.1 Infraspecific units identified for	r the evaluated oak	trees in the four	populations and in the
FAN comparative trial.			

		Q. J	o. ssp.	petrae	a.	sa	<i>Q. p.</i> ssp.	. dalechampii		Q. p. ssp. polycarpa		
	f. platy	phylla	f. lad	ciniata	f. longifolia	l etra			o. ssp mpii		o. ssp rpa	Ļ
Population	subf. <i>normalis</i>	subf. angulata	subf. Iobulosa	subf. <i>pinnata</i>	subf. angustifolia	Tota <i>Q. p.</i> ssp. <i>p</i>	f. lancifolia	f. pinnatifida	Total <i>Q.  </i> dalechai	var. <i>typica</i>	Total <i>Q. J</i> polycar	тота
FUN	2	0	2	5	1	10	4	14	18	6	6	34
OLT	10	0	6	9	0	25	6	17	23	2	2	50
SAT	13	0	9	15	1	38	0	11	11	1	1	50
HEL	13	0	12	13	3	41	0	2	2	0	0	43
Comparativ	e trial											
FAN	5	1	7	34	3	50	0	0	0	0	0	50
Total	43	1	36	76	8	164	10	44	54	9	9	227

Making a comparative analysis at the level of the intraspecific taxonomic structure of the sessile oak among the four populations, it can be observed that the peripheral populations FUN, OLT, and SAT exhibit higher intraspecific variability compared to the HEL population. Additionally, in the peripheral populations, especially in FUN and OLT, there is a higher proportion of the subspecies *dalechampii*. This fact may result from the action of natural selection, which has favored the subspecies *dalechampii* due to its adaptation to local xerophytic conditions, which are more pronounced in the peripheral populations (Martonne aridity indices < 28.0) compared to the reference population HEL (Martonne aridity index = 34.1).

At the level of peripheral populations, it is observed that the FUN population contains the highest number of infraspecific units (approximately 77%), with 7 out of the 9 units recorded in the specialized literature being present here. The OLT and SAT populations have an equal proportion of infraspecific units (66.6% each), but they differ in the fact that in the OLT population, there are no specimens corresponding to *Q. p.* ssp. *petraea* f. *longifolia* subf. *angustifolia*, whereas specimens of *Q. p.* ssp. *dalechampii* f. *lancifolia* are present. On the other hand, in the SAT population, the situation is reversed.

The HEL population and the FAN comparative trial have the same number of infraspecific units (55% of the total number of units described in the literature). In both cases, *Q. p.* ssp. *dalechampii* f. *lancifolia* and *Q. p.* ssp. *polycarpa* var. *typica* were absent. Furthermore, specific to the FAN comparative trial is the fact that we only find specimens related to *Q. p.* ssp. *petraea*, as well as the fact that here was identified the only specimen assimilated to f. *platyphylla* subf. angulata.





Figure 4.4.1.1 Intraspecific units identified for Q. petraea sensu lato

4.4.2 Intrapopulational and interpopulational variability of macromorphological and micromorphological foliar descriptors

## 4.4.2.1 Phenotypic variability evaluation based on foliar macromorphological descriptors

## > Dimensional variables (measurable)

At the subspecies level, *Q. p.* ssp. *petraea* and *Q. p.* ssp. *dalechampii* had relatively similar mean LA values (about 50 cm<sup>2</sup>), but higher compared to *Q. p.* ssp. *polycarpa* (about 45 cm<sup>2</sup>) (Figure 4.4.2.1.1). In addition, the mean LA values are considerably higher than the mean value (33.3 cm<sup>2</sup>) reported by Fortini et al. (2015) for *Q. petraea*.

As with LA, values for LP were obviously lower in *Q. p.* ssp. *polycarpa* (38.5 cm) (Figure 4.4.2.1.1), but between *Q. p.* ssp. *petraea* and *Q. p.* ssp. *dalechampii*, the latter has a mean value (47.7 cm) 1.7 cm higher.

Regarding the lamina length (LL), *Q. p.* ssp. *petraea* and *Q. p.* ssp. *dalechampii* showed average values of 11.5 cm and 11.2 cm, respectively, and *Q. p.* ssp. *polycarpa* of 9.9 cm (Figure 4.4.2.1.1). For *Q. petraea* similar studies found lower values, respectively 9.5-10.4 (Bruschi et al., 2000; Boratynski et al., 2008; Fortini et al., 2015). However, the dimensions of the lamina are influenced by the degree of shading, thus Ponton et al. (2004) obtained for sessile oak average lengths of 8.8 cm for leaves fully exposed to light. It should be noted that the average value obtained for *Q. p.* ssp. *dalechampii* is obviously higher than the values identified in similar studies for *Q. pubescens*, a species that some studies consider to be closer morphologically (Camus, 1934-1954; Brullo et al., 1999; Di Pietro et al., 2012). Thus, in native stands the average length of the lamina for *Q. pubescens* is around 8.0-8.4 cm (Sofletea et al., 2011; Enescu et al., 2013), while foreign studies report values between 6.9-9.4 cm (Bruschi et al., 2000; Di Pietro et al., 2016), in some situations even below 6 cm (Franjić et al. al., 2006).



From the point of view of the descriptor PL (petiole length), the average values for the three sessile oak subspecies are approximately equal, reaching 1.7-1.8 cm, with individual average values between 0.9 – 2.8 cm (Figure 4.4.2.1.1). The average value is close to those found for sessile oak in Italy where the average size of the petiole was 1.5 cm (Fortini et al., 2015), respectively 1.43-1.79 cm (Bruschi et al., 2003), and France (Dupouey & Badeau, 1993), where a size of 1.6 cm was recorded. For sessile oak stands in the northern part of Europe, Kremer et al. (2002) reported a much lower average value of PL (of 1.13 cm), while for stands in the central, southern and western parts of Europe, PL reaches 1.52 cm.

The LW parameter (lamina width) had identical average values for *Q. p.* ssp. *petraea* and *Q. p.* ssp. *dalechampii*, more precisely both subspecies presented an average leaf width of 3.6 cm (Figure 4.4.2.1.1). Also, the average value of LW for *Q. p.* ssp. *polycarpa* is very close, reaching the value of 3.4 cm. These values are lower than those found for sessile oak in Italy, where the maximum lamina width was 5.89 cm (Bruschi et al., 2000). Also in Italy, for the same parameter, Fortini et al. (2015) identified a lower value of 2.84 cm.

Analyzing the mean values for the SW descriptor (sinus width) it is observed that *Q. p.* ssp. *petraea* and *Q. p.* ssp. *polycarpa* have sinus widths of 2.0 cm, while *Q. p.* ssp. *dalechampii* has slightly deeper sinuses (1.8 cm) (Figure 4.4.2.1.1). When using the same measurement method for SW, Fortini et al. (2015) obtained a lower average value of 1.33 cm in sessile oak, as did Yücedağ and Gailing (2013) who found values between 1.13 - 1.36. On the other hand, also in sessile oak, Bruschi et al. (2000) obtained a value close to those in the present case, namely 1.7 cm. Comparing the mean values of sinus width with those of maximum lamina width for the three sessile oak subspecies, it can be seen that SW is approximately twice as small as LW.

At the WP descriptor level (the length of the lamina from the base to the maximum width area), the average values recorded are significantly higher for *Q. p.* ssp. *petraea* compared to the other two sessile oaks (Figure 4.4.2.1.1). This aspect was expected, since the common sessile oak is distinguished from the other two subspecies by the obovate shape of the leaves. Thus, for *Q. p.* ssp. *dalechampii* and *Q. p.* ssp. *polycarpa*, the average value of WP is below ½ of the average value of LL, and for *Q. p.* ssp. *petraea* the WP exceeds ½ of the average value of LL, which means that ssp. *dalechampii* and ssp. *polycarpa* have maximum widths in the lower half of the lamina, while ssp. *petraea* in the upper half. These aspects are, moreover, characteristic for the differentiation of sessile oak subspecies, being also recorded in the literature (Georgescu & Morariu, 1948; Beldie, 1952; Şofletea & Curtu, 2007; Clinovschi, 2005).



Figure 4.4.2.1.1 Boxplot diagram for the dimensional variables of the leaves assessed in sessile oak (Qpe - *Q. p. ssp. petraea*; Qda - *Q. p. ssp. dalechampii*; Qpo - *Q. p. ssp. polycarpa*). The bottom and top of the boxes indicate the 25% and 75% quartiles, and the line in the inner part indicates the median. The whiskers represent the minimum and maximum values of the evaluated descriptor. The symbol 'x' indicates the average value. Values outside the distribution (outliers) are represented by the symbol 'o'.

#### > Observable and countable variables

Regarding the degree of pubescence on the abaxial surface of the leaf (AB PU descriptor), *Q. p. ssp. petraea* has an average value of 2.7, being lower than the other two subspecies, which exceed the 3 on the evaluation scale, namely 3.1 for *Q. p.* ssp. *dalechampii*, respectively 3.3 for *Q. p.* ssp. *polycarpa* (Figure 4.4.2.1.2). The higher pubescence in the case of *Q. p.* ssp. *dalechampii* and *Q. p.* ssp. *polycarpa* can be explained by the more xerophytic and thermophilic character of the two subspecies (Constantinescu, 1973), this being an important adaptive trait in more arid climatic conditions (Morales



et al., 2002). These values are higher compared to the one found in sessile oak in another study, namely Kremer et al. (2002) reported a value of 1.4 for AB PU.

The degree of pubescence on the adaxial surface (AD PU) is significantly lower compared to AB PU. Thus, for all three sessile oak subspecies, the average AD PU values were between 1.4 for *Q. p. ssp. petraea* and *Q. p. ssp. dalechampii*, and 1.6 for *Q. p. ssp. polycarpa* (Figure 4.4.2.1.2). Most morphological studies in sessile oak have only evaluated pubescence on the lower face of the lamina (Dupouey & Badeau, 1993; Bruschi et al., 2000, 2003; Kremer et al., 2002, Fortini et al., 2015), because the upper part is considered apparently glabrous (Gellini et al., 1992), although some studies indicate the existence of a low degree of pubescence (Milletti et al., 1982).

Moreover, as with AD PU, petiole pubescence (PE PU) is a descriptor less frequently evaluated in taxonomic studies in oaks, but which has been shown to be useful in certain situations (Fortini et al., 2015). In the present case, a higher degree of pubescence of the petiole is observed for the individuals of *Q. p.* ssp. *dalechampii* (of 2.1), followed by those attributed to *Q. p.* ssp. *polycarpa* (of 1.9) and *Q. p.* ssp. *petraea* (of 1.8) (Figure 4.4.2.1.2).

The base shape of the lamina (BSL) is frequently considered a diagnostic element in the identification of species of the genus *Quercus* (Beldie 1952; Schartz 1993; Christensen, 1997). Thus, at the subspecies level, the basal shape of the lamina is wide-cuneate or subcordate with an average value of 2.9 for *Q. p.* ssp. *petraea*, 3.1 for *Q. p.* ssp. *dalechampii* and 3.4 for *Q. p.* ssp. *polycarpa* (Figure 4.4.2.1.2). In a study for sessile oak, the mean BSL value was 2.0 (Fortini et al., 2015).

