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Phenotypic variability of common beech along an altitudinal gradient

SUMMARY

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PhD Thesis

Title: Phenotypic variability of common beech along an altitudinal gradient

Titlu: Variabilitatea fenotipică a fagului de-a lungul unui profil altitudinal

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Contents

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In this PhD thesis, I presented the results of my research unfolding within the "Marin Drăcea" National Institute for Research and Development in Forestry, Brașov Station, and Interdisciplinary Doctoral School from Brașov. My research focused on evaluating common beech phenotypic variability along an altitudinal gradient through spring and autumn phenology monitoring based on field observations and remote sensing devices. I further associated these phenological data with genetic diversity quantified as a heterozygosity index.

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LIST OF ABBREVIATIONS

%CFL – Percentage of colored and fallen leaves

%CL – Percentage of colored leaves

%FL – Percentage of fallen leaves

(EST)-SSRs - gene-based Expressed Sequence Tag

ADD - Autumn degree days

BBCH – ger. “**B**iologische **B**undesanstalt, Bundessortenamt und **Ch**emische Industrie” phenological scale

CDD - Cumulated degree days

DBH – Diameter at breast height

DNA – Deoxyribonucleic acid

DOY – Day of Year

DSM – Digital Surface Model

GDD - Growing degree days

GPS - Global Positioning System

GSD - Ground Sampling Distance

IFN – Inventarul forestier național

IPCC – Intergovernmental Panel on Climate Change

Mpx – Megapixels

m – mean

me - median

MSE - Mean Squared Error

NDVI - Normalized difference vegetation index

NIR - Near-Infrared Light

nPD – number of point divisions

RGB – Red Green Blue

RMSE - Root Mean Square Error

SD - Standard Deviation

SDD - Senescence degree days

SLC – Slenderness coefficient

SNPs - Single nucleotide polymorphism

SSRs - Simple Sequence Repeats

TLS – Terrestrial laser scanning

UAVs – Unnamed aerial vehicle

UNESCO – United Nations Educational, Scientific and Cultural Organization

VI - Vegetation Index

INTRODUCTION

The trend of increasing temperatures has become more and more perceptible. It can be directly associated with desertification processes, melting glaciers, reducing snow-covered areas, rising sea and ocean levels, intensifying storms, and indirectly with soil erosion (Aitken et al. 2008). As a result of climate change, species in forest ecosystems are forced to adapt to new conditions and react through their regulatory mechanisms (physiological adaptation), being forced to restrict and reposition their habitats (Barbu et al. 2016; Prasad et al. 2024) or are even pushed to their survival limit.

In the current context of climate change, forest management must focus on species that have proven high adaptability and phenotypic plasticity to new conditions, resistance to entomological and phytopathological pests, and diversified use of wood material. These arguments/needs justify the choice of common beech as a viable solution and the need to obtain as much information as possible regarding its adaptability potential.

Common beech (*Fagus sylvatica* L.) is the most widespread forest species in Europe (Rigo et al. 2016), including in Romania (IFN-Cycle II Results 2018). This species has a high ecological and economic value in recent decades, becoming a species of interest at the European level (Leuschner & Ellenberg 2017; Leuschner 2020). Common beech has a high carbon storage capacity in its biomass (Jagodziński et al. 2020), a high adaptability potential to new ecological conditions (Kramer et al. 2010), a wide range of wood uses (Pramreiter & Grabner 2023), and good resistance to entomological and phytopathological pathogens (Chira et al. 2014; Biriș 2014). Being a mesothermal and mesophilic species (Șofletea & Curtu 2007), in the context of climate change, its competitive capacity may be reduced (Geßler et al. 2007), preferring a moderate climate, without extreme temperatures (Zlatník 1978). For a better understanding of the adaptation potential of this species, it is necessary to monitor the phenological responses to the effects of climate change (Schieber 2006).

Leaf phenology represents a bioindicator of local adaptability (McKown et al. 2018) and can be considered a response to climate change, which is very sensitive to temperature variation. Through phenology monitoring, it is possible to quantify changes in processes in terrestrial ecosystems covered by vegetation, such as photosynthesis and transpiration (Klosterman & Richardson 2017), allowing, at the same time, to assess the impact of climate change effects on them (Atkins et al. 2020). At the same time, the timing of tree initiation in spring and autumn phenology can be associated with susceptibility to various pathogens in the stand (van Asch et al. 2007; Krokene et al. 2011; McKinney et al. 2011).

Time series observations of spring and autumn phenology can contribute to a better understanding of the effects of climate variability and climate change on biological processes at the level of plants and trees in correlation with meteorological factors (Budeanu et al. 2016). Images collected through various remote sensing devices (drones or satellites) facilitate the precise delineation and quantification of the different phenophases of spring and autumn phenology. Using various vegetation indices calculated based on the pixel values extracted from these images can provide information about the health status of a stand and other phenotypic characteristics, in this case, phenology. Improving the resolution of these images allows the extraction of various information, even at the individual/tree level.

The architecture of a tree depends on many factors, including those that make up seasonal (climate, exposure, slope) and stand conditions (density, competition, access to light), which can be controlled, to some extent, by silvicultural interventions, being also considered as a response to them (Tomșa et al. 2021). This phenotypic trait results from the interaction between an individual's genotype and environmental conditions (Chéné et al. 2012). Each branch responds to microrelief conditions (Abegg et al. 2021). The degree of forking of a tree is an important aspect that particularly determines its economic value. However, the nature of the appearance of this phenotypic trait is still uncertain, being associated both with late spring frosts (Jouve et al. 2007) and with the presence of specific pathogens of an entomological (Kerr & Boswell 2001) and phytopathological nature (Lebeda 1983) or of genetic diversity (Wilcox 1982; Xiong et al. 2010; Callister et al. 2011).

The growth of a tree is a trait of its fitness, with growth rates being directly associated with individual reproductive success (Avanzi et al. 2020), and often, it is also used as a predictive indicator for survival rate (Biegler & Bugmann 2004). In the case of common beech, the growth rate and the start time in the growing season are hereditary traits (Gauzere et al. 2016). However, in natural conditions and uneven age stands, these growth indicators, such as height and DBH, may reflect variations in microclimate conditions and their age.

Silvicultural interventions implicitly reduce the density within a stand of competition (Kirk & Berrill 2016). The competition exerted on a tree by neighboring specimens depends on the species, size, and crown shape (Barbeito et al. 2017) but also its access to resources (water, light) (Fichtner et al. 2012). Competition affects juvenile specimens (Palaghianu 2009) and mature trees (Duduman et al. 2010). The plasticity of a tree's crown in terms of access to light is a limiting factor for its growth (Penanhoat et al. 2024), and slenderness is often used as an index for resistance to windfall (Wang et al. 1998).

Phenotypic characteristics are determined and controlled by the interaction between genotype and environment (Li et al. 2017; Bian et al. 2022). Heterozygosity is one of the most important indicators of genetic variation influencing tree growth rate (Mitton et al. 1980). Low heterozygosity also implies a reduction in the fitness of a tree (Rodríguez-Quilón et al. 2015). However, the association of heterozygosity with various phenotypic traits is still intensely debated (Szulkin et al. 2010; Mueller et al. 2011; Gkafas et al. 2019). In the context of climate change, beech had the ability to specialize genotypically, thus leading to the emergence of certain locally adapted varieties and ecotypes (Božič et al. 2013). The genotypes of a species have the ability to adapt to new environmental conditions, showing different phenological behavior in low and high-altitude areas, directly determining the length of the growing season (Menamo et al. 2021).

In this thesis, the phenotypic variability of the beech was evaluated by monitoring the spring and autumn phenology during three years along an altitudinal profile with an amplitude of 900 m, which also included study sites located in the rough terrain, both through field observations and different remote sensing devices (drone and satellite images), to analyze the degree of adaptation to the different local environmental conditions. Also, using remote sensing, more precisely the use of the Terrestrial Laser Scanning (TLS) method, various biometric characteristics of the analyzed beech specimens were extracted, which were subsequently associated with the genetic diversity of each individual, quantified as the degree of heterozygosity. The statistical analyses were performed by approaching classical methods (the *Pearson* correlation coefficient) and new machine learning techniques (*random forest* algorithm) or linear and non-linear models, which were applied to the calibration data set and the test one.

1. THE CURRENT STATE OF KNOWLEDGE

1.1. The common beech, the most important broadleaves forest tree species from Romania

The common beech (*Fagus sylvatica* L.) ("European beech") is one of the most important and widespread deciduous forest species in Europe (Rigo et al. 2016), with a wide range in terms of environmental conditions (Leuschner et al. 2006). It is a very competitive species, often being found in the form of pure stands or mixed stands with silver fir (*Abies alba*), Norway spruce (*Picea abies*), and sessile oak (*Quercus petraea*) (Doniță et al. 1990; Donita et al. 2005). Beech forests, especially old stands, have an important role in biodiversity conservation and are of particular economic interest (Moning & Müller 2009).

Of the total area of forests in Romania (6.9 mil. ha), beech occupies an area of 2.1 mil. ha (approximately 31%), being the most widespread species in the national forest fund (IFN Results - Cycle II 2018). Of the total volume of standing timber in Romania's forests, beech occupies approx. 38%, having the largest share. In Romania, beech is mainly found in mountainous areas, its area largely overlapping with the Carpathian Mountains. The lower limit of the beech, also called the xeric limit, is represented by altitudes of 300 m, also found isolated on narrow and humid valleys in Banat and the Moldavian Plateau, at altitudes of 100-200 m (Stănescu et al. 1997). The upper limit of the beech trees in Romania is located at altitudes between 1200-1400 m in the Western Carpathians but is also found at higher altitudes by 100-200 m in the Southern Carpathians (Șofletea & Curtu 2007). The altitudinal optimum in which this species is found in Romania is 600-1200 m (Budeanu et al. 2016); however, the altitudinal amplitude it is distributed is 200-1600 (Budeanu et al. 2015). Low temperatures generally determine the upper limit of the beech's distribution area, and the lower limit by high temperatures and low amounts of precipitation (Grace et al. 2002; Nedealcov et al. 2018). Although this species is considered to have high adaptability and tolerance, demonstrated by the vast area and wide altitudinal amplitude in which it is found, it becomes sensitive to climatic conditions both in the lower and upper limits of its distribution (Mátyás et al. 2010). It can be found at low altitudes in the north of the continent, so that towards its south, it can be found at altitudes of over 1000 m, sometimes even close to 2000 (von Wühlisch 2008), according to the requirement for precipitation and an optimal temperature found on the latitudinal gradient.