Regarding the number of lobes (NL), it had average values of 13.6 for *Q. p.* ssp. *petraea*, 13.4 for *Q. p.* ssp. *dalechampii* and 11.1 for *Q. p.* ssp. *polycarpa* (Figure 4.4.2.1.2), values that fall within the limits specified in the literature (Beldie, 1952; Şofletea & Curtu). Analyzing this descriptor, Fortini et al. (2015) obtained an average number of lobes of 12.39, thus an intermediate value compared to the present study. In contrast, a close value was reported by Dupouey and Badeau (1993), with NL being 13.2. Close values of the number of lobes were identified by Yücedağ and Gailing (2013) in sessile oak stands from Turkey, where the values of this descriptor varied between 12.3 - 14.1.

The number of intercalary veins (NV) is very low for all three subspecies. However, leaves of *Q. p.* ssp. *dalechampii* tend to have a higher number of NV (on average 0.4) compared to *Q. p.* ssp. *polycarpa* (on average 0.2) and *Q. p.* ssp. *petraea* (on average 0.1). The values obtained are much lower than those found by Fortini et al. (2015) (of 2.86) or Yücedağ and Gailing (2013) (of 0.8 - 1.5).



Figure 4.4.2.1.2 Boxplot diagram for observable and countable leaf variables assessed in sessile oak (Qpe - *Q. p. ssp. petraea*; Qda - *Q. p. ssp. dalechampii*; Qpo - *Q. p. ssp. polycarpa*). The bottom and top of the boxes indicate the 25% and 75% quartiles, and the line in the inner part indicates the median. The whiskers represent the minimum and maximum values of the evaluated descriptor. The symbol 'x' indicates the average value. Values outside the distribution (outliers) are represented by the symbol 'o'.



## > Transformed (calculated) variables

Regarding the lamina shape (OB), for *Q. p.* ssp. *petraea* this descriptor has an average value of 56%, while for *Q. p.* ssp. *dalechampii* the average value is around 50%, and for *Q. p.* ssp. *polycarpa* below 50% (Figure 4.4.2.1.3). Therefore, it can be said that for *Q. p.* ssp. *petraea* the leaves predominantly denote an obovate shape, and for the other two subspecies an elliptic-ovate shape. In the study by Fortini et al. (2015), this descriptor had a value of 49.2, being closer to that found here for *Q. p.* ssp. *dalechampii* and *Q. p.* ssp. *polycarpa*.

For petiole ratio (PR), *Q. p.* ssp. *polycarpa* recorded an average value of 15.2, while for the other two subspecies an average PR value of 13.2 was obtained (Figure 4.4.2.1.3). These values are higher compared to the study by Fortini et al. (2015), where PR had an average value of 12.69.

The lobe depth ratio (LDR) had an average value of 40.5 for *Q. p.* ssp. *polycarpa*, 44.8 for *Q. p.* ssp. *petraea* and 48.9 for *Q. p.* ssp. *dalechampii* (Figure 4.4.2.1.3). According to these values, it is found that the leaves are generally pinnate-lobed to pinnate-fidate. In the study by Fortini et al. (2013) the lobe depth ratio was higher, 52.4%.

The lobe width ratio (LWR) recorded values between 31.6 for *Q. p.* ssp. *dalechampii* and 34.5 for *Q. p.* ssp. *polycarpa* (Figure 4.4.2.1.3), higher values than that reported by Fortini et al. (2015), of 27.47.



Figure 4.4.2.1.3 Boxplot diagram for the transformed variables of the leaves asseased in sessile oak (Qpe - *Q. p. ssp. petraea*; Qda - *Q. p. ssp. dalechampii*; Qpo - *Q. p. ssp. polycarpa*). The bottom and top of the boxes indicate the 25% and 75% quartiles, and the line in the inner part indicates the median. The whiskers represent the minimum and maximum values of the evaluated descriptor. The symbol 'x' indicates the average value. Values outside the distribution (outliers) are represented by the symbol 'o'.

#### 4.4.2.2 Phenotypic variability evaluation based on foliar micromorphological descriptors

At the level of the three subspecies, the highest average density of stomata was 317 and corresponds to *Q. p.* ssp. *dalechampii*, and the lowest was 306 in the case of *Q. p.* ssp. *petraea* (Figure 4.4. 2.2.1). These average values are close to those reported in sessile oak in a recent study carried out in northern Turkey (Yücedağ et al., 2019), where a number of 333 stomata·mm<sup>-2</sup> were found, and by Bruschi et al. (2000) in stands in northern and central Italy, where they found 335 stomata·mm<sup>-2</sup>. However, in a later more complex study, Bruschi et al. (2003) analyzed both unexposed and light-exposed leaves, and found mean StoD values ranging from 329 - 498.

In terms of stomatal dimensions, the differences between the three sessile oak subspecies were reduced. Thus, for the SRL descriptor (stomata rim length) the average values were similar (approximately 23  $\mu$ m) (Figure 4.4.2.2.1). Small differences were obtained for stomata rim width (SRW), the average values being slightly higher for *Q. p.* ssp. *dalechampii* (of 17.3  $\mu$ m) compared to the other two subspecies (*Q. p.* ssp. *petraea* – 16, 8  $\mu$ m, respectively *Q. p.* ssp. *polycarpa* – 16.9  $\mu$ m). On average, the stomata are longer and wider than in the sessile oak stands analyzed by Fortini et al. (2015), where the stomata were 13.1  $\mu$ m long and 11.29  $\mu$ m wide, respectively. In contrast, the dimensions are similar to those reported by Şofletea et al. (2001) in the sessile oak stands from Felmer (22.4  $\mu$ m length, 15.2  $\mu$ m width, respectively).



For *Q. p.* ssp. *petraea* the FSR was 3.5  $\mu$ m, for *Q. p.* ssp. *dalechampii* 3.6  $\mu$ m, and for *Q. p.* ssp. *polycarpa* 3.9  $\mu$ m (Figure 4.4.2.2 .1). For the same descriptor, Bruschi et al. (2000) mention an almost double value of 7.13  $\mu$ m, and Fortini et al. (2009), of 4.8  $\mu$ m.

The mean values for SRL among the three sessile oak subsections were similar, and the StoD was slightly higher for *Q. p.* ssp. *dalechampii*, and the mean value of SAI was slightly higher in its case, respectively of 7313 (Figure 4.4 .2.2.1). The other two subspecies having closer values of the density and length of the stomata, and the SAI values were close, for *Q. p.* ssp. *polycarpa* SAI being 7103, and for *Q. p.* ssp. *petraea* 7030 (Figure 4.4.2.2.1). Micromorphological studies for sessile oak reported average values of SAI above those found in the present study, being between 7828-11343 (Bruschi et al., 2003).

All three subspecies show a relatively abundant and close number of glandular trichomes (NGT). *Q. p.* ssp. *dalechampii* has an average number of 65.2, *Q. p.* ssp. *petraea* of 61.9, and *Q. p.* ssp. *polycarpa* of 60.9 (Figure 4.4.2.2.1). For sessile oak Fortini et al. (2015) reported a mean value of 60.37, while Bruschi et al. (2003) found mean values ranging from 56-125.

As in the case of NGT, also NST (number of stellate trichomes) showed close values between the three subspecies. Specifically, *Q. p.* ssp. *petraea* had a value of 11.4, *Q. p.* ssp. *dalechampii* of 12.0, and *Q. p.* ssp. *polycarpa* of 13.0 (Figure 4.4.2.2.1).

Regarding the length of the rays of stellate trichomes (LRS), the average values are around 100  $\mu$ m, an aspect also confirmed by other studies in sessile oak (Gellini et al., 1992; Bruschi et al., 2003). Moreover, LRS and NGT proved to be descriptors with high discriminating power between *Q. petraea* and *Q. pubescens*, in the latter species LSR having values above 300  $\mu$ m, and NTG being significantly lower (Bruschi et al., 2000). *Q. p.* ssp. *dalechampii* and *Q. p.* ssp. *petraea* presented the longest stellate trichomes, of 101.1  $\mu$ m and 99.0  $\mu$ m, respectively), and in *Q. p.* ssp. *polycarpa* they measured on average only 91.8  $\mu$ m (Figure 4.4.2.2.1).

Analyzing the average values of the number of rays of stellate trichomes (NR) it is observed that in *Q. p.* ssp. *petraea* and *Q. p.* ssp. *dalechampii* stellate-bifurcated trichomes tend to predominate for these NR having a value of 2.7 (Figure 4.4.2.2.1). In *Q. p.* ssp. *polycarpa* the proportion of stellate and stellate-bifurcated trichomes was equal, NR having an average value of 3.0. Bruschi et al. (2000) found for NR an average value of 3.33.



Figure 4.4.2.2.1 Boxplot diagram for the micromorphological variables of the leaves assessed in sessile oak (Qpe - *Q. p. ssp. petraea*; Qda - *Q. p. ssp. dalechampii*; Qpo - *Q. p. ssp. polycarpa*). The bottom and top of the boxes indicate the 25% and 75% quartiles, and the line in the inner part indicates the median. The whiskers represent the minimum and maximum values of the evaluated descriptor. The symbol 'x' indicates the average value. Values outside the distribution (outliers) are represented by the symbol 'o'.



# 4.4.3 Assessment of macromorphological and micromorphological variability using multivariate statistical analyses

## Analiza varianței (ANOVA)

Among the three sessile oak subspecies, the descriptors that significantly contributed to their differentiation were leaf perimeter (LP), lamina length (LL), WP (length of lamina at largest width), abaxial laminar pubescence (AB PU), petiole pubescence (PE PU), NL (lobe number), BSL (basal shape of the lamina), number of intercalary veins (NV), leaf shape (OB), lobe depth ratio (LDR), percentage venation (PV), lobe width ratio (LWR) and length of rays of stellate trichomes (LRS) (Table 4.4.3.1). If we refer to the average values of the three subspecies for the descriptors that determined differences, it is observed that the description matches that of the literature, respectively: the subspecies *dalechampii* and *polycarpa* compared to the subspecies petraea have smaller lamina lengths, the maximum width in the half lower part of the lamina, a lower number of lobes, more ovate shapes, with a subcordate leaf base. In addition, for the subspecies *dalechampii* and *polycarpa*, a higher tendency to present intercalary veins was observed.