This species achieves maximum performance in areas with a humid, mild and sunny climate (Horgan et al. 2003), being demanding on thermal and water conditions. At the European level, the distribution of beech covers a wide range of climatic conditions, with an amplitude of 4-12 ˚C in mean annual temperature and an annual rainfall of 450-2000 mm (Konnert 1995). The thermal optimum of beech in Romania is 6-9 ˚C (yearly average temperature) (Șofletea & Curtu 2007), showing its mesothermal behavior, but the tolerance limit is 3 °C. The start of beech in the growing season occurs when the average daily temperature exceeds the threshold of 10 °C (Popescu & Sofletea 2020).

Like any other forest species, the common beech is forced to adapt and react through its regulatory mechanisms in response to climate change, sometimes even being pushed to its survival limit. Beech adaptation depends on the species' genetic diversity and ecological plasticity to new seasonal conditions (Westergren et al. 2023).

The phenotypic plasticity and adaptability of beech to various environmental conditions are due to its genetic composition (Schaberg et al. 2008; Nicolescu et al. 2017). Beech specimens originating from the southeast of the continent are not affected by drought to the same extent as those from the center of the natural range (Fotelli et al. 2009). However, they have high genetic diversity (Vornam et al. 2004). On a larger scale, this genetic differentiation between populations becomes even more substantial (Magri et al. 2006), justifying the different responses of this species to various environmental conditions (Knutzen et al. 2015).

1.2. Phenological observation on beech

The phenology of forest species is one of the most representative, sensitive, and relatively easy-to-observe indicators that respond to climate effects. The dynamics of phenology affect the survival rate, reproductive capacity, persistence, and, implicitly, the distribution area of forest species (Chuine 2010). The phenotypic plasticity of a species allows it to survive in various environmental conditions (Preite et al. 2015). The onset of the growing season is considered an indicator of adaptability, with important implications for forest management (Chuine et al. 2000; Casmey et al. 2022).

Temperature is an important factor in the growth and development of forest species. It influences the rate of chemical reactions within the physiological processes of a plant or tree (Badeck et al. 2004), differently at the level of each organ (Delpierre et al. 2016). In many cases, it has been demonstrated that an increase in temperature accelerates the development of trees; more precisely, an increase of one degree in spring temperature can cause an advance start of the growing season by 2-7 days (Zohner et al. 2021), implicitly, a faster transition to the next phenophase (Badeck et al. 2004). A longer growing season implies an earlier start of the bud growth and development process, respectively, as leaf emergence is caused by global warming (Chmielewski et al. 2018). Instead, early onset in the growing season combined with high temperatures and low precipitation (drought) causes premature leaf yellowing, implicitly an early onset of senescence (Menzel 2003). The start of the beech growing season is highly influenced by temperature dynamics in late winter and early spring (Bigler & Vitasse 2019). The low temperatures cause an early onset of senescence; a one-degree increase in autumn temperature delays senescence by up to 8 days for some forest species (Zohner et al. 2021).

Based on observations from the field or those obtained with the help of *remote sensing* techniques (drones, satellite images), phenology can provide algorithms and models that explain specific forest reactions to the effects of climate change and their ability to adapt to new environmental conditions. Although field observations of forest species phenology are demanding and time-consuming, they provide valuable and accurate information at the individual level (Park et al. 2019). Time series observations of both spring and autumn phenology can contribute to a better understanding of climate variability or the effects of climate change and the responses of forest species to them in correlation with meteorological data, especially temperature (Budeanu et al. 2016). Images collected with the help of UAV devices and satellites facilitate the precise delineation and quantification of the different phenophases of phenology (Fig. 1.1). However, certain factors can affect the quality of data/images obtained, especially those of a meteorological nature (clouds, wind, rain, fog), but also their technical specifications (resolution, autonomy, height and maximum overflight distance), especially in the case of monitoring at the individual level (Jacobsen 2017; Sánchez-Azofeifa et al. 2011).

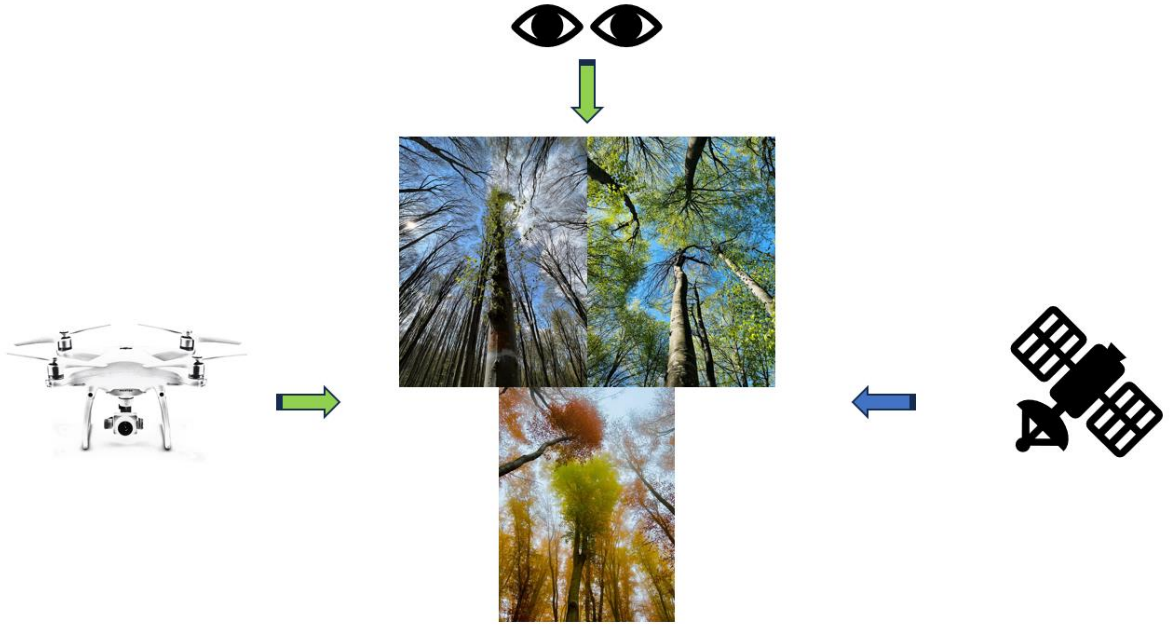


Fig. 1.1 - Illustration of the three methods adopted to assess *F. sylvatica* phenology: classic observations in the field, drone, and satellite images

1.3. The relationship between phenotypic characteristics of trees and the degree of heterozygosity

Terrestrial laser scanning (TLS) methods have shown potential in forestry applications. TLS methods can successfully estimate a wide range of structural indices of a stand (Pascu et al. 2019). Apostol et al. (2018) demonstrated that using TLS methods could provide results as accurate as conventional ones in estimating the values of tree diameters. However, they still recommend the latest for the determination of heights. In recent years, TLS methods have been improved, Capalb et al. (2023) stating that data obtained from point clouds processed by these devices are of sufficient resolution to extract both diameter and tree height values from an equine and relative-equine spruce stand (however, some limitations were reported in the case of individuals of reduced height). However, the accuracy with which the data regarding the architecture of the trees is obtained is crucial in their subsequent processing and interpretation (Tomșa et al. 2021).

Genetic markers have already been used to test the association of phenology with the genetic diversity of a tree, which is determined through SSRs and SNPs (Hall et al. 2007). Heterozygosity was also associated with the phenology of different plant species (Kitamoto et al. 2006; Suni & Whiteley 2015) and trees (Kembrytė et al. 2022). Both EST (Tomșa et al. 2021), SSR (Kembrytė et al. 2022), and SNPs (Hall et al. 2007) genetic markers were used to calculate heterozygosity. Most studies on beech genetic variability initially used the isozymes proposed by Müller-Starck & Starke (1993). Later, genetic analyses performed on beech individuals that used microsatellites (SSRs-simple sequence repeats) provided important information regarding the distribution of genetic variability (Vornam et al. 2004; Cvrčková et al. 2017), the influence of glacial refugia (Buiteveld et al. 2007), population dynamics (Rajendra et al. 2014) and evolutionary relationships at the inter- and intraspecifically between different interfertile beech subspecies (Budde et al. 2023). Babushkina et al. (2016) stated that individuals who have a higher degree of heterozygosity show a more stable growth pattern and a higher tolerance to the influences of environmental factors, such as temperature or amount of precipitation. Leimu et al. (2006) demonstrated that an individual's low level of heterozygosity (e.g., caused by the mating of related specimens or genetic drift within a small population) could reduce its fitness. Harmful genetic mutations (mainly recessive mutations with adverse effects on fitness) are (partially) masked in heterozygous individuals. Therefore, trees showing a low level of heterozygosity may exhibit considerably lower fitness than specimens with a high degree of heterozygosity. Few genetic markers in specific genes with significant effects (local effect) could cause such correlations between heterozygosity and fitness (Hansson et al. 2002; Szulkin et al. 2010). The combination of several genetic markers, assumed to be neutral, can be used to calculate individual heterozygosity at the genome level (Forstmeier et al. 2012).

2. AIM AND OBJECTIVES

The research presented in this work aimed to evaluate the phenotypic variability of beech along an altitudinal transect and analyze its degree of adaptation to local environmental conditions.

To achieve the proposed aim, relative to the current state of knowledge, the following specific objectives were established:

1. Observation of spring and autumn phenology in beech populations located at different altitudinal levels

2. Testing some remote sensing techniques for performing phenological observations in rough terrain

3. Analysis of the relationship between common beech phenotypic characteristics and their heterozygosity index

3. MATERIAL AND METHODS

3.1. Research location

The research was performed along an altitudinal transect with an amplitude in the range of 550 – 1450 m and with five study areas (Fig. 3.1). The lower limit of the altitudinal transect is found on Lempeș Hill, in "Țara Bârsei" depression. The other populations are found along the Postăvaru massif. A total of 150 mature trees were selected, 30 from each plot, located at least 25 m apart, mainly situated on the northern exposure, from the upper canopy of the stand (Kraft classes I, II, and III) (Kraft 1884). The age of the analyzed specimens was between 80-120 years. This altitudinal profile with an amplitude of 900 m overlaps with the natural area of the beech in the Brașov area, where it forms mixtures with other species of deciduous (such as *Acer pseudoplatanus* or *Carpinus betulus*) and resinous (*Picea abies* or *Abies alba*) (Irimin & Ungureanu 2015; Mărgălinescu & Irimin 2016; Ștefan 2018).

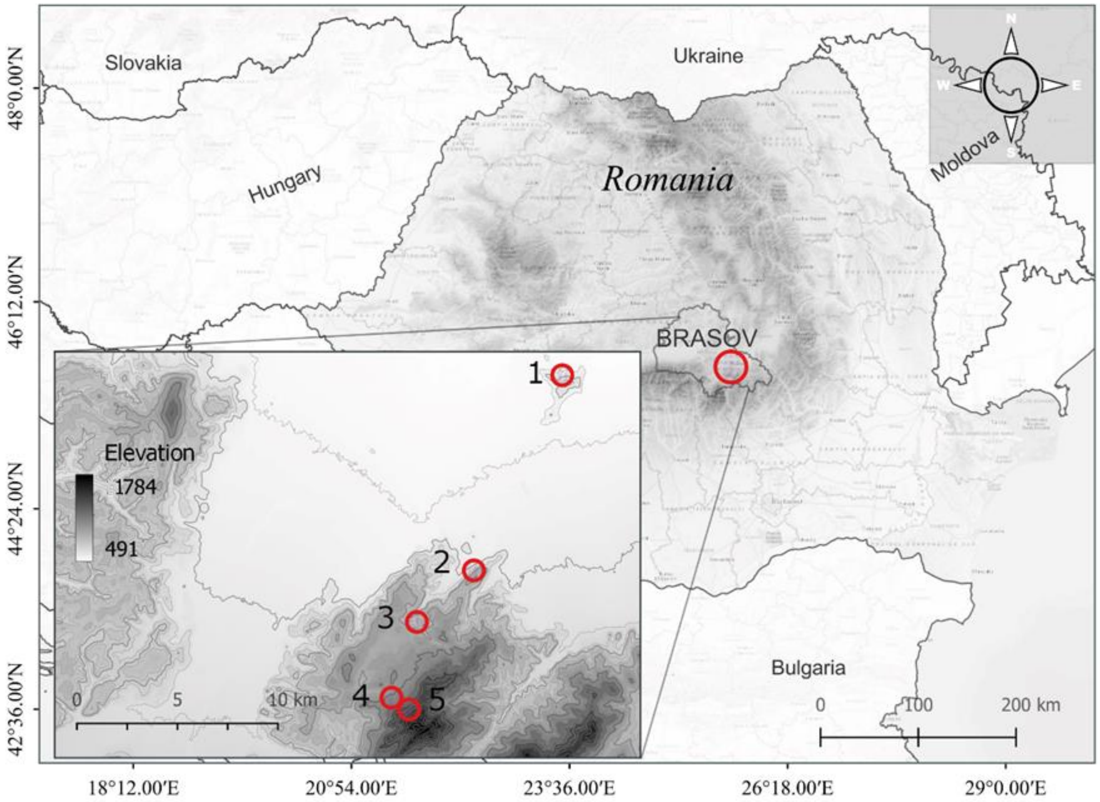


Fig. 3.1 - Geographical location of the study sites distributed along the altitudinal transect (1 – Lempeș, 2 – Tâmpa, 3 – Solomon, 4 – P. Lupului and 5 – Ruia; QGis 2024)

3.2. Phenological observations

Phenological field observations were carried out according to the methodology proposed by Vitasse et al. (2009). Each population in each study site was monitored at a frequency of twice a week, from April to June, in the case of spring phenology (even three times a week, to be able to capture rapid transitions from one phenophase to another), and from September to November, in the case of senescence. These observations were always made by the same operator, with the naked eye or with the help of 15x binoculars, from an average distance of 15 m from the tree.

Spring phenology was evaluated according to Vitasse's scale, which includes four stages of development (Table 3.1). A tree was assigned a stage based on the majority weight (>50%) of buds in the crown's upper third. A population was assigned a stage based on the mean of the 30 individuals in each study area. Depending on the percentage of coverage of the green color, this qualitative scale for evaluating the process of bud development and leaf appearance was converted into a quantitative one to have a higher precision in the delimitation of the stages and to bring it to the exact measurement unit as that of senescence. This method also proved effective in the study of Alberto et al. (2011), obtaining promising results.

Table 3.1 – Phenological stages of bud development and leaf unfolding

Code	0	1	2	3
Phenological stage in the field				
Phenological stage	Dormant winter bud	Bud swollen	Bud burst	At least one leaf unfolding
BBCH correspondent	BBCH 00	BBCH 01	BBCH 07	BBCH 09

Green Color Coverage Range (%)	<25	26-50	51-75	>75

Senescence (%CFL) was calculated based on two variables evaluated in the field, the percentage of yellowed/colored leaves and the percentage of fallen/missing leaves, according to formula (1):

$$\%CFL = (\%CL \times (100 - \%FL))/100 + \%FL,$$ *Formula 3.1*

where: %CFL – percentage of yellowed and fallen leaves

%CL – percentage of yellowed leaves

%FL – percentage of fallen leaves

A tree was assigned a percentage of %CL and %FL based on the majority proportion (>50%) of leaves in the crown's upper third. A study area was assigned a percentage of %CL and %FL based on the mean of the 30 individuals.

The beginning of an individual's growing season was associated with reaching stage 2 according to the scale of bud development, respectively, that of bud burst (Chesnoiu & Sofletea 2009, Schieber et al. 2013). This association is justified because the field operator can precisely identify this stage than the first (bud swollen) during the phenological observations. Its equivalent stage in the BBCH scale is that of BBCH 09, sharing the exact name of the "bud burst" (Hack et al. 1992). The start of senescence was associated with the yellowing of the leaves in the upper third of the crown, in a proportion of 50% (Čufar et al. 2012, Schieber et al. 2017). The length of the growing season was associated with the period between the initiation into the growing season (buds burst) and the 50% yellowing of the leaves (Drobyshev et al. 2010).

3.3. Meteorological data

The process of acquiring meteorological data differed for each of the 3 years of conducting the studies. In the first year, 2021, raw data were extracted from weather stations near the study sites. In the second year, 2022, 3 sensors were installed in each study site, inside the stands, to record the values of temperature (◦C) and relative humidity (%) in the air (two *HOBO* model loggers and one *iButton*. Later, these meteorological data were downloaded through stations and software dedicated to each model (*HOBOware*, respectively *OneWireViewer*).

Each sensor was configured to record temperature and relative humidity values at a frequency of 30 minutes (48 values/day) and then compared with those downloaded from the same meteorological stations. Meteorological data for 1970 – 2000 were also downloaded from the WorldClim platform (Fick & Hijmans 2017) to compare the data sets and capture a possible trend of temperature increase. Later, these raw data were processed and processed, obtaining several meteorological indices: daily average temperature (◦C), daily maximum temperature value (◦C), daily minimum temperature value (◦C), daily average relative humidity (%), daily maximum relative humidity (%) and daily minimum relative humidity (%). For the spring phenology, the GDD ("growing degree days") index was calculated, extracting the thermal thresholds of 5 ◦C for GDD_5 and 10 ◦C for GDD_10 from the average daily temperature values (McMaster & Wilhelm 1997) and CDD ("cumulated degree days"), extracting the 0 ◦C thermal thresholds (Grigorieva et al. 2010). Based on the concept of the specific accumulation of GDD required for the start of each phenophase approached by Zafarian et al. (2019), the meteorological indicator ADD (eng. "autumn degree days") for autumn phenology was also designed, being calculated by extracting the thermal threshold of 0 ◦C from the average daily temperature value and accumulating the remaining values.

3.4. Phenological observations performed through remote sensing

3.4.1. Time series data collection and drone image processing

Obtaining images through drone

Simultaneously with the field phenological observations, aerial images were collected using a remote sensing device, a drone. These overflights were carried out only in two of the five study areas, Solomon and P. Lupului, and targeted all 60 trees. For this, retrieving the exact GPS coordinates of target trees was necessary, using the *Global Navigation Satellite System* (GNSS 2008) navigation equipment.

The remote sensing device model used was a *Phantom 4 Pro v2* drone (Fig. 3.2), equipped with a 1-inch CMOS RGB (Red - 660 nm, Green – 550 nm, and Blue – 460 nm) sensor, 20 Mpx resolution (effective pixels) and an 84° FOV lens with 8.8/24 mm lens (equivalent to 35 mm format), f/2.8 – f/11 aperture (Bârliba et al. 2020). Overflights were timed to cover all 60 target individuals in the two study areas using *PIX4Dcapture* software (2022).

Fig. 3.2 - The drone used, the *Phantom 4 Pro v2* model (photo on the left), and establishing the overflight route over the target trees (photo on the right) (Ciocîrlan 2022)

Thirty-two overflights were carried out (16 for each study area) during the 2021 vegetation season, 14 of which were validated. Thus, a time series that captures the dynamics of spring and autumn phenophases was obtained.

Processing of images obtained with drone

The collected images were processed using *OpenDroneMap* (OpenDroneMap v1.1.0), an open-access software, resulting in orthorectified images for each flight. These products were subsequently georeferenced using field control points and *QGis* software (QGis v3.26.3. 2017). In each of these images, the shaded portions were identified by applying a filter on the pixels so that they correspond to a certain range of values specific to the RGB model (initially estimated from the sample identification), later being replaced by null values, using the *raster* package (Hijmans & van Etten 2012) from the *R* software (R Project v4.1.4. 2022) (Fig. 3.3).