Variabla*		Q. p	. ssp. pet	raea			Q. p. ss	p. dalech	nampii		Q. p. ssp. polycarpa						
Variable	Med	Min	Max	SD	CV%	Med	Min	Max	SD	CV%	Med	Min	Max	SD	CV%	Lweich	ρ
LA	50.2	31.5	81.8	10.0	19.9	49.9	30.7	68.7	9.1	18.2	45.4	35.0	57.2	7.5	16.5	1.67	0.210
LP	46.0	33.7	61.1	6.2	13.4	47.7	34.3	60.2	5.5	11.6	38.5	35.6	42.4	3.0	7.8	29.01	0.000
LL	11.4	9.0	14.8	1.1	9.9	11.2	9.2	13.0	0.9	8.4	9.9	8.6	10.8	0.8	7.6	51.19	0.000
PL	1.7	0.9	2.8	0.3	19.6	1.7	1.0	2.7	0.3	18.1	1.8	1.0	2.6	0.5	28.3	0.63	0.541
LW	3.6	2.7	4.8	0.4	12.2	3.6	2.7	4.3	0.3	9.7	3.4	2.9	3.8	0.3	9.8	1.39	0.270
SW	2.0	1.2	3.3	0.3	16.3	1.8	1.0	2.7	0.4	22.0	2.0	1.6	2.4	0.3	13.7	3.22	0.060
WP	6.4	4.4	9.5	0.8	13.2	5.6	3.7	6.7	0.7	11.6	4.9	3.0	6.2	0.9	19.1	10.22	0.000
AB PU	2.7	1.7	4.0	0.6	21.0	3.1	2.0	4.0	0.6	20.5	3.3	2.8	4.0	0.4	13.5	11.87	0.000
AD PU	1.4	1.0	2.2	0.4	27.4	1.4	1.0	2.2	0.4	30.9	1.6	1.0	2.8	0.6	38.9	1.07	0.361
PE PU	1.8	1.0	3.2	0.5	26.1	2.1	1.0	3.6	0.5	22.6	1.9	1.2	3.0	0.7	36.5	6.10	0.008
NL	13.6	10.8	17.3	1.4	9.9	13.2	9.3	15.3	1.3	9.5	11.1	8.8	13.6	1.3	12.1	16.65	0.000
BSL	2.9	2.2	3.9	0.2	8.5	3.1	2.0	4.0	0.4	12.7	3.4	2.7	3.8	0.4	10.8	12.11	0.000
NV	0.1	0.0	1.8	0.3	242.7	0.4	0.0	2.5	0.6	156.1	0.1	0.0	0.6	0.2	143.2	4.55	0.005
OB	56.1	37.4	65.4	4.6	8.2	50.7	31.6	60.0	5.6	11.0	49.8	28.3	57.9	8.9	17.9	21.96	0.000
PR	13.2	8.3	21.8	2.1	16.0	13.2	7.4	19.0	2.2	16.8	15.2	9.5	19.4	3.2	20.9	1.70	0.165
LDR	44.8	7.0	66.0	8.9	20.0	49.0	19.0	71.0	11.0	22.5	40.4	29.0	50.0	7.8	19.3	4.92	0.001
PV	0.9	0.0	14.4	2.3	246.9	3.1	0.0	23.1	5.2	166.4	1.2	0.0	4.4	1.6	134.4	4.40	0.002
LWR	31.6	23.3	38.8	2.7	8.4	32.3	26.2	39.9	3.0	9.1	34.5	31.5	36.8	2.0	5.7	9.49	0.000
StoD	305.7	220.0	492.0	44.7	14.6	316.8	205.0	524.0	71.5	22.6	310.4	226.0	437.0	77.6	25.0	0.57	0.830
SRW	16.8	10.9	21.1	1.5	8.7	17.3	14.5	19.7	1.2	6.9	16.9	13.3	18.8	1.6	9.3	2.11	0.061
SRL	23.1	15.1	28.2	1.9	8.2	23.3	18.2	30.0	1.9	8.0	23.3	20.6	27.2	2.1	8.9	0.30	0.987
FSR	3.5	2.1	5.9	0.6	16.3	3.6	2.6	4.6	0.4	12.1	3.9	3.1	4.9	0.6	14.4	1.74	0.128
SAI	7030.4	4485.0	11119.0	1068.9	15.2	7313.3	5027.0	10742.0	1449.7	19.8	7168.9	5243.0	9177.0	1516.6	21.2	0.86	0.627
LRS	99.0	62.4	135.6	12.0	12.1	101.1	73.8	123.5	11.8	11.7	91.8	75.3	107.8	10.9	11.9	2.68	0.019
NGT	61.9	17.0	133.0	21.1	34.0	65.2	25.0	98.0	19.8	30.4	60.1	25.0	108.0	27.9	46.4	0.55	0.882
NST	11.4	3.0	25.0	5.5	48.1	12.0	3.0	28.0	5.9	49.3	13.0	8.0	17.0	4.7	36.5	0.578	0.875
NR	2.7	1.5	4.0	0.7	27.5	2.7	2.0	4.0	0.7	25.3	3.0	2.0	4.0	0.8	25.5	0.71	0.767

Table 4.4.3.1 ANOVA analysis between the three sessile oak subspecies

\*The variable codes are presented in tables 3.2.4.1 and 3.2.4.2. Bold variables show significant differences (p<0.05).

#### Principal components analysis

The variable LA (leaf area) is redundant and was removed from the PCA analysis, having very close correlations with some dimensional variables. Therefore, from the PCA graph for the 26 micro- and macromorphological variables (Figure 4.4.3.1), an overlap of the three subspecies is found. However, *Q. p.* ssp. *polycarpa* was better associated with the evaluated variables, having negative values on factor 1.



This tendency is due to the lower values of the dimensional variables LP, LL, LW and WP, which have a high and positive contribution for factor 1 (Figure 4.4.3.2).

Subsequently, for the PCA analysis, only the variables for which it was previously demonstrated, by means of ANOVA, that present significant differences between the three subspecies were considered. In this case, the two main components accounted for 24.58% (component 1) and 19.20% (component 2) of the total variance (Figure 4.4.3.3). Even in this case, a clear separation of the subspecies was not achieved. The most discriminating variables identified along component 1 were LL (lamina length) and WP (length of lamina at largest width), which were lower for *Q. p.* ssp. *polycarpa*, while the presence of intercalary veins (NV) and the percentage of venation (NV) were discriminating traits for *Q. p.* ssp. *dalechampii* individuals at the level of component 2. At the same time, the variables AB PU (abaxial laminar pubescence) and BSL (basal shape of lamina) had almost zero contribution for factor 2, but higher for factor 1 (Figure 4.4.3.4).



Figure 4.4.3.1 PCA plot with the full set of variables included in the analysis



Figure 4.4.3.2 Contribution of variables to the first two factors



Figure 4.4.3.4 Contribution of variables to the first two factors

Figure 4.4.3.3 PCA graph including the set formed by the significant variables of the ANOVA analysis (Qpe -*Q. p. ssp. petraea*; Qda - *Q. p.* ssp. *dalechampii*; Qpo -*Q. p.* ssp. *polycarpa*)

To verify whether there is indeed a grouping of subspecies, a PCA analysis was conducted by including only the variables that generated significant differences within the discriminant analysis. Thus, in the PCA based on the 11 significant variables obtained through the DA analysis, the two principal components accounted for 42.18% of the total variance, with 23.78% for component 1 and 18.40% for component 2 (Figure 4.4.3.5). A relatively continuous distribution pattern of individuals along PCA component 2 was identified, with the variable LDR making a significant contribution (41.4%) (Figure 4.4.3.6). However, individuals of *Q. p.* ssp. *polycarpa* exhibited negative values on factor 1.







Figure 4.4.3.5 PCA graph including the set formed by the significant variables of the discriminant analysis (Qpe - *Q. p.* ssp. *petraea*; Qda - *Q. p.* ssp.

dalechampii; Qpo - Q. p. ssp. polycarpa)

Figure 4.4.3.6 Contribution of variables to the first two factors

In a recent study carried out in Italy (Proietti et al., 2021) the discrimination between *Q. petraea*, *Q. pubsescens* and *Q. dalechampii* was attempted by means of multivariate analysis methods (including PCA). The PCA results showed that *Q. petraea* and *Q. dalechampii* overlap almost completely, so according to the features of the leaves and stems, individuals of *Q. dalechampii* are morphologically close to *Q. petraea*, the differences between the two being considered insignificant.

## > Cluster analysis

Cluster analysis was applied both for the entire set of trees and at the level of the populations and the FAN comparative trial.

Initially, a cluster analysis was conducted using the entire set of macro- and micromorphological variables (Figure 4.4.3.7). The analysis revealed the differentiation of two main subclusters, each further divided into two other subclusters. Theoretically, three subclusters corresponding to the three subspecies were expected, but the division was not evident at all. As a result, the analysis was reperformed, this time using only the variables that showed significance according to the ANOVA (Figure 4.4.3.8) and DA (Figure 4.4.3.9) analyses. However, neither classification demonstrated a clear separation of the three sessile oak subspecies; instead, it produced a significantly larger number of lower subclusters.

When the analysis was reperformed for the entire set of variables, the similarities and differences between the four populations and the FAN comparative trail were observed, leading to their separation into two main subclusters. One subcluster comprised the peripheral populations FUN and SAT, while the second subcluster consisted of the FAN comparative trial, the peripheral population OLT, and the reference population HEL (Figure 4.4.3.10). The closer grouping of the FAN comparative trial with the OLT population could be a consequence of their similar values in terms of dimensional variables. Additionally, the differences between the two main subclusters are accentuated by a large Euclidean distance.

In the case where only the variables that generated significant differences were retained, two subclusters were also obtained. However, this time, one subcluster was represented by the FAN comparative trial, the reference population HEL, and the peripheral population FUN, while the second subcluster consisted of the OLT and SAT populations (Figure 4.4.3.11). Moreover, this time the Euclidean distance was considerably shorter.





Figure 4.4.3.7 Cluster dendrogram based on the entire set of variables. The horizontal axis represents the evaluated trees (Qpe - Q. p. ssp. petraea represented in black; Qda - Q. p. ssp. dalechampii represented in green; Qpo - Q. p. ssp. polycarpa represented in red), and the vertical axis the Euclidean distance.





Figure 4.4.3.8 Cluster dendrogram based on significant variables generated by ANOVA analysis. The horizontal axis represents the evaluated trees (Qpe - Q. p. ssp. *petraea* represented in black; Qda - Q. p. ssp. *dalechampii* represented in green; Qpo - Q. p. ssp. *polycarpa* represented in red), and the vertical axis the Euclidean distance.





Figure 4.4.3.9 Cluster dendrogram based on significant variables generated by DA analysis. The horizontal axis represents the evaluated trees (Qpe - Q. p. ssp. petraea represented in black; Qda - Q. p. ssp. dalechampii represented in green; Qpo - Q. p. ssp. polycarpa represented in red), and the vertical axis the Euclidean distance.



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Figure 4.4.3.10 Cluster dendrogram based on the entire set of variables. The horizontal axis represents the Euclidean distance and the vertical axis represents the analyzed populations and the FAN comparative trial.



Figure 4.4.3.11 Cluster dendrogram based on significant variables generated by the ANOVA analysis. The horizontal axis represents the Euclidean distance and the vertical axis represents the analyzed populations and the FAN comparative trial.

## > Discriminant analysis

For the discriminant analysis (DA), the variable LA (leaf area) was excluded due to its high correlation with other dimensional variables.

The variables that significantly contributed (p<0.05) to differentiate the three subspecies of sessile oaks were LP (leaf perimeter), WP (length of lamina at largest width), BSL (basal shape of lamina), NL (number of lobes), LW (leaf width), AB PU (abaxial laminar pubescence), PV (percentage venation), SW (sinus width), and LDR (lobe depth ratio). Thus, by applying the analysis with only these 11 variables that showed the highest discriminatory power among the three subspecies, the two discriminant functions were established:

Function 1: -759,5 - 95,8·WP + 19,3·LP + 115,8·BSL - 33,0·NL - 501,4·LW - 68,8·PL + 50,7·ABPU - 0,8·LRS + 7,2·PV + 709,6·SW + 22,4·LDR

Function 2: 1127,5 - 0,2·WP - 19,0·LP + 112,0·BSL - 26,6·NL + 465,6·LW – 104,7·PL - 14,7·ABPU - 2,8·LRS - 9,9·PV - 500,4·SW - 17,9·LDR

The first function generates the minus sign (-) for *Q. p.* ssp. *petraea* and plus (+) for *Q. p.* ssp. *dalechampii*, respectively *Q. p.* ssp. *polycarpa*, and the second function generates the minus sign (-) for *Q. p.* ssp. *dalechampii*, respectively plus (+) for the other two subspecies.