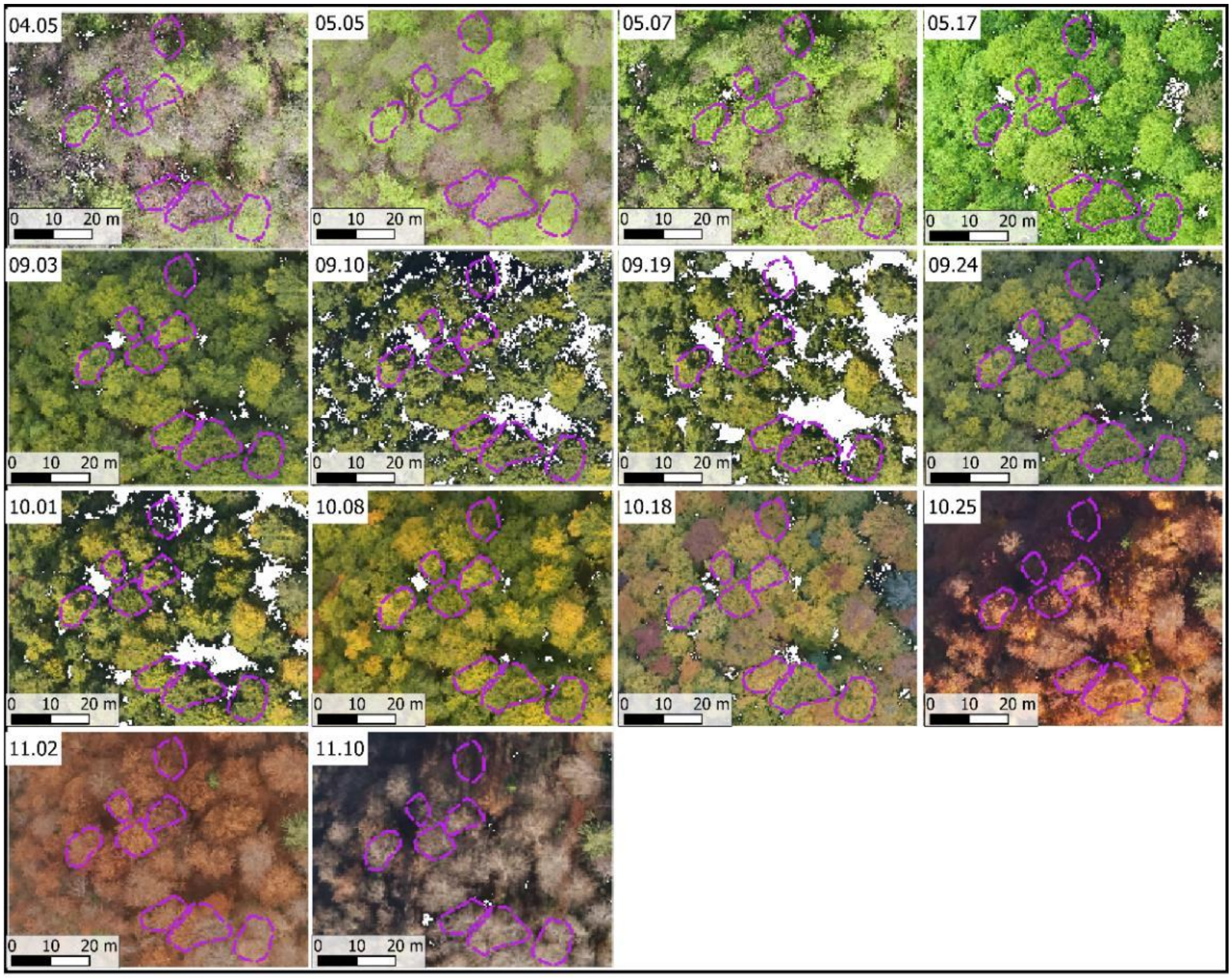


Fig. 3.3 - Time series captures of individuals monitored in the Solomon study site at different phenological stages (month and day in the upper left corner) after removing shaded portions and converting their pixel values to null values. The dashed magenta portions represent the delineation of the crown and were drawn to retrieve the wavelength values of the RGB band.

Crown delineation was done manually for each of the 60 target trees; then, using the *rgdal* package (Bivand et al. 2023), several vegetation indices were calculated (Table 3.2), and the mean, median, and standard error values were extracted for each pixel in each image.

Table 3.2 – Vegetation indices used

No.	Name	Abbreviation	Formula	Reference
1	Digital number for red band	R	red/ 255	Pearson et al. 1976
2	Digital number for green band	G	green/255	Pearson et al. 1976
3	Digital number for blue band	B	blue/255	Pearson et al. 1976
4	Green Red Ratio Index	GRRI	G/R	Fuentes et al. 2001
5	Blue Green Ratio Index	BGRI	B/G	Zarco-Tejada et al. 2005
6	Green Blue Difference Index	GBDI	$G - B$	Kawashima & Nakatani 1998
7	Red Blue Difference Index	RBDI	$R - B$	Kawashima & Nakatani 1998
8	Excess of green index	ExG	$2G - R - B$	Woebbecke et al. 1995
9	Grayscale index	GRAY	$(R + G + B)/ 3$	Wang et al. 2014
10	Chromatic coordinates for red/ Normalized red of RGB	NRRGB	$R/ (R + G + B)$	Woebbecke et al. 1995
11	Chromatic coordinates for green/ Normalized green of RGB	NGRGB	$G/ (R + G + B)$	Woebbecke et al. 1995
12	Chromatic coordinates for blue/ Normalized blue of RGB	NBRGB	$B/ (R + G + B)$	Woebbecke et al. 1995
13	Normalized Green Red Difference Index	NGRDI	$(G - R)/ (G + R)$	Rouse et al. 1974
14	Kawashima index	KAW	$(R - B)/ (R + B)$	Kawashima & Nakatani 1998
15	Normalized Green Blue Difference Index	NGBDI	$(G - B)/ (G + B)$	Hunt et al. 2005
16	Green Leaf Index	GLI	$(2G - R - B)/ (2G + R + B)$	Louhaichi et al. 2001
17	Modified Green Red Vegetation Index	MGVRI	$(G^2 - R^2)/ (G^2 + R^2)$	Bendig et al. 2015
18	Red Green Blue Vegetation Index	RGVBI	$(G - B \times R)/ (G^2 + B \times R)$	Bendig et al. 2015
19	Visible Atmospherically Resistant Index	VARI	$(G - R)/ (G + R - B)$	Gitelson et al. 2002

The dataset used for phenology prediction consists of a matrix of 60 individuals during 16 drone overflights throughout the growing season (of which 14 were validated), 960 observations, and 48 predictors.

Based on the orthorectified images, each tree was evaluated according to the same methodology described in Table 3.1. This allowed us to obtain quantified data in the same unit of measure and compare the phenological data from the field with those obtained by the drone.

3.4.2. Time series data collection and image processing obtained through the *Copernicus* platform

Data collection

The products in raster format, available on the *Copernicus Land Monitoring* portal, were collected in 19 captures for each study site from April to November 2021. The *Sentinel-3 OLCI* satellite provides biophysical parameters at a resolution of 300 m at a frequency of 10 days. To estimate the phenological stage at the level of each study site, the pixel values of some biophysical parameters were extracted (Table 3.3) for similar periods in which the phenological observations were made in the field.

Table 3.3 – Biophysical parameters extracted from the *Copernicus* platform used in phenology monitoring.

No.	Denumire	Abreviere	Descriere
1	Dry Matter Productivity	DMP	The overall growth rate or dry biomass increase of the vegetation (kg/ha/day)
2	Fraction of Absorbed Photosynthetically Active Radiation	FAPAR	Quantifies the fraction of the solar radiation absorbed by live leaves for photosynthesis activity
3	Fraction of Vegetation Cover	FCover	Fraction of ground covered by green vegetation
4	Leaf Area Index	LAI	Half the total area of green elements of the canopy per unit of the horizontal ground area
5	Normalized Difference Vegetation Index	NDVI	Indicator of the greenness of the biomass

Processing of biophysical parameters and satellite images obtained through the *Copernicus* platform

The data set related to the biophysical parameters collected during the vegetation season of 2021 consisted of 95 rasters (5 study areas x 19 captures), from which average pixel values were extracted for each study site using the *rgdal* package (Bivand et al. 2023) in the *R* software (R Project v4.1.4. 2022).

3.5. Evaluation of the architectural characteristics of trees

3.5.1. Assessment of architectural characteristics of trees through remote sensing (TLS)

For the evaluation of the architectural characteristics of 55 beech individuals from the first four study areas (Fig. 3.1), the following non-destructive terrestrial laser scanning (TLS) method was adopted. The *GeoSlam Horizon Mobile Scanner* was used to capture the 3D model of the target tree structures in detail. For each target tree, GPS coordinates were extracted to georeference the 3D model of the entire scanned surface.

The scanning procedure consisted of performing a 360° tour around the target tree, obtaining a consistent point cloud. This minimized interference and occlusion caused by neighboring trees and the angle at which the field operator took the scans, ensuring complete coverage of the structure of a target tree.

After the field scans, the point clouds' raw data were downloaded and processed in the *CloudCompare* software. The following steps were performed to prepare the data analysis: separation of vegetation and soil points, manual segmentation of target trees, and noise filtering (Fig. 3.4).

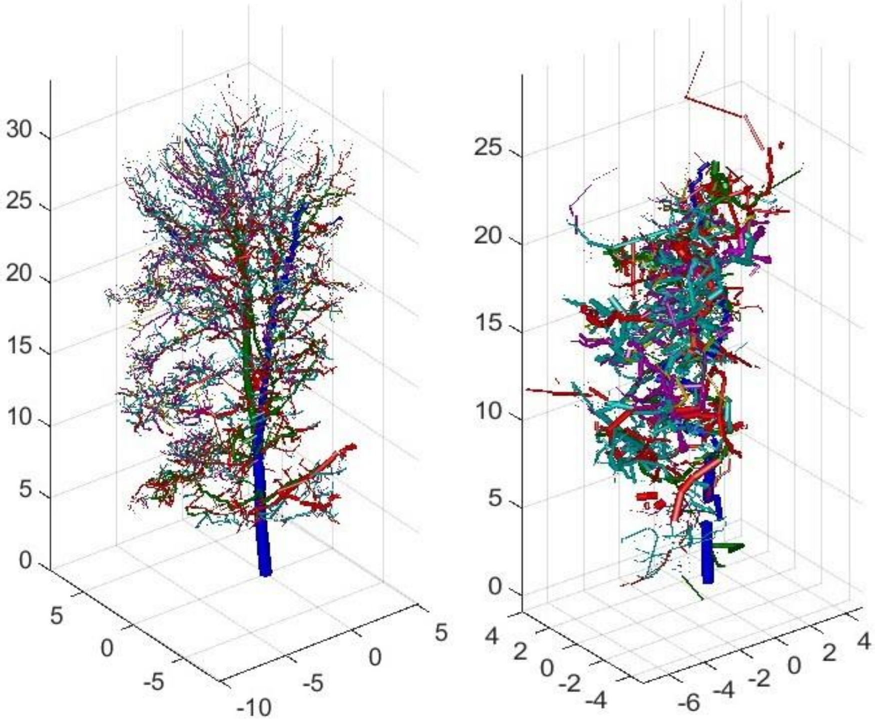


Fig. 3.4 - Noise segmentation and filtering to obtain the architectural characteristics of the target trees, as detailed as possible (examples of a validated and an unvalidated case) (Ciocîrlan 2024)

The processed point clouds were subsequently imported into the *Tree Quantitative Structure Models* (TreeQSM) software, implemented in *MATLAB* (Toh et al. 1999). Thus, the quantitative extraction of the tree's architectural characteristics was allowed. The extracted tree architectural features are total volume, trunk volume, branch volume, tree height, trunk height, branch length, maximum branching order, DBH, and crown diameter (mean and maximum value) (Table 3.4).