In addition, the two classification functions obtained for each subspecies can be used to determine for each case to which subspecies it most likely belongs. In the present case, only 84.6% of the cases were correctly classified (Table 4.4.3.2). The high percentage of incorrectly classified specimens in the case of *Q. p.* ssp. *dalechampii* and *Q. p.* ssp. *polycarpa* may be a consequence of the fact that the differences at the level of the leaves are relatively subtle, often being supplemented by those of the twigs and fruits.



Table 4.4.3.2 Matrix of classifications according to discriminant analysis (Qpe - *Q. p.* ssp. *petraea*; Qda - *Q. p.* ssp. *dalechampii*; Qpo - *Q. p.* ssp. *polycarpa*)

Identified	Correctly classified cases (%)	Predicted classification						
subspecies	Correctly classified cases (%)	Qpe	Qda	Qpo				
Qpe	94,5	155	9	-				
Qda	57,4	22	31	1				
Qpo	66,7	3	-	6				
Total	84,6	180	40	7				

For representations of the *Quercus* genus, such functions have been commonly tested and validated at the interspecific level and to a lesser extent at the infraspecific level. At the interspecific level, there are studies concerning the discrimination between *Quercus petraea* (sessile oak) and *Quercus robur* (pedunculate oak) (Bacillieri et al., 1996; Kremer et al., 2002; Boratynski et al., 2008; Yücedağ & Gailing, 2013; Jurkšienė & Baliuckas, 2014), between *Quercus petraea* and *Quercus pubescens* (downy oak) (Dupouey & Badeau, 1993), between *Quercus frainetto* (Hungarian oak) and *Quercus pubescens* (Fortini et al., 2015), or between several species of the subgenus *Lepidobalanus* (Viscosi et al., 2009b).

Among the evaluated micromorphological descriptors, only LRS (length of rays of stellate trichomes) had a significant contribution in distinguishing the three subspecies. At the infraspecific level, using discriminant functions, it was possible to differentiate between *Q. brantii* var. *belangeri* and *Q. brantii* var. *brantii*, although the separation was not entirely complete (Panahi et al., 2012).



# 5. FINAL CONCLUSIONS. ORIGINAL CONTRIBUTIONS. DISSEMINATION OF RESULTS. FUTURE RESEARCH DIRECTIONS

## 5.1. Final conclusions

- **\*** Regarding the assessment of spring and autumn phenology in order to establish correlations between phenophases and local climatic conditions
- Peripheral populations are characterized by a higher interannual variability and larger ranges of variation in the onset of bud burst compared to the non-peripheral population.
- In peripheral populations, the time of bud burst had mean values of DOY < 100, which suggests that, in these areas, sessile oak may be more susceptible to late frosts.</p>
- A typical mesothermal species, the sessile oak from peripheral populations in eastern Romania requires accumulated temperatures, from January 1<sup>st</sup> until bud burst (DOY), of over 173°C, and from senescence (DOY) until bud burst (DOY), of approximately 438-447°C, a value that can exceed 500°C for sessile oak that vegetates in the sub-Carpathian region, non-peripheral.
- The leaf development phenophase lasted on average 13-16 days, and that of the flowering of male catkins 7-10 days, both phenophases having a more accelerated dynamic in the peripheral populations.
- ➢ In 2017, during the onset of spring phenophases, temperatures below 0⁰C along with abundant snow partially affected the maturation process of male flowers but not the leaf development.
- The pollen release process occurred when the average temperatures during this phenophase were above 10°C, and the cumulative daily average temperatures above the threshold of 5°C were relatively similar.
- ➢ In the FAN comparative trial, the bud burst of the sessile oak provenances showed a clear altitudinal/longitudinal tendency, with a delay from east to west of 0.5-1.4 days per degree of longitude.
- In the FAN comparative trial, variations were found in the flowering time with the latitude and longitude of the origin of the provenances, with earlier flowering occurring in northern and eastern provenances compared to southern and western ones.
- ➢ By assessing the spring phenophases and autumn senescence, it was observed that the synchronization of spring phenophases is more consistent than the leaf senescence phenophase.
- The growing season in the non-peripheral population ends one to almost two weeks earlier compared to peripheral populations.

## **\*** Regarding the analysis of the influence of the phenological category on different wood traits

- > The basic wood density for early trees was higher than for late trees, an aspect that can be attributed to the growth differences between the two phenological categories.
- Geographical variations of the basic density were highlighted both in the natural stands and at the level of provenances from the FAN comparative trial.
- Compared to peripheral populations, basic wood density was higher in the non-peripheral population due to wider annual rings.
- Peripheral populations showed a higher number of rings in the sapwood, suggesting a delay in the transition to heartwood formation.



- For the provenances from the comparative trial, a decreasing trend in the number of sapwood rings was found from west to east, in the sense that peripheral provenances had a lower number of sapwood rings.
- In the FAN comparative trial, for intermediate trees, the formation of annual rings in 2021 was more advanced than for early flushing trees. This fact can be related either to the phases of the anthesis process, or to the origin of the provenances with intermediate flushing.
- > PCA analysis revealed a better association for trees with intermediate flushing.

## Regarding the analysis of phenotypic variability in a comparative trial of sessile oak provenances installed in the research area

- Comparative evaluations in the FAN sessile oak provenance trial indicated that non-local provenances were better adapted to the test site conditions and showed superior growth performance compared to local provenances.
- Under the seasonal conditions specific to the test site, provenances 29 Râmnicu Sărat, 32 Cluj, and 33 Zalău were the top performers in terms of growth, but exhibited inferior stem forms.

## Regarding the assessment of phenotypic variability at the level of foliar macro and micromorphological descriptors and comparison of the results using multivariate statistical analyses

- > At the intraspecific level, all the infraspecific units described in the specialized literature have been identified, indicating the existence of high morphological variability at the leaf level.
- Q. p. ssp. petraea f. platyphylla subf. angulata was not found in the natural stands, suggesting that its frequency is likely lower compared to other infraspecific units.
- Q. p. ssp. dalechampii was more frequently encountered in peripheral populations, probably due to the prolonged action of natural selection, favoring this infrataxon in ecosystems frequently affected by drought from eastern Romania. Moreover, within the analyzed north-south transect, the frequency of this subspecies decreased from south to north, which is also related to the known general precipitation regime in the vegetation season of eastern Romania.
- Unlike ssp. petraea, the other two subspecies are characterized by shorter leaves, with the maximum width in the lower half of the lamina, fewer lobes, ovate shapes, and subcordate basal leaf shapes, as also reported in the literature. Additionally, ssp. dalechampii and ssp. polycarpa showed a higher frequency of intercalary veins.
- > The only micromorphological descriptor that demonstrated a significant contribution in differentiating the three subspecies of sessile oak was LRS (length of rays of stellate trichomes).
- PCA results revealed that the three taxa overlap almost completely, suggesting morphological similarity at the level of macro and micromorphological leaf descriptors.
- The cluster analysis did not capture a classification of trees into subspecies, implying the existence of a unique morphological entity.
- > The generated discriminant functions provided the possibility of correctly separating the three subspecies in approximately 85% of cases.



- Regarding the identification by phenotypic and phenological criteria of valuable populations or provenances with high potential for adaptation to local climatic conditions.
- Following the evaluations at the interpopulation level, the peripheral populations FUN and OLT show high morphological variability so is recommended their *in situ* conservation.
- From the analysis of the phenological and phenotypic behavior of the provenances from the FAN comparative trial, the provenance 32-Cluj was identified as a superior provenance both through the point of view of later bud burst and high bioaccumulation capacity, characteristics that recommend it to be promoted in the culture, in similar site conditions to those of the test site.

### 5.2 Original contributions

- The realization for the first time of a multi-year phenological study of Quercus petraea sensu lato in peripheral populations from the eastern proximity of the species' general distribution range in Romania, both in natural stands and in a comparative trial installed within the research area, where provenances from different parts of the species' distribution in our country are tested.
- Highlighting for the first time of correspondences between phenological categories and different wood traits in sessile oak
- Accomplishment for the first time of a comprehensive and detailed leaf morphological analysis in the studied area, encompassing both macro- and micromorphological descriptors
- Identification of stands exhibiting high morphological variability, suitable to be classified as forest genetic resource
- Identification of leaf macro- and micromorphological descriptors with the highest capacity to differentiate between the three subspecies
- Identification for the first time, based on leaf macro- and micromorphological descriptors, of discriminant functions to determine the three subspecies.

#### **5.3 Dissemination of results**

#### A. Papers published in ISI journals:

**Gafenco (Pleşca), I. M**., Pleşca, B. I., Apostol, E. N., & Şofletea, N. (2022). Spring and Autumn Phenology in Sessile Oak (*Quercus petraea*) Near the Eastern Limit of Its Distribution Range. Forests, 13(7), 1125, <u>https://doi.org/10.3390/f13071125</u>, IF=3.282.

## B. Papers published in journals indexed in international databases (BDI):

**Gafenco (Pleșca), I. M**., Pleșca, B. I., Șofletea, N. (2023). The taxonomic structure of sessile oak (*Quercus petraea* (Matt.) Liebl.) in marginal populations from Eastern Romania – a bibliographic study – accepted for publication in Revista de Silvicultură și Cinegetică

**Gafenco (Pleşca), I. M**., Pleşca, B. I., Apostol, E. N.,, Ivan, R.C.; Ungureanu, A.M., Şofletea, N. (2023). Phenotypic variability of sessile oak (*Quercus petraea* (Matt.) Liebl.) in a comparative trial from Eastern Romania – accepted for publication in Revista de Silvicultură și Cinegetică

#### C. Papers presented at national or international symposia and conferences:

**Gafenco (Pleşca), I. M**., Pleşca, B. I., Apostol, E. N., & Şofletea, N. (2020). Spring and Autumn Phenology in Sessile Oak (*Quercus petraea*) Near the Eastern Limit of Its Distribution Range, 9th International Symposium "Forest and Sustainable Development" Braşov, Romania 16-18 October.



#### **5.4 Future research directions**

Climate change presents unique challenges for peripheral populations of sessile oak, which require specific responses and demand further research and investigation.

Given the current and anticipated climate aridification, subspecies *dalechampii* and *polycarpa* could potentially offer promising solutions for sessile oak cultivation in dry and warm areas. Identifying these subspecies and establishing their precise distribution should be a priority. In the future, modeling the distribution ranges of oaks using ecological niche models that incorporate complex processes (phenology, plasticity, dispersal, etc.) must be a highly active research direction.

Furthermore, investigations based on a combined assessment of molecular markers and leaf and fruit morphology are necessary to determine the best approach for distinguishing between the three subspecies.

Additional studies are also needed to explore and interpret the obtained results in relation to the anatomical and chemical characteristics of the wood. Moreover, expanding research to include dendrochronological analysis can provide valuable insights into cambial phenology in relation to tree leafing.

Moreover, investigations should continue in evaluating and monitoring the effects of late frosts on spring phenology to identify the most resistant provenances.

Furthermore, to gain insights into the adaptive plasticity of the tested sessile oak provenances, it is crucial to compare their relative performances under different experimental conditions (e.g., in Mihăiești and Râmnicu Sărat comparative trials).



## BIBLIOGRAPHY

- 1. Alberto, F., Bouffier, L., LOUVET, J.M., LAMY, J.B., Delzon, S., & Kremer, A. (2011). Adaptive responses for seed and leaf phenology in natural populations of sessile oak along an altitudinal gradient. Journal of Evolutionary Biology, 24(7), 1442-1454.
- 2. Alberto, F.J., Derory, J., Boury, C., Frigerio, J.M., Zimmermann, N.E., & Kremer, A. (2013). Imprints of natural selection along environmental gradients in phenology-related genes of *Quercus petraea*. Genetics, 195(2), 495-512.
- 3. Apostol, E. N., Curtu, A. L., Daia, L. M., Apostol, B., Dinu, C. G., & Şofletea, N. (2017). Leaf morphological variability and intraspecific taxonomic units for pedunculate oak and grayish oak (genus *Quercus* L., series *Pedunculatae* Schwz.) in Southern Carpathian Region (Romania). Science of The Total Environment, 609, 497-505.
- 4. Apostol, E., Curtu, A., Șofletea, N. (2015). Structura taxonomică intraspecifică într-un complex de cvercinee din estul României, la contactul cu zona silvostepei externe. Revista de Silvicultură și Cinegetică, 37, 47-51.
- 5. Arend, M., Brem, A., Kuster, T. M., & Günthardt-Goerg, M. S. (2012). Seasonal photosynthetic responses of three European oak species (*Quercus robur, Q. petraea* and Q. *pubescens*) to severe drought and elevated daytime temperature. Plant Biology, 1-8.
- 6. Bacilieri, R., Ducousso, A., Kremer. A. (1995). Genetic, morphological, ecological and phenological differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. in a mixed stand of northwest of France. Silvae genetica, 44(1), 1-9.
- 7. Badea, O. (1998). Fundamente dendrometrice și auxologice pentru monitoringul forestier. Rezumatul Tezei de doctorat, Suceava, 35p.
- 8. Bălănică, T., Tomescu, A. (1953). Dare de seamă asupra observațiilor fenologice forestiere efectuate în anul 1949. Analele ICAS, 13(1), 71-80.
- 9. Bartha, D. (2014). *Quercus polycarpa* Schur, 1851. In book: Enzyklopädie der Holzgewächse. Handbuch und Atlas der Dendrologie. Band III/2/35 Chapter: *Quercus polycarpa*. Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie.
- 10. Bartlet, J. E., Kotrlik, J. W., & Higgins, C. C. (2001). Determining appropriate sample size in survey research appropriate sample size in survey research. Information Technology, Learning, and Performance Journal, 19(1), 43-50.
- 11. Beldie, A. (1952). Flora R.P.R. vol I. (Genus *Quercus*). Editura Academiei RPR, 224-261.
- 12. Beldie, A., Cretzoiu, P. (1941). Studiu sistematic al gorunului din România. Analele ICAS, 7(1), 38-49.
- 13. Bergès, L., Dupouey, J. L., & Franc, A. (2000). Long-term changes in wood density and radial growth of *Quercus petraea* Liebl. in northern France since the middle of the nineteenth century. Trees, 14, 398-408.
- 14. Bergès, L., Nepveu, G., & Franc, A. (2008). Effects of ecological factors on radial growth and wood density components of sessile oak (*Quercus petraea* Liebl.) in Northern France. Forest Ecology and Management, 255(3-4), 567-579.
- 15. Bertin, R. I. (2008). Plant phenology and distribution in relation to recent climate change. The Journal of the Torrey Botanical Society, 135(1), 126-146.
- 16. Bessa, F., Sousa, V., Quilhó, T., & Pereira, H. (2022). An Integrated Similarity Analysis of Anatomical and Physical Wood Properties of Tropical Species from India, Mozambique, and East Timor. Forests, 13(10), 1675.



- 17. Beuker, E., Raspe, S., Bastrup-Birk, A., Preuhsler, T., & Fleck, S. (2016). Part VI: Phenological Observations., In: UNECE ICP Forests Programme Co-ordinating Centre (ed.): Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. Thünen Institute of Forest Ecosystems, Eberswalde, Germany. 12 p. + Annex
- 18. Boratynski, A., Marcysiak, K., Lewandowska, A., Jasinska, A., Iszkulo, G., & Burczyk, J. (2008). Differences in leaf morphology between *Quercus petraea* and *Q. robur* adult and young individuals. Silva Fennica, 42(1), 115.
- 19. Borazan, A., & Babaç, M.T. (2003). Morphometric leaf variation in oaks (*Quercus*) of Bolu, Turkey. Annales Botanici Fennici, JSTOR, 233-242.
- 20. Borovics, A., & Mátyás, C. (2013). Decline of genetic diversity of sessile oak at the retracting (xeric) limits. Annals of Forest Science, 70, 835-844.
- 21. Brullo, S., Guarino, R., Siracusa, G. (1999). Revisione tassonomica delle querce caducifoglie della Sicilia. Webbia 54(1):1-72.
- 22. Bruschi, P., Grossoni, P., & Bussotti, F. (2003). Within-and among-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. natural populations. Trees-structure and function, 17(2), 164-172.
- 23. Bruschi, P., Vendramin, G.G., Bussotti, F., Grossoni, P. (2000). Morphological and molecular differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Willd. (*Fagaceae*) in northern and central Italy. Annals of Botany, 85(3), 325-333.
- 24. Bussotti, F., & Grossoni, P. (1997). European and Mediterranean oaks (*Quercus L.; Fagaceae*): SEM characterization of the micromorphology of the abaxial leaf surface. Botanical Journal of the Linnean Society, 124(2), 183-199.
- 25. Campbell, R. K., & Sugano, A. I. (1975). Phenology of bud burst in Douglas-fir related to provenance, photoperiod, chilling, and flushing temperature. Botanical Gazette, 136(3), 290-298.
- 26. Camus, A. (1934–1954). Les chênes. Monographie du genre Quercus. Editura Lechevalier, Paris.
- 27. Chaar, H., & Colin, F. (1999). Impact of late frost on height growth in young sessile oak regenerations. Annals of Forest Science, 56(5), 417-429.
- 28. Chen, X., & Xu, L. (2012). Phenological responses of *Ulmus pumila* (Siberian Elm) to climate change in the temperate zone of China. International Journal of Biometeorology, 56, 695-706.
- 29. Chesnoiu, E.N. (2017). Discriminarea taxonomică prin descriptori ai frunzelor și analiza fenologiei de primăvară la stejarul pedunculat (*Quercus robur* L.) și stejarul brumăriu (*Quercus pedunculiflora* K. Koch). Rezumatul Tezei de doctorat, Brașov, 67.
- 30. Chesnoiu, E.N., Şofletea, N., Curtu, A.L., Toader, A., Radu, R., & Enescu, M. (2009). Bud burst and flowering phenology in a mixed oak forest from Eastern Romania. Annals of Forest Research, 52, 199-206.
- 31. Chmura, D. J., & Rozkowski, R. (2002). Variability of beech provenances in spring and autumn phenology. Silvae genetica, 51(2-3), 123-127.
- 32. Chuine, I. (2010). Why does phenology drive species distribution?. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1555), 3149-3160.
- 33. Chuine, I., & Beaubien, E. G. (2001). Phenology is a major determinant of tree species range. Ecology Letters, 4(5), 500-510.
- 34. Ciocîrlan, E., Sofletea, N., Ducci, F., & Curtu, A. L. (2017). Patterns of genetic diversity in European beech (*Fagus sylvatica* L.) at the eastern margins of its distribution range. iForest-Biogeosciences and Forestry, 10(6), 916.
- 35. Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A., & Schwartz, M.D. (2007). Shifting plant phenology in response to global change. Trends in Ecology & Evolution, 22(7), 357-365.



- 36. Clinovschi, F. (2005). Dendrologie, Editura Universității Suceava, 299 p.
- 37. Cole, E. F., & Sheldon, B. C. (2017). The shifting phenological landscape: Within-and between-species variation in leaf emergence in a mixed-deciduous woodland. Ecology and Evolution, 7(4), 1135-1147.
- 38. Constantinescu, N. (1973). Regenerarea arboretelor (Ediția a II-a). Editura Ceres, București, 667 p.
- 39. Crabbe, R.A., Dash, J., Rodriguez-Galiano, V.F., Janous, D., Pavelka, M., & Marek, M.V. (2016). Extreme warm temperatures alter forest phenology and productivity in Europe. Science of the Total Environment, 563, 486-495.
- 40. Crăciunesc, I. (2013). Evaluarea nivelului de hibridare naturala la specii autohtone de cvercinee: analiza de caz în rezervația Bejan-Deva. Teza de doctorat: 175 p.
- 41. Crăciunesc, I., Enescu, C.M., Şofletea, N., & Curtu, A.L. (2012). Floral phenology in a mixed species, natural oak forest of Romania. Conferința IUFRO "Genetics of *Fagaceae* and *Nothofagaceae*" 9-12 octombrie, Bordeaux (Franța).
- 42. Curtu, A. L., Sofletea, N., Toader, A. V., & Enescu, M. C. (2011). Leaf morphological and genetic differentiation between *Quercus robur* L. and its closest relative, the drought-tolerant *Quercus pedunculiflora* K. Koch. Annals of Forest Science, 68, 1163-1172.
- 43. Czúcz, B., Gálhidy, L., & Mátyás, C. (2011). Present and forecasted xeric climatic limits of beech and sessile oak distribution at low altitudes in Central Europe. Annals of Forest Science, 68, 99-108.
- 44. Dantec, C. F., Vitasse, Y., Bonhomme, M., Louvet, J. M., Kremer, A., & Delzon, S. (2014). Chilling and heat requirements for leaf unfolding in European beech and sessile oak populations at the southern limit of their distribution range. International Journal of Biometeorology, 58, 1853-1864.
- 45. de Sauvage, J. C., Vitasse, Y., Meier, M., Delzon, S., & Bigler, C. (2022). Temperature rather than individual growing period length determines radial growth of sessile oak in the Pyrenees. Agricultural and Forest Meteorology, 317, 108885.
- 46. Deans, J. D., & Harvey, F. J. (1995). Phenologies of sixteen European provenances of sessile oak growing in Scotland. Forestry: An International Journal of Forest Research, 68(3), 265-274.
- 47. Delpierre, N., Dufrêne, E., Soudani, K., Ulrich, E., Cecchini, S., Boé, J., François, C. (2009). Modelling interannual and spatial variability of leaf senescence for three deciduous tree species in France. Agricultural and Forest Meteorology, 149(6-7), 938-948.
- 48. Delpierre, N., Guillemot, J., Dufrêne, E., Cecchini, S., & Nicolas, M. (2017). Tree phenological ranks repeat from year to year and correlate with growth in temperate deciduous forests. Agricultural and Forest Meteorology, 234, 1-10.
- 49. Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., & Rathgeber, C. B. (2016). Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. Annals of Forest Science, 73(1), 5-25.
- Denéchère, R., Delpierre, N., Apostol, E. N., Berveiller, D., Bonne, F., Cole, E., Delzon S., Dufrêne, E., Gressler, E., Jean, F., Lebourgeois, F., Liu, G., Louvet, J. M., Parmentier, J., Soudani, K., & Vincent, G. (2021). The within-population variability of leaf spring and autumn phenology is influenced by temperature in temperate deciduous trees. International Journal of Biometeorology, 65, 369-379.
- 51. Di Pietro, R., Di Marzio, P., Medagli, P., Misano, G., Silletti, G. N., Wagensommer, R. P., & Fortini, P. (2016). Evidence from multivariate morphometric study of the *Quercus pubescens* complex in southeast Italy. Botanica Serbica, 40(1), 83-100.
- 52. Di Pietro, R., Viscosi, V., Peruzzi, L., & Fortini, P. (2012). A review of the application of the name *Quercus dalechampii*. Taxon, 61(6), 1311-1316.
- Diaz-Maroto, I. J., & Tahir, S. (2018). Testing of wood physical properties in oak species (*Quercus Robur* L., *Q. Petraea* (Matts) Liebl. and *Q. Pyrenaica* Willd.) for cooperage. Part II: wood grain. Wood Research, 63(6), 959-969.