Table 3.4 – The analyzed architectural characteristics of beech trees

Tree characteristics	Formula/ Method	Reference
Total volume	The sum of all cylinder volumes (m^3)	TreeQSM
Trunk volume	Volume of the stem (m^3)	TreeQSM
Branch volume	The volume of all the branches (m^3)	TreeQSM
Tree height (H)	The distance between the base and the top of the highest branch of the tree (m)	TreeQSM
Trunk height	The distance between the base and the top of the highest first-order branch of the tree (m)	TreeQSM
Branch length	The sum of the length of all the branches (m)	TreeQSM
Number of branches	The sum of all the branches (pcs.)	TreeQSM
Max branching order	Maximum branching order	TreeQSM
DBHqsm (DBH)	Diameter at breast height in m, the diameter of the cylinder	TreeQSM
Crown diameter (average)	The projection reduced to the horizon of the crown, the average value of two perpendicular directions, in m	TreeQSM
Crown diameter (maximum)	The projection reduced to the horizon of the crown, the maximum value of two perpendicular directions, in m	TreeQSM

3.5.2. The forking of the trees

Tree forking was assessed according to the *TreeBreeDex* protocol (Ducci et al. 2012). Each individual was assigned a class based on the presence or absence of branching and their number and position on the trunk.

3.5.3. The slenderness of the trees

The slenderness coefficient (SLC) was calculated as the ratio of the total height of a tree to its diameter measured at 1.30 m (Wang et al. 1998). The two biomass indicators were quantified in the same unit of measure (m) (Formula 3.2).

$$SLC = \frac{H}{DBH},$$ *Formula 3.2*

where: *SLC* - slenderness coefficient; *H* – total height of the tree (m); *DBH* – diameter measured at 1,30 m (m).

3.5.4. Competition assessment

The intensity of competition exerted on target trees was quantified using the *Hegyi* index (Hegyi 1974). The variables used in calculating this competition indicator were the diameters of the target trees' neighbors, with a diameter bigger than 7 cm (DBH), and the distance between the trees of interest and their competitors within a radius of 15 m (Formula 3.3).

$$(z) = \sum_{i=1}^{n} \frac{D_i}{D_j} \times \frac{1}{Dis_{ij}},$$ *Formula 3.3*

unde: *j* – arborele țintă; *i* – competitor al *j*, pe o rază de 15 m *;* D – DBH (cm); D*is* – distanța dintre *j* și *i* (m).

3.6. Heterozygosity index

DNA was isolated from the buds of 55 beech individuals, collected in 2020, using the DNeasy Plant Mini Kit extraction kit (Qiagen, Hilden, Germany). The genetic composition of the samples was expressed using six nuclear microsatellites (simple repetitive sequences, SSRs) (Asuka et al. 2004; Pastorelli et al. 2003; Vornam et al. 2004) and six replicated gene-based sequences (EST)-SSRs (Kubisiak et al. 2009; Burger et al. 2018). The genetic composition and marker analysis of more individuals, including the 55 ones for which architectural characteristics were also collected (described in subsection 3.5.1), are described in more detail by Grigoriadou Zormpa et al. (2024). Heterozygosity was calculated at the individual level as the number of heterozygous markers relative to the total number of analyzed markers (in the current case, 12).

3.7. Statistical analyses

3.7.1. Phenological data

For the variance study, the changes along the altitudinal gradient were analyzed at the level of each phenological stage (bud swollen, bud burst, at least one leaf unfolding, the beginning of leaf yellowing, the end of leaf yellowing, the beginning of leaf fall, the end of leaf fall) as well as for the length of the growing season, for all 3 monitored years. They were graphically illustrated using *R* software (v. 4.3.1; R Core Team 2021) and the packages *dplyr* (Wickham et al. 2023) and *ggplot2* (Wickham 2016). The *Shapiro-Wilk* normality test (Shapiro & Wilk 1965) and *Kruskal-Wallis* (Kruskal & Wallis 1952) non-parametric comparison of the mean between groups were performed to identify differences between populations/study sites during spring and autumn phenology, illustrated using packages *ggsignif* (Kassambara 2023), *ggsignif* (Constantin & Patil 2021) and *tidy* (Wickham & Girlich 2022). Subsequently, *Dunn*'s test (Dunn 1964) was applied to reveal significant differences between populations/ study sites. Testing the correlation between the dynamics of phenology and the previously mentioned meteorological data was performed using a correlogram, using *R* software with the packages *ggplot2* (Wickham 2016), *dplyr* (Wickham et al. 2023), *factoextra* (Kassambara & Mundt 2020), *ggcorrplot* (Wei & Simko 2021), *Hmisc* (Harrell 2023), *PerformanceAnalytics* (Peterson et al. 2018) and *RColorBrewer* (Neuwirth 2014).

3.7.2. Statistical analyses of phenological data obtained through remote sensing devices

Phenology prediction using linear regression analysis

Two methods were used to build a reproducible model to predict phenology. The first model predicts phenology as the proportion of leafy tree cover at the individual level through vegetation indices calculated from drone imagery and using location (study area) and date (time of field phenological observations and drone overflights) as predictors. The second model assumed the prediction of an average value for each study site related to the biophysical parameters collected from the *Copernicus* platform. Subsequently, multiple regression models were tested to identify the best-fit predictors and their interaction (Duncan et al. 1986).

Phenology prediction using non-linear regression analysis

A machine learning algorithm of the *random forest* type was implemented to predict phenological stages at the individual level using aerial images. This algorithm uses random samples from the initial data set and builds a model that identifies the most appropriate function, repeating this operation to maximize efficiency. In this case, the algorithm based on non-linear regression refers to the original data set and provides results based on average predictions.

Before processing, the data series were converted to obtain values in the range (0 – 1), and seasonal activity, the transition between seasons, was chosen as a predictor. This algorithm was implemented using the *caret* package (Kuhn 2008) in *R* software, building the model relative to the original calibration data set (at 80%, 48 trees) and tested on the remaining 12 individuals.

3.7.3. Statistical analyses of the association of phenological, genetic, and architectural data obtained by terrestrial laser scanning

The significance thresholds of the correlation coefficients for the 55 individuals analyzed, calculated based on the limit values of the significant correlation coefficient (Table 12) (Giurgiu 1972) from * ($p < 0.05$) - $r > 0.266$, ** ($p < 0.01$) $r > 0.3633$ and *** ($p < 0.001$) - $r > 0.432$.

RStudio software (version 4.2.2.) was used to extract the R^2 and *p* values of the architectural characteristics of the analyzed individuals after processing the raw data in the *TreeQSM* software.

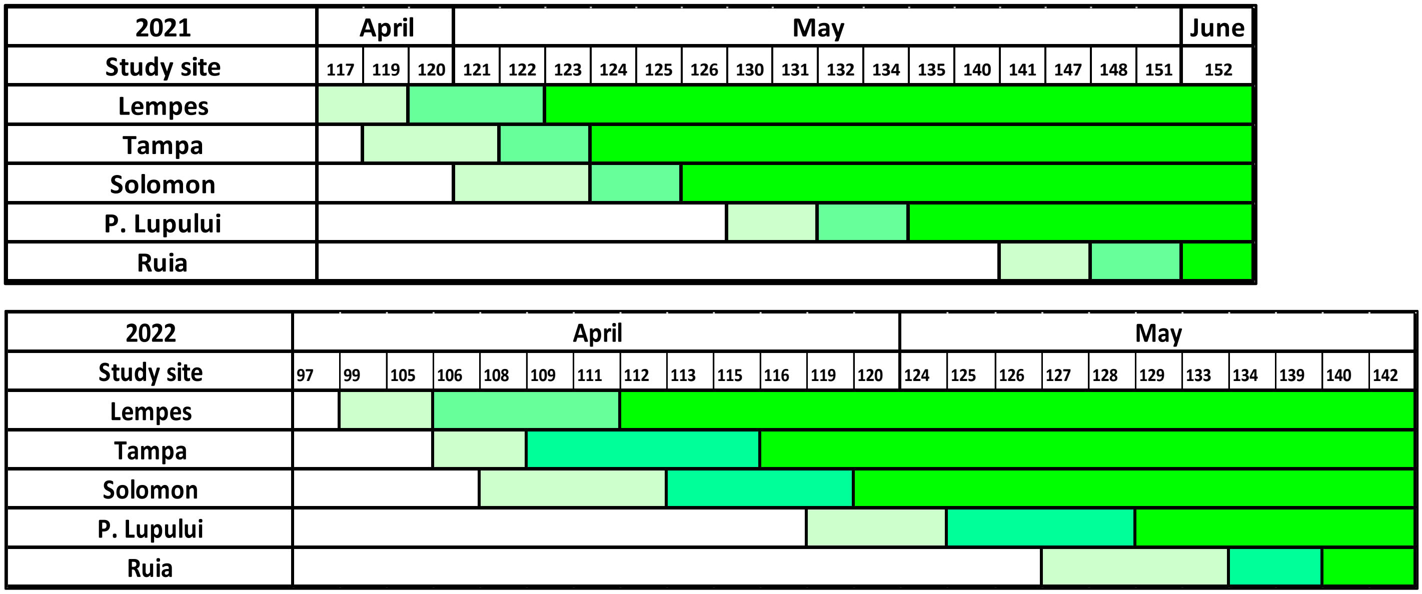
Also, the *RStudio* interface was used to correlate the data on phenological, genetic, and architectural characteristics through the function *rcorr* (*R* package *corrplot*). The use of this function led to the design of a *matrix-type* correlogram, which allows both the visualization of the values of the correlation coefficients and their significance (according to a color gradient), as well as the testing of the null hypothesis (the value of the correlation coefficient $r = 0$). The obtained results were graphically illustrated using the *R* package *ggplot2*, in the form of a correlation matrix.

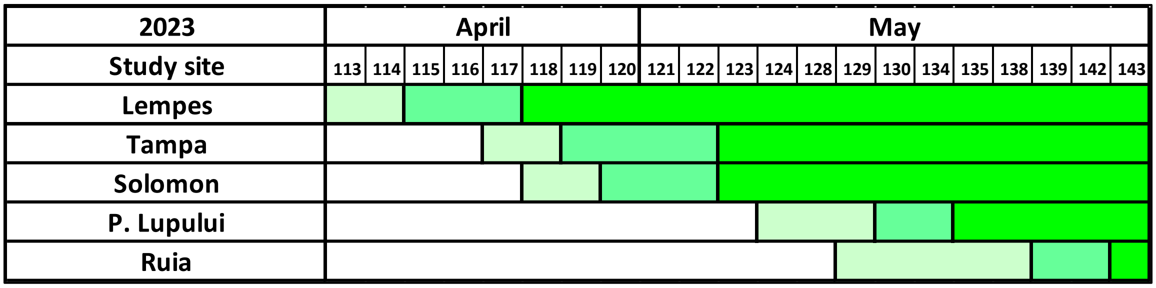
4. RESULTS AND DISCUSSION

4.1. Phenological observations

4.1.1. Spring phenology

According to the adopted methodology, the dynamics of the phenophases of spring phenology were monitored during the 3 years (2021, 2022, and 2023) in the 5 study sites. A difference of 14 days was recorded between the first two years, within the Lempeș population, located at the lowest limit of the altitudinal gradient, respectively of 9 days between 2022 and 2023 (Fig. 4.1), regarding the start of the growing season (moment associated with obtaining the stage of bud burst). Within the population located at the highest limit of the gradient, Ruia, a difference of 14 days was recorded between the first two years, 2021 and 2022, respectively, of 5 days between 2022 and 2023. During the 3 years, the populations obtained stage 2 (bud burst) differently. In the first two years, a difference of 29 days was recorded between the populations located at the extremes of the altitudinal profile, respectively 25 days in the third. In the first year, the duration of the transition between the first three phenophases, from bud swollen to bud burst, respectively from bud burst to at least one leaf unfolding, varied from 6-7 days (12 days in the case of the population located at the highest limit of the transect, Ruia), 11-13 days in 2022 (14 days in Ruia) and 6-12 days in 2023 (15 days in Ruia), resulting in a more accelerated spring phenology dynamics in 2021 compared to the other two. However, the start of the growing season occurred later (120 D.O.Y. in 2021, compared to 106 D.O.Y. in 2022 and 115 D.O.Y. in 2023, the average values for the population located at the lowest limit of the altitudinal profile, Lempeș).





Dormant winter bud (0)	Bud swollen (1)	Bud burst (2)	At least one leaf unfolding (3)

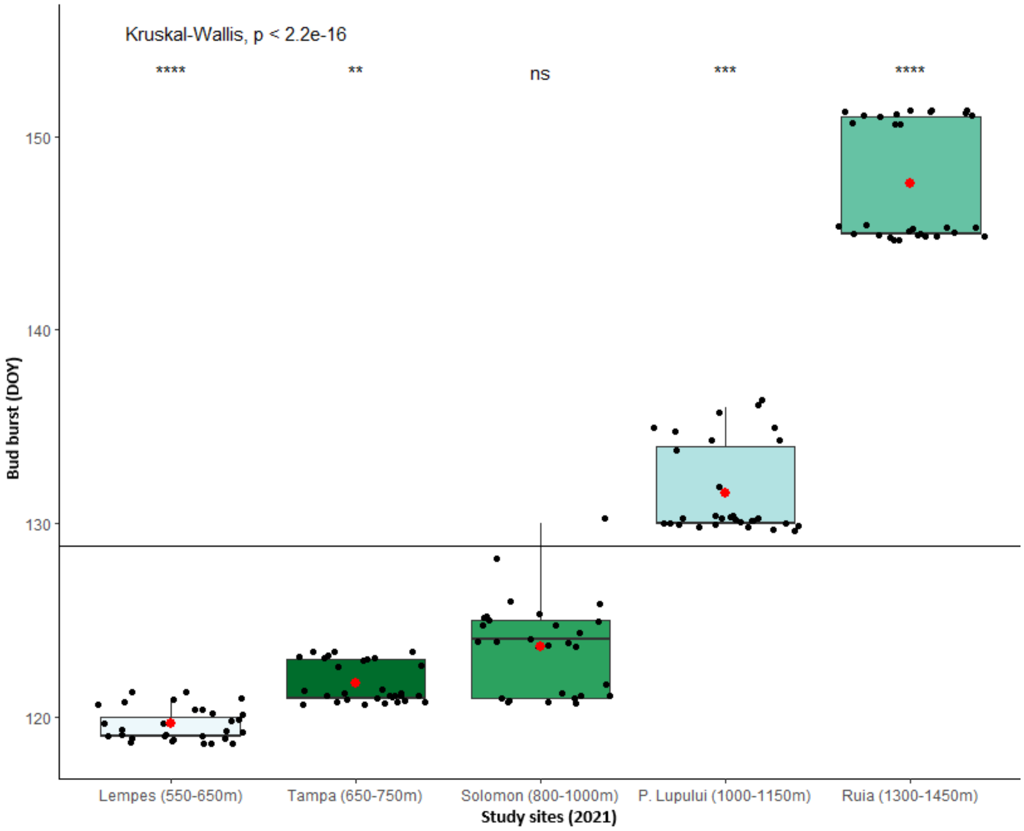
Fig. 4.1 - The dynamics of the spring phenology phenophases of the three monitored years in each study site, based on average values during the D.O.Y. (Day of the year).

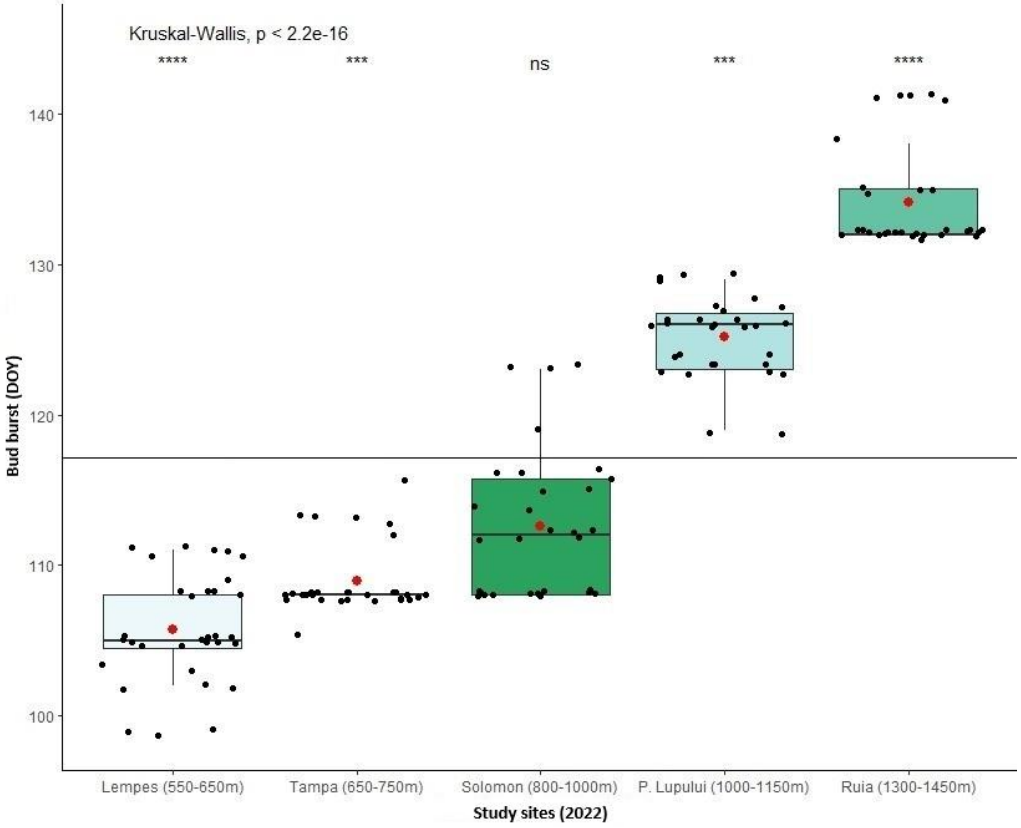
Bud burst

Following the normality testing of the data set related to the phenological stage of bud burst, using the *Shapiro-Wilk* test, significant differences were obtained during the three monitored years in all five study sites (Table 4.1). As a result, the non-parametric *Kruskal-Wallis* test was applied, and the intra- and inter-population variation (Fig. 4.4.) in obtaining stage 2 was significant in all cases. The start of this phenophase varied according to the altitudinal gradient, its onset directly proportional to the increase in altitude. In 2021 and 2022, a difference of 28 days was recorded between the individuals located on the extreme surfaces within this altitudinal profile and 24 days in 2023 regarding the start of the bud burst phenophase.

Table 4.1 – Testing for normality on the data set related to the phenological stage of bud burst during the 3 years monitored in the 5 study sites (*Shapiro Wilk* Test).