- 54. Ducci, F., & Donnelly, K. (2017). Forest tree marginal populations in Europe-report on the state of knowledge on forest tree marginal and peripheral populations in Europe. Annals of Silvicultural Research, 41(3), 1-12.
- 55. Ducousso, A., Guyon, J., & Kremer., A. (1996). Latitudinal and altitudinal variation of bud burst in western populations of sessile oak (*Quercus petraea* (Matt) Liebl). Annales des Sciences Forestières: 775-782.
- 56. Dumitriu-Tătăranu, I., Ghelmeziu, N., Florescu, I., Milea, I., Moş, V., & Tocan, M. (1983). Estimarea calității lemnului prin metoda carotelor de sondaj (Estimation of Wood Quality Using the Method of Cores Sampling). Editura Tehnică, București, 69-82.
- 57. Dupouey, J., & Badeau., V., (1993). Morphological variability of oaks (*Quercus robur L, Quercus petraea* (Matt) Liebl, *Quercus pubescens* Willd) in northeastern France: preliminary results. Annales des Sciences Forestières. EDP Sciences, 35s-40s.
- 58. Dytham, C. (2011). Choosing and Using Statistics. A Biologist's Guide; Wiley-Blackwell: Oxford, UK.
- 59. Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. Molecular ecology, 17(5), 1170-1188.
- 60. Eliades, N. G. H., Papageorgiou, A. C., Fady, B., Gailing, O., Leinemann, L., & Finkeldey, R. (2019). An approach to genetic resources conservation of peripheral isolated plant populations: the case of an island narrow endemic species. Biodiversity and Conservation, 28, 3005-3035.
- 61. Enescu, C. M., Curtu, A. L., & Şofletea, N. (2013). Is *Quercus virgiliana* a distinct morphological and genetic entity among European white oaks?. Turkish Journal of Agriculture and Forestry, 37(5), 632-641.
- Fady, B.; Aravanopoulos, F.A.; Alizoti, P.; Mátyás, C.; von Wühlisch, G.; Westergren, M.; Belletti, P.; Cvjetkovic, B.; Ducci, F.; Huber, G.; Kelleher, C. T., Khaldi, A., Dagher Kharrat M. B. I., Kraigher, H., Kramer, K., Mühlethaler, U., Peric, P., Perry, A., Rousi, M., Sbay, H., Stojnic, S., Tijardovic, M., Tsvetkov, I., Varela, M. C., Vendramin, G. G., & Zlatanov, T. (2016). Evolution-based approach needed for the conservation and silviculture of peripheral forest tree populations. Forest Ecology and Management, 375, 66-75.
- Fedorova, B., Kadavý, J., Adamec, Z., Knott, R., Kučera, A., Kneifl, M., Drápela, K., & Inurrigarro, R. O. (2018). Effect of thinning and reduced throughfall in young coppice dominated by *Quercus petraea* (Matt.) Liebl. and *Carpinus betulus* L. Austrian Journal of Forest Science, 1, 1-17.
- 64. Filipova, E., & Asenov, A. (2016). Review on *Quercus dalechampii* Ten. and (Mattuschka) Liebl. in the vegetation of Bulgaria. Annual of Sofia University "St. Kliment Ohridski" Faculty of Biology, book 2-botany, Volume 100.
- 65. Firmat, C., Delzon, S., Louvet, J. M., Parmentier, J., & Kremer, A. (2017). Evolutionary dynamics of the leaf phenological cycle in an oak metapopulation along an elevation gradient. Journal of Evolutionary Biology, 30(12), 2116-2131.
- 66. Fober, H. (1998). Provenance experiment with pedunculate (*Quercus robur* L.) and sessile (*Q. petraea* [Matt.] Liebl.) oaks established in 1968. Arboretum Kórnickie, 43, 67-78.
- 67. Fonti, P., Heller, O., Cherubini, P., Rigling, A., & Arend, M. (2013). Wood anatomical responses of oak saplings exposed to air warming and soil drought. Plant Biology, 15, 210-219.
- 68. Fortini, P., Antonecchia, G., Di Marzio, P., Maiuro, L., & Viscosi, V. (2015). Role of micromorphological leaf traits and molecular data in taxonomy of three sympatric white oak species and their hybrids (*Quercus* L.). Plant Biosystems, 149(3), 546-558.
- 69. Fortini, P., Viscosi, V., Maiuro, L., Fineschi, S., Vendramin, G. (2009). Comparative leaf surface morphology and molecular data of five oaks of the subgenus *Quercus* Oerst (*Fagaceae*). Plant Biosystems 143(3):543-554.



- 70. Franjić, J., Liber, Z., Skvorc, Z., Idzojtic, M., Sostaric, R., & Stancic, Z. (2006). Morphological and molecular differentiation of the Croatian populations of *Quercus pubescens* Willd.[Fagaceae]. Acta Societatis Botanicorum Poloniae, 75(2), 123-130.
- 71. **Gafenco** (**Pleşca**), **I. M.**, Pleşca, B. I., Apostol, E. N., & Şofletea, N. (2022). Spring and Autumn Phenology in Sessile Oak (*Quercus petraea*) Near the Eastern Limit of Its Distribution Range. Forests, 13(7), 1125.
- 72. **Gafenco (Pleșca),** I.M., Pleșca, B.I., Apostol, E.N., Ivan, R.C., Ungureanu, A.M., Șofletea, N. (2023b). Phenotypic variability of sessile oak (*Quercus petraea* (Matt.) Liebl.) in a comparative trial from Eastern Romania. Revista de Silvicultură și Cinegetică, 52.
- 73. **Gafenco (Pleşca),** I.M., Pleşca, B.I., Şofletea, N. (2023a). The taxonomic structure of sessile oak (*Quercus petraea* (Matt.) Liebl.) in marginal populations from Eastern Romania a bibliographic study. Revista de Silvicultură și Cinegetică, 52.
- 74. Gancz V., Doniță, N., Biriș, I.A., Bandiu, C., Apostol, J., Marcu, C. (2008). Harta Pădurilor pe Unități Ecosistemice 1:100.000, Editura Silvică (ISBN 978-973-88379-2-8).
- 75. Gapare, W. J., Aitken, S. N., & Ritland, C. E. (2005). Genetic diversity of core and peripheral Sitka spruce (*Picea sitchensis* (Bong.) Carr) populations: implications for conservation of widespread species. Biological Conservation, 123(1), 113-123.
- 76. Garca-Mozo, H., Pablo, J. H., Galn, C., Gmez-Casero, M. T., & Domnguez, E. (2001). Catkin frost damage in Mediterranean cork-oak (*Quercus suber* L.). Israel Journal of Plant Sciences, 49(1), 42-47.
- 77. Georgescu, C.C., & Morariu, I. (1948). Monografia stejarilor din România. Tipografia Universul SA, 26 p+11planșe.
- 78. Govaerts, R., & Frodin, D.G. (1998). World checklist and bibliography of *Fagales*. Kew: Royal Botanic Gardens, Kew vii, 407 p.
- 79. Gričar, J., De Luis, M., Hafner, P., & Levanič, T. (2013). Anatomical characteristics and hydrologic signals in tree-rings of oaks (*Quercus robur* L.). Trees, 27, 1669-1680.
- 80. Gričar, J., Jevšenak, J., Hafner, P., Prislan, P., Ferlan, M., Lavrič, M., Vodnik, D., & Eler, K. (2022). Climatic regulation of leaf and cambial phenology in *Quercus pubescens*: their interlinkage and impact on xylem and phloem conduits. Science of the Total Environment, 802, 149968.
- 81. Grotehusmann, H., & Schönfelder, E. (2011). Comparison of French and German sessile oak ((Matt.) Liebl.) provenances. Silvae Genetica, 60(1-6), 186-196.
- 82. Grundström, M., Adams-Groom, B., Pashley, C. H., Dahl, Å., Rasmussen, K., de Weger, L. A., Thibaudon, M., Fernández-Rodríguez, S., Silva-Palacios, I., & Skjøth, C. A. (2019). Oak pollen seasonality and severity across Europe and modelling the season start using a generalized phenological model. Science of the Total Environment, 663, 527-536.
- 83. Guada, G., Vázquez-Ruiz, R. A., & García-González, I. (2019). Response patterns of xylem and leaf phenology to temperature at the southwestern distribution boundary of *Quercus robur*: A multi-spatial study. Agricultural and Forest Meteorology, 269, 46-56.
- 84. Guilley, E., Hervé, J. C., & Nepveu, G. (2004). The influence of site quality, silviculture and region on wood density mixed model in *Quercus petraea* Liebl. Forest Ecology and Management, 189(1-3), 111-121.
- 85. Hänninen, H. (1995). Effects of climatic change on trees from cool and temperate regions: an ecophysiological approach to modelling of bud burst phenology. Canadian Journal of Botany, 73(2), 183-199.
- 86. Hardie, D. C., & Hutchings, J. A. (2010). Evolutionary ecology at the extremes of species' ranges. Environmental Reviews, 18(NA), 1-20.