Bud burst			
Study site	2021	2022	2023
Lempes	0.06359 x 10^{-4}	0.01304	0.05659 x 10^{-3}
Tampa	0.01023 x 10^{-5}	0.07699 x 10^{-6}	0.0442 x 10^{-8}
Solomon	0.001183	0.03572 x 10^{-2}	0.03816 x 10^{-2}
P. Lupului	0.04093 x 10^{-5}	0.01375	0.0451 x 10^{-3}
Ruia	0.01823 x 10^{-5}	0.01628 x 10^{-5}	0.01951 x 10^{-5}


Kruskal-Wallis, p < 2.2e-16
****
**
ns
***
****
Bud burst (DOY)
150
140
130
120
Lempes (550-650m)
Tampa (650-750m)
Solomon (800-1000m)
P. Lupului (1000-1150m)
Ruia (1300-1450m)
Study sites (2021)


Kruskal-Wallis, p < 2.2e-16
****
***
ns
***
****
Bud burst (DOY)
140
130
120
110
100
Lempes (550-650m)
Tampa (650-750m)
Solomon (800-1000m)
P. Lupului (1000-1150m)
Ruia (1300-1450m)
Study sites (2022)

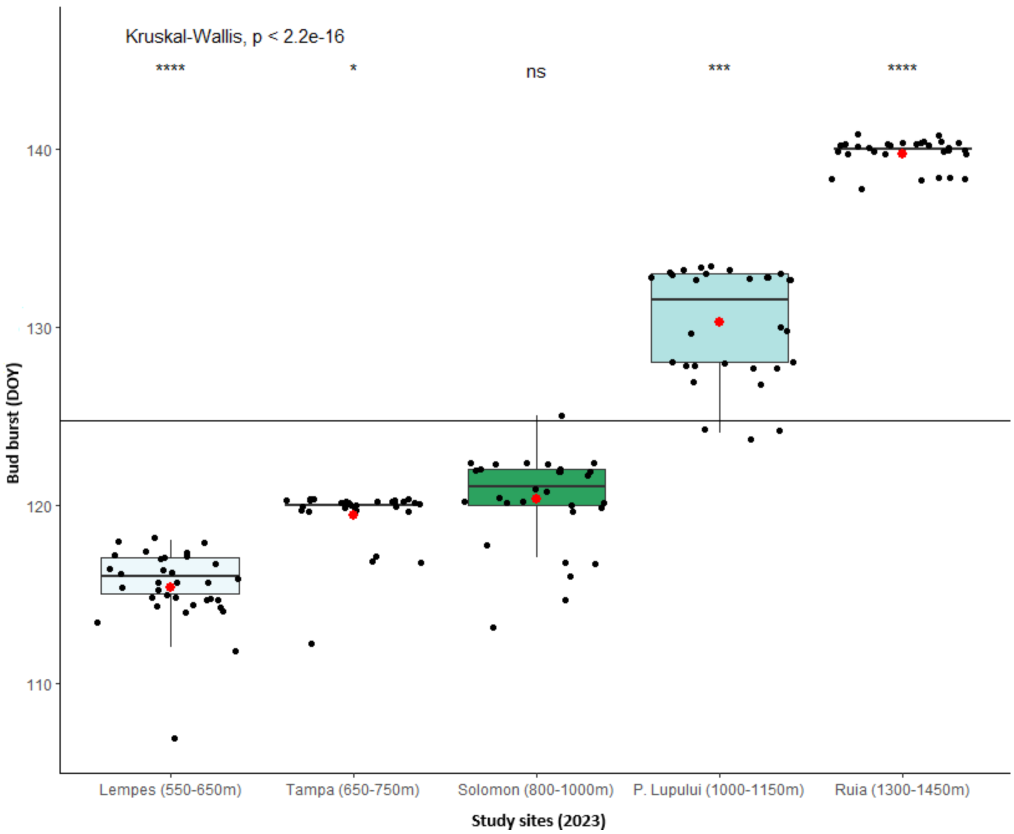


Fig. 4.2 - The intra- and inter-population variation of beech in obtaining stage 2 (bud burst), associated with the start of the growing season, in the five study sites during the three monitored years (*Kruskal-Wallis* test; **** - p ≤ 0.0001, *** - p 0.001, *** - p.01, ≤ 0.0 p. * - p ≤ 0.05 and ns – p > 0.05).

The three-year phenological monitoring reveals significant differences in the beginning of the bud burst phenophase at the interpopulation level, with significant differences between the individuals located in the five study areas, except for those located in the Tampa and Solomon areas in the years 2021 and 2023 (Table 4.2).

Table 4.2 – The interpopulation variation based on the differences between the averages of the five study sites during the three monitored years on the data set related to the phenological stage of bud burst (*Dunn*'s test).

2021	Lempes	Tampa	Solomon	P. Lupului
Tampa	0.0018	X	X	X
Solomon	0	**0.0648**	X	X
P. Lupului	0	0	0.0004	X
Ruia	0	0	0	0.0032
2022	**Lempes**	**Tampa**	**Solomon**	**P. Lupului**

Tampa	0.0314	X	X	X
Solomon	0.0002	0.0496	X	X
P. Lupului	0	0	0.0001	X
Ruia	0	0	0	0.0034
2023	Lempes	Tampa	Solomon	P. Lupului
Tampa	0.0007	X	X	X
Solomon	0	**0.1685**	X	X
P. Lupului	0	0	0.0001	X
Ruia	0	0	0	0.0042

With the increase in altitude, the dynamics of the phenophase of burst buds varied during the three monitored years. For every 100 m increase in altitude, the individuals located along the altitudinal profile required, on average, in the year 2021, approx. 3.5 days more to start in the growing season (reaching phenological stage 2), approx. 3.5 days in 2022 and approx. 3 days in 2023. The values of the coefficients of determination R^2 were very high in the case of the 3 years, 0.92 (2021), 0.9182 (2022), and 0.886 (2023), reflecting a very good fit with some linear regression models (Fig. 4.3).

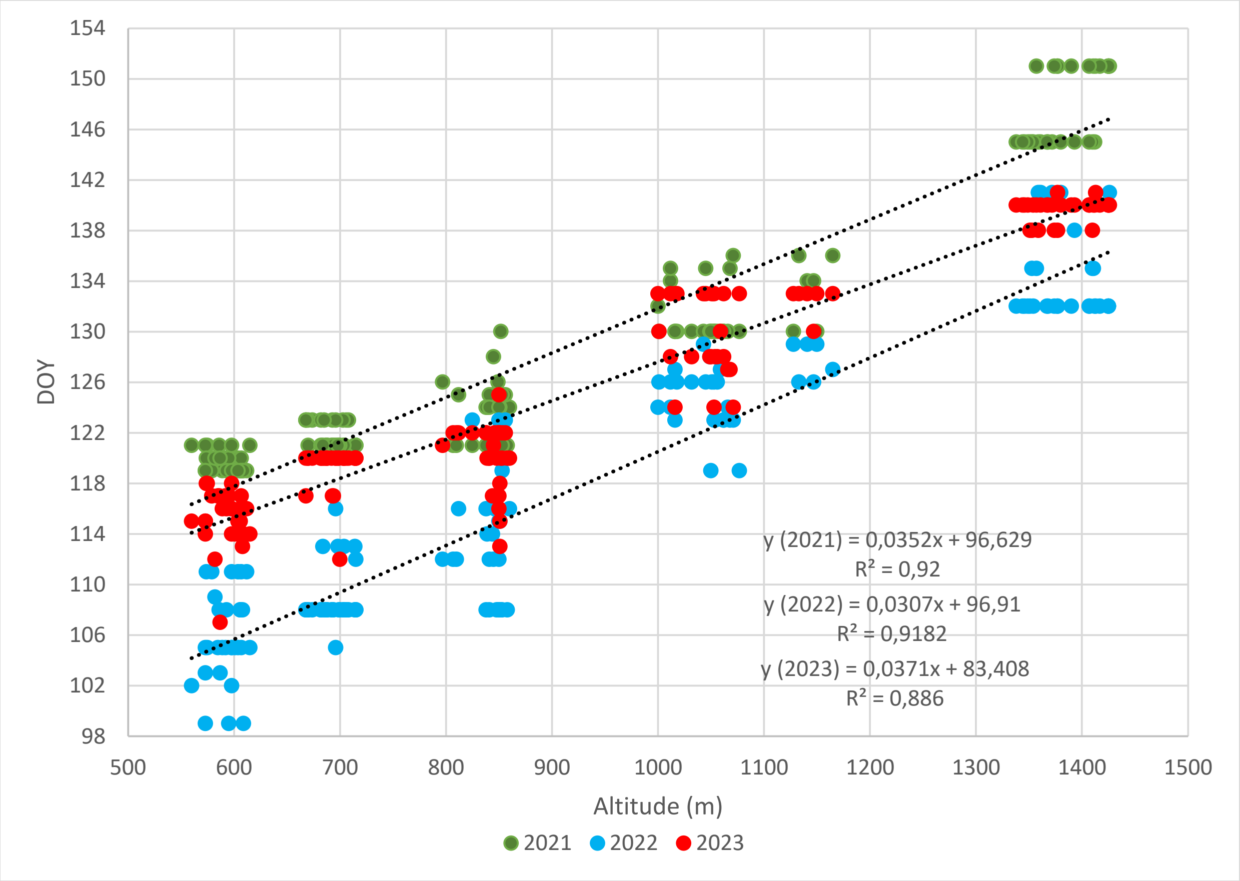


Fig. 4.3 - The dynamics of the phenophase of bud burst in relation to the altitudinal gradient at the individual level for the three monitored years.

4.1.2. Autumn phenology (senescence)

The dynamics of autumn phenology in common beech varied during the three monitored years (Fig. 4.4). Senescence was estimated by quantifying two indicators, which simultaneously represent the two phenophases of autumn phenology: yellowing and leaf falling. The yellowing phenophase of beech leaves extended over a longer period in 2021 (21-32 days) compared to 2022 (18-25 days), respectively 2023 (5-9 days). The second phenophase lasted longer in 2021 (17-27) compared to 2022 (12-18) but less than in 2023 (19-28). The onset of these two phenophases was inversely proportional to the increase in altitude, except in 2021, where the influence of the other seasonal conditions (exposure, air currents, wind, and stand density) was greater.