- 87. Harmer, R. (1992). Relationships between shoot length, bud number and branch production in *Quercus petraea* (Matt.) Liebl. Forestry: An International Journal of Forest Research, 65(1), 61-72.
- 88. Hodžić, M. M., & Ballian, D. (2020). Quality of pedunculate oak Provenances in Bosnian--Herzegovinian provenance test based on branching angle and stem form. Forestist, 70(2), 95-105.
- 89. IFN (Inventarul Forestier Național). (2019). Disponibil online: http://roifn.ro/site/rezultate-ifn-2/(Accesat pe 5 Decembrie 2020).
- 90. Jensen, J.S. (2000). Provenance variation in phenotypic traits in *Quercus robur* and *Quercus petraea* in Danish provenance trials. Scandinavian Journal of Forest Research, 15(3), 297-308.
- 91. Jensen, J.S., & Hansen, J.K. (2008). Geographical variation in phenology of *Quercus petraea* (Matt.) Liebl and *Quercus robur* L. oak grown in a greenhouse. Scandinavian Journal of Forest Research, 23(2), 179-188.
- 92. Jevšenak, J., Goršić, E., Stojanović, D. B., Matović, B., & Levanič, T. (2019). Sapwood characteristics of *Quercus robur* species from the south-western part of the Pannonian Basin. Dendrochronologia, 54, 64-70.
- 93. Jurkšienė, G., & Baliuckas, V. (2014). Leaf morphological variation of sessile oak (*Quercus petraea* (Matt.) Liebl.) and pedunculate oak (*Quercus robur* L.) in Lithuania. Research for Rural Development, 2, 63-69.
- 94. Kint, V., Hein, S., Campioli, M., & Muys, B. (2010). Modelling self-pruning and branch attributes for young *Quercus robur* L. and *Fagus sylvatica* L. trees. Forest Ecology and Management, 260(11), 2023-2034.
- 95. Kissling, P. (1977). Les poils des quatre espèces de chênes du Jura (*Quercus pubescens, Q. petraea, Q. robur* et *Q. cerris*). Ber. Schweiz. Bot.
- 96. Kleinschmit, J. (1993). Intraspecific variation of growth and adaptive traits in European oak species. In Annales des Sciences Forestières (Vol. 50, No. Supplement, pp. 166s-185s). EDP Sciences.
- 97. Knapic, S., Louzada, J. L., Leal, S., & Pereira, H. (2007). Radial variation of wood density components and ring width in cork oak trees. Annals of Forest Science, 64(2), 211-218.
- 98. Kolář, T., Giagli, K., Trnka, M., Bednářová, E., Vavrčík, H., & Rybníček, M. (2016). Response of the leaf phenology and tree-ring width of European beech to climate variability. Silva Fennica, 50(2).
- 99. Kramer, K. (1994). A modelling analysis of the effects of climatic warming on the probability of spring frost damage to tree species in The Netherlands and Germany. Plant, Cell & Environment, 17(4), 367-377.
- 100. Kramer, K. (1995). Modelling comparison to evaluate the importance of phenology for the effects of climate change on growth of temperate-zone deciduous trees. Climate Research, 119-130.
- 101. Kramer, K. (1996). Phenology and growth of European trees in relation to climate change, 210 p.
- 102. Kramer, K., Leinonen, I., & Loustau, D. (2000). The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forests ecosystems: an overview. International Journal of Biometeorology, 44(2), 67-75.
- 103. Kremer, A., Dupouey, J.L., Deans, J.D., Cottrell, J., Csaikl, U., Finkeldey, R., Espinel, S., Jensen, J., Kleinschmit, J., & Van Dam, B. (2002). Leaf morphological differentiation between *Quercus robur* and *Quercus petraea* is stable across western European mixed oak stands. Annals of Forest Science, 59(7), 777-787.
- 104. Krüssmann, G. (1986). Manual of cultivated broad-leaved trees & shrubs. Batsford, London, 621p.
- 105. Kučera, P. (2018). New name for Central Europaean oak formerly labelled as *Quercus dalechampii*. Biologia, 73(4), 313-317.



- 106. Kuster, T. M., Arend, M., Günthardt-Goerg, M. S., & Schulin, R. (2013). Root growth of different oak provenances in two soils under drought stress and air warming conditions. Plant and Soil, 369, 61-71.
- 107. Kuster, T. M., Dobbertin, M., Günthardt-Goerg, M. S., Schaub, M., & Arend, M. (2014). A phenological timetable of oak growth under experimental drought and air warming. PloS one, 9(2), e89724.
- 108. Lázaro-Nogal, A., Matesanz, S., García-Fernández, A., Traveset, A., & Valladares, F. (2017). Population size, center–periphery, and seed dispersers' effects on the genetic diversity and population structure of the Mediterranean relict shrub *Cneorum tricoccon*. Ecology and Evolution, 7(18), 7231-7242.
- 109. Lechowicz, M. J. (1984). Why do temperate deciduous trees leaf out at different times? Adaptation and ecology of forest communities. The American Naturalist, 124(6), 821-842.
- 110. Liepe, K. (1993). Growth-chamber trial on frost hardiness and field trial on flushing of sessile oak (*Quercus petraea* Liebl). In Annales des Sciences Forestières (Vol. 50, No. Supplement, pp. 208s-214s). EDP Sciences.
- 111. Liu, G., Chuine, I., Denéchère, R., Jean, F., Dufrêne, E., Vincent, G., Berveiller, D., & Delpierre, N. (2021). Higher sample sizes and observer inter-calibration are needed for reliable scoring of leaf phenology in trees. Journal of Ecology, 109(6), 2461-2474.
- 112. Macdonald, S. L., Llewelyn, J., Moritz, C., & Phillips, B. L. (2017). Peripheral isolates as sources of adaptive diversity under climate change. Frontiers in Ecology and Evolution, 5, 88.
- 113. Marchand, L. J., Dox, I., Gričar, J., Prislan, P., Leys, S., Van den Bulcke, J., Fonti, P., Lange, H., Matthysen, E., Peñuelas, J., Zuccarini, P., & Campioli, M. (2020). Inter-individual variability in spring phenology of temperate deciduous trees depends on species, tree size and previous year autumn phenology. Agricultural and Forest Meteorology, 290, 108031.
- 114. Matula, R. (2008). Comparison of general tree characteristics of less known oak species *Quercus dalechampii* Ten. and *Quercus polycarpa* Schur. Journal of Forest Science, 54(8), 333-339.
- 115. Mátyás, C. (2021). Adaptive pattern of phenotypic plasticity and inherent growth reveal the potential for assisted transfer in sessile oak (*Quercus petraea* L.). Forest Ecology and Management, 482, 118832.
- 116. Mátyás, C., Berki, I., Bidló, A., Csóka, G., Czimber, K., Führer, E., Gálos, B., Gribovszki, Z., Illés, G., Hirka, A., Somogyi, Z. (2018). Sustainability of forest cover under climate change on the temperatecontinental xeric limits. Forests, 9(8), 489.
- 117. Mátyás, C., Vendramin, G. G., & Fady, B. (2009). Forests at the limit: Evolutionary–genetic consequences of environmental changes at the receding (xeric) edge of distribution. Report from a research workshop. Annals of Forest Science, 66(8), 800.
- 118. Menzel, A., & Fabian, P. (1999). Growing season extended in Europe. Nature, 397(6721), 659-659.
- 119. Menzel, A., Estrella, N., Fabian, P. (2001). Spatial and temporal variability of the phenological seasons in Germany from 1951 to 1996. Global Change Biology, 7(6), 657-666.
- 120. Menzel, A., Estrella, N., Heitland, W., Susnik, A., Schleip, C., & Dose, V. (2008). Bayesian analysis of the species-specific lengthening of the growing season in two European countries and the influence of an insect pest. International Journal of Biometeorology, 52, 209-218.
- 121. Michelot, A., Simard, S., Rathgeber, C., Dufrêne, E., & Damesin, C. (2012). Comparing the intraannual wood formation of three European species (*Fagus sylvatica, Quercus petraea* and *Pinus sylvestris*) as related to leaf phenology and non-structural carbohydrate dynamics. Tree physiology, 32(8), 1033-1045.



- 122. Morin, X., Roy, J., Sonié, L., Chuine, I. (2010). Changes in leaf phenology of three European oak species in response to experimental climate change. New Phytologist, 186(4), 900-910.
- 123. Nanson, A. (2004). Genetic and forest trees breeding. Editura Gembloux, Belgium.
- 124. Nielsen, J.L.; Scott, J.M.; & Aycrigg, J.L. (2001). Endangered species and peripheral populations: Cause for conservation. Endanger. Species Update, 18, 194–197.
- 125. Nizinski, J.J., & Saugier, B. (1988). A model of leaf budding and development for a mature *Quercus forest*. Journal of Applied Ecology, 643-652.
- 126. Nölte, A., Yousefpour, R., & Hanewinkel, M. (2020). Changes in sessile oak (*Quercus petraea*) productivity under climate change by improved leaf phenology in the 3-PG model. Ecological Modelling, 438, 109285.
- 127. Panahi, P., Jamzad, Z., Pourmajidian, M., Fallah, A., Pourhashemi, M., & Sohrabi, H. (2012). Taxonomic revision of the *Quercus brantii* complex (*Fagaceae*) in Iran with emphasis on leaf and pollen micromorphology. Acta Botanica Hungarica, 54(3-4), 355-375.
- 128. Panchen, Z. A., Primack, R. B., Nordt, B., Ellwood, E. R., Stevens, A. D., Renner, S. S., Willis, C. G., Fahey, R., Whittemore, A., Du, Y., & Davis, C. C. (2014). Leaf out times of temperate woody plants are related to phylogeny, deciduousness, growth habit and wood anatomy. New Phytologist, 203(4), 1208-1219.
- 129. Pâques, L.E. (coord.) (2016). TREES4FUTURE Designing Trees for the Future. Alternative Methodologies for Phenology Assessment in Forest Trees, pp. 4–6.
- 130. Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Global Change Biology, 13(9), 1860-1872.
- 131. Pârnuța, G., Lorent, A., Tudoroiu, M., & Petrila, M. (2010). Regiuni de Provenienta Pentru Materiale de Baza din Care se Obtin Materiale Forestiere de Reproducere în România, Editura Silvica, București, Romania.
- 132. Pașcovschi, S., & Doniță, N. (1967). Vegetația lemnoasă din silvostepa României. Editura Academiei Republicii Socialiste România, București, 294 p.
- 133. Pásztory, Z., Börcsök, Z., Ronyecz, I., Mohácsi, K., Molnár, S., & Kis, S. (2014). Oven dry density of sessile oak, turkey oak and hornbeam in different region of Mecsek Mountain. Wood Research, 59(2), 683-694.
- 134. Peñuelas, J., Rutishauser, T., & Filella, I. (2009). Phenology feedbacks on climate change. Science, 324(5929), 887-888.
- 135. Perot, T., Balandier, P., Couteau, C., Delpierre, N., Jean, F., Perret, S., & Korboulewsky, N. (2021). Budburst date of *Quercus petraea* is delayed in mixed stands with Pinus sylvestris. Agricultural and Forest Meteorology, 300, 108326.
- 136. Ponton, S., Dupouey, J. L., & Dreyer, E. (2004). Leaf morphology as species indicator in seedlings of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl.: modulation by irradiance and growth flush. Annals of Forest Science, 61(1), 73-80.
- 137. Popescu, R., & Şofletea, N. (2020). Spring and autumn phenology in sub-mesothermal beech stands from the southwestern extremity of the Carpathians. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48(2), 1057-1069.
- 138. Pretzsch, H., Biber, P., Schütze, G., Kemmerer, J., & Uhl, E. (2018). Wood density reduced while wood volume growth accelerated in Central European forests since 1870. Forest Ecology and Management, 429, 589-616.
- 139. Preushler, T. (1999). Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forest. In Part IX, Phenological Observatio; UNECE: Geneva, Switzerland.



- 140. Proietti, E., Filesi, L., Di Marzio, P., Di Pietro, R., Masin, R., Conte, A. L., & Fortini, P. (2021). Morphology, geometric morphometrics, and taxonomy in relict deciduous oaks woods in northern Italy. Rendiconti Lincei. Scienze Fisiche e Naturali, 32(3), 549-564.
- 141. Puchałka, R., Koprowski, M., Gričar, J., & Przybylak, R. (2017). Does tree-ring formation follow leaf phenology in Pedunculate oak (Quercus robur L.)?. European Journal of Forest Research, 136(2), 259-268.
- 142. Raab-Straube, E.V., Raus, T. (2013). Euro+Med-Checklist Notulae, 1. Willdenowia, 43(1), 151-164.
- 143. Rao, R. V., Aebischer, D. P., & Denne, M. P. (1997). Latewood density in relation to wood fibre diameter, wall thickness, and fibre and vessel percentages in *Quercus robur* L. IAWA Journal, 18(2), 127-138.
- 144. Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O., & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology, 169, 156-173.
- 145. Sanda, V., Barabaş, N., & Ştefănuţ, S. (2004). Atlas *Florae Romaniae*. III-Genul *Quercus* Editura Ion Borcea, Bacău. 173 p.
- 146. Savage, J. A., Kiecker, T., McMann, N., Park, D., Rothendler, M., & Mosher, K. (2022). Leaf out time correlates with wood anatomy across large geographic scales and within local communities. New Phytologist, 235(3), 953-964.
- 147. Schwarz, O. (1964). *Quercus* L. Pp. 61-64. In: Tutin, T.G., Burges. N.A., Chater A.O., Edmondson J.R., Heywood V.H., Moore D.M., Valentine D.H., Walters S.M. & Webb, D.A. Flora Europaea. Cambridge, University Press 1.
- 148. Schwarz, O. (1993). *Quercus* L. In: Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M. & Webb, D.A. Flora Europaea, 2nd rev.ed. Cambridge, University Press, 72-76.
- 149. Sheskin, D. J. (2004). Handbook of parametric and nonparametric statistical procedures. 3rd ed. Chapman and Hall/CRC, Boca Raton, London, New York, Washington, 1182 p.
- 150. Smith, D. M. (1954). Maximum moisture content method for determining specific gravity of small wood samples. US Department of Agriculture, Forest Service, Forest Products Laboratory: Madison, WI, USA, 1954; 110–120.
- 151. Şofletea, N., & Curtu, L. (2000). Dendrologie. Vol. II, Editura Pentru Viață, 300 p.
- 152. Şofletea, N., & Curtu, L. (2007). Dendrologie. Editura Universității Transilvania, 418 p.
- 153. Șofletea, N., Moldovan, I.C., Enescu, M.C., Crăciunesc, I., & Curtu, A.L. (2011). Considerații privind identificarea hibrizilor între speciile autohtone de cvercinee. Revista pădurilor, 126(1), 6-11.
- 154. Şofletea, N., Târziu, D., Spârchez, Gh., & Curtu, A.L., (2001). Cercetări privind ecotipul de gorun submezoterm de mare altitudine de la Poiana Brașov. Analele ICAS, 44(1), 17-22.
- 155. Soularue, J. P., & Kremer, A. (2014). Evolutionary responses of tree phenology to the combined effects of assortative mating, gene flow and divergent selection. Heredity, 113(6), 485-494.
- 156. Stănescu, V., Şofletea, N., & Popescu, O.C., (1997). Flora forestieră lemnoasă a României. Editura Ceres, București, 450 p.
- 157. Struck, G., & Dohrenbusch, A. (2000). Development of a new branchiness index Asix-A simple tool to describe branchiness in young deciduous forest stands. Annals of Forest Science, 57(8), 811-818.
- 158. Stuparu, E. (2009). Variația genetică a principalelelor specii de arbori forestieri: evaluări în culturi comparative. Gorunul (*Quercus petraea* L). pp. 201-220. In: Surse de semințe testate pentru principalele specii de arbori forestieri din România, editat de G. Mihai. Editura Silvică, București.


- 159. Stuparu, E., Guiman, G., & Scărlătescu, V. (2003). Cercetări privind diversitatea genetică a gorunului din populații naturale din subregiunea Făgăraș-Sud. Analele ICAS, 45, 39-46.
- 160. Subhasis, M., Kaiser, I., Showkat, A., Aatif, H., Sabyasachi, D., & Todaria, N. P. (2019). Wood specific gravity of temperate forest species of Garhwal Himalaya, India. Indian Forester, 145(11), 1035-1038.
- 161. Sun, S., & Frelich, L. E. (2011). Flowering phenology and height growth pattern are associated with maximum plant height, relative growth rate and stem tissue mass density in herbaceous grassland species. Journal of Ecology, 99(4), 991-1000.
- 162. Teodosiu, M., Guiman, G., Bujilă, M., Frăţilă, E., Coandă, C., Hăruţă, O., & Dorog, S. (2005). Observaţii fenologice la specii forestiere în sezonul de vegetaţie 2004. Analele ICAS, 48(1), 73-83.
- 163. Thomas, F.M., & Gausling, T. (2000). Morphological and physiological responses of oak seedlings (*Quercus petraea* and *Q. robur*) to moderate drought. Annals of Forest Science, 57(4), 325-333.
- 164. Tomescu, A. (1957). Fazele periodice de vegetație la speciile forestiere Sinteza pentru perioada 1946-1955. Editura Agro-Silvică de Stat, București, 123 p.
- 165. Tomescu, A. (1967). Cercetări fenologice la principalele specii forestiere autohtone din RSR-Sinteza pentru perioada 1956-1965. Centrul de documentare tehnică pentru economia forestieră, București, 100 p.
- 166. Torres-Ruiz, J. M., Kremer, A., Carins Murphy, M. R., Brodribb, T., Lamarque, L. J., Truffaut, L., Bonne, F., Ducousso, A., & Delzon, S. (2019). Genetic differentiation in functional traits among European sessile oak populations. Tree physiology, 39(10), 1736-1749.
- 167. Ursenbacher, S., Guillon, M., Cubizolle, H., Dupoué, A., Blouin-Demers, G., & Lourdais, O. (2015). Postglacial recolonization in a cold climate specialist in western Europe: patterns of genetic diversity in the adder (*Vipera berus*) support the central–marginal hypothesis. Molecular Ecology, 24(14), 3639-3651.
- 168. Vander Mijnsbrugge, K., Turcsán, A., Maes, J., Duchêne, N., Meeus, S., Van der Aa, B., Steppe, K., & Steenackers, M. (2017). Taxon-independent and taxon-dependent responses to drought in seedlings from *Quercus robur* L., *Q. petraea* (Matt.) Liebl. and their morphological intermediates. Forests, 8(11), 407.
- 169. Vavrčík, H., & Gryc, V. (2012). Analysis of the annual ring structure and wood density relations in English oak and Sessile oak. Wood research, 57(4), 573-580.
- 170. Viscosi, V., Fortini, P., Slice, D., Loy, A., & Blasi, C. (2009a). Geometric morphometric analyses of leaf variation in four oak species of the subgenus *Quercus* (*Fagaceae*). Plant Biosystems, 143(3), 575-587.
- 171. Viscosi, V., Lepais, O., Gerber, S., & Fortini, P. (2009b). Leaf morphological analyses in four European oak species (*Quercus*) and their hybrids: A comparison of traditional and geometric morphometric methods. Plant Biosystems An International Journal Dealing with all Aspects of Plant Biology, 143(3), 564-574.
- 172. Vitasse, Y., Bresson, C.C., Kremer, A., Michalet, R., & Delzon, S. (2010). Quantifying phenological plasticity to temperature in two temperate tree species. Functional Ecology, 24(6), 1211-1218.
- 173. Vitasse, Y., Delzon, S., Bresson, C.C., Michalet, R., & Kremer, A., (2009). Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. Canadian Journal of Forest Research 39(7):1259-1269.
- 174. Vitasse, Y., François, C., Delpierre, N., Dufrêne, E., Kremer, A., Chuine, I., Delzon, S. (2011). Assessing the effects of climate change on the phenology of European temperate trees. Agricultural and Forest Meteorology, 151(7), 969-980.



- 175. Vitasse, Y., Porté, A.J., Kremer, A., Michalet, R., & Delzon, S., (2009). Responses of canopy duration to temperature changes in four temperate tree species: relative contributions of spring and autumn leaf phenology. Oecologia 161(1), 187-198.
- 176. Wang, L., Han, X., Yin, Q., Wang, G., Xu, J., Chai, Y., & Yue, M. (2021). Differences in leaf phenological traits between trees and shrubs are closely related to functional traits in a temperate forest. Acta Oecologica, 112, 103760.
- 177. Wiersma, J. H. (1962). Enkete kwantitatieve aspecten van het exotenvraagstuk. Nederlands Bosbouw Tijtschrift, 34(5), 175-184.
- 178. Yücedağ, C., & Gailing, O. (2013). Morphological and genetic variation within and among four *Quercus petraea* and *Q. robur* natural populations. Turkish Journal of Botany, 37(4), 619-629.
- 179. Yücedağ, C., Müller, M., & Gailing, O. (2021). Morphological and genetic variation in natural populations of *Quercus vulcanica* and *Q. frainetto*. Plant Systematics and Evolution, 307, 1-15.
- 180. Yücedağ, C., Sanders, J., Musah, M., & Gailing, O. (2019). Stomatal density in *Quercus petraea* and *Q. robur* natural populations in Northern Turkey. Dendrobiology, 81.
- 181. Zar, J.H. (2010). Biostatistical Analysis, 5th ed.; Prentice Hall: Upper Saddle River, NJ, USA.
- 182. Zhang, X., Friedl, M. A., & Schaaf, C. B. (2009). Sensitivity of vegetation phenology detection to the temporal resolution of satellite data. International Journal of Remote Sensing, 30(8), 2061-2074.
- 183. Zobel B.J., & Sprague, J.R., (1998). Juvenile Wood in Forest Trees. Springer-Verlag Berlin Heidelberg. Germany, 1-19.
- 184. Zobel, B. J., & van Buijtenen, J. P. (2012). Wood variation: its causes and control. Springer Science & Business Media, USA, New York, 1-32.
- 185. Zobel, B. J., van Buijtenen, J. P., Zobel, B. J., & van Buijtenen, J. P. (1989). Wood variation and wood properties. Wood variation: its causes and control, 1-32.
- 186. \*\*\* QuickPHOTO MICRO 3.2.
- 187. \*\*\* STATISTICA v. 8.0 StatSoft Inc., Tulsa, OK, USA.
- 188. \*\*\* WinFOLIA PRO 2020, Regent Instruments.
- 189. \*\*\*XLFolia, Regent Instruments.



## Short summary

The aim of the research was to evaluate the phenological and taxonomic structure in populations of the lower altitudinal limit representative of the eastern area of the species Quercus petraea (Matt.) Liebl. in Romania.

Based on the phenological analyses, small variations were identified between the reference population and peripheral populations associated with climatic conditions. The timing of spring phenophases was found to be more constant than leaf senescence. Moreover, bud burst in the comparative trial showed an obvious longitudinal tendency, with an east–west delay of 0.5–1.4 days for each degree of longitude.

Regarding the analysis of the influence of phenological category on different wood traits, it was statistically confirmed that, at comparable ages and radial growths, late flushing trees had lower basic wood density and showed greater density differences between sapwood and heartwood compared with trees with early flushing (F = 3.18, p = 0.04). In the comparative trail, intermediate flushing trees have wider rings, the formation of which is much more advanced than early flushing trees.

In the comparative trial statistically significant differences were detected between provenances for most of the considered traits (except diameter at breast height and pruned height), which were confirmed by the Duncan test. Generally, the non-local provenances were amongst the most performant in terms of growth. The correlation analysis revealed that provenances from western longitudes and southern latitudes, as well as from low altitudes had better survival rates. Trunk shape and branch diameter were positively and significantly correlated with altitude ( $r = 0,120^*$ ;  $r=0,169^{**}$ ), meaning that the lower altitudes provenances had straighter trunks and sharper insertion angles.

Through phenotypic analyses, the foliar descriptors that significantly contributed to the differentiation of the three sessile oak subspecies were determined. Thus, the subthermophilic sessile oaks (ssp. *dalechampii* and ssp. *polycarpa*) compared to the common sessile oak have shorter leaves, with the maximum width in the lower half of the lamina, a lower number of lobes, ovate shapes, with the base of the leaf subcordate. In addition, a higher tendency to present intercalary veins is observed for the subthermophilic sessile oaks.