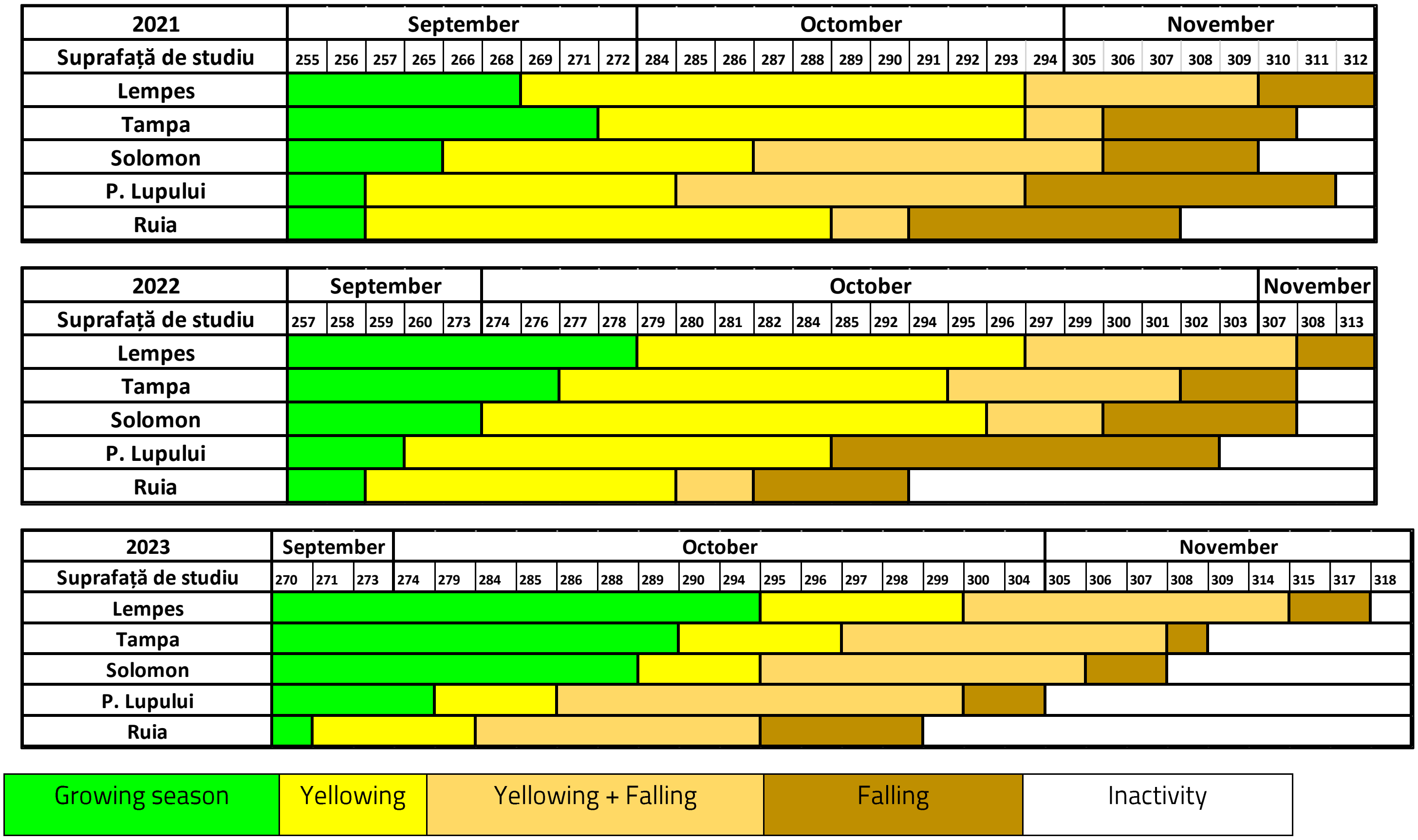


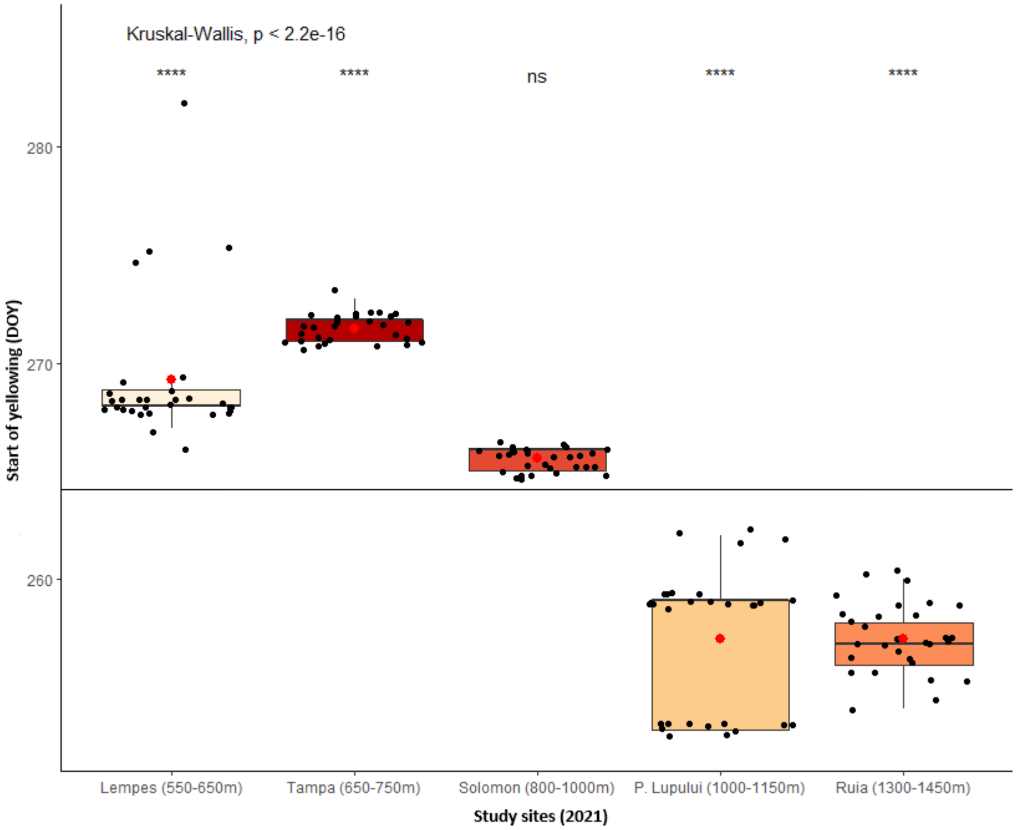
Fig. 4.4 - The dynamics of the phenophases in the autumn phenology of the three monitored years in each study site, based on average values during the D.O.Y. (Day of the year).

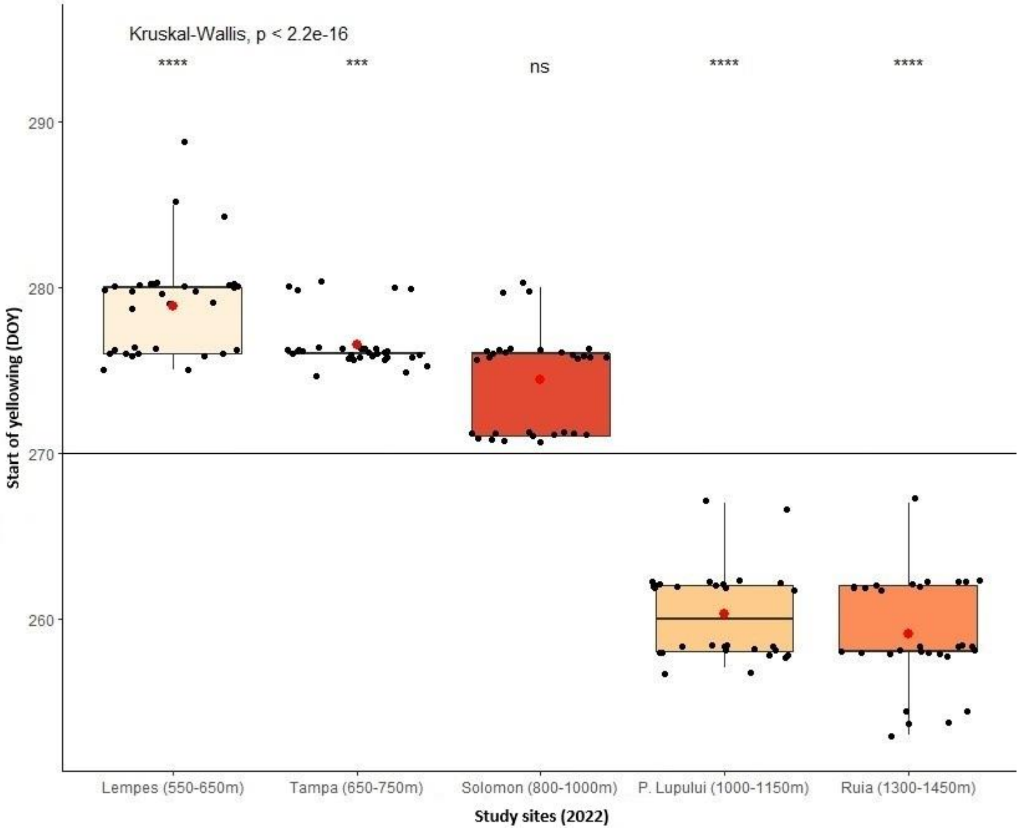
Yellowing of the leaves

Following normality testing on the data set related to the onset of the phenological stage of leaf yellowing, using the *Shapiro-Wilk* test, significant differences were obtained during the three monitored years in all five study areas (Table 4.3). As a result, the non-parametric *Kruskal-Wallis* test was applied, and the intra- and inter-population variation (Fig. 4.5.) at the start of this phenophase was significant in all cases. The onset of this phenophase varied according to the altitudinal gradient, being directly proportional to the increase in altitude, except in 2021, the individuals located in the second sample area within this profile, Tampa, were the ones that had the latest onset of leaf yellowing. Also, in the same year, individuals located on the surfaces of P. Lupului and Ruia, located at the upper altitudinal limit, started simultaneously in this phenophase. In 2021, a difference of 12 days was recorded between the individuals situated on the extreme surfaces within this altitudinal profile, of 20 days in 2022 and 24 days in 2023, regarding the start of the leaf yellowing phenophase.

Table 4.3 – Testing for normality on the data set related to the beginning of the phenological stage of leaf yellowing during the 3 years monitored in the 5 study areas (*Shapiro Wilk* Test).

The onset of yellowing of the leaves			
Study site	2021	2022	2023
Lempeș	0.01602 x 10^{-6}	0.07752 x 10^{-3}	0.06083 x 10^{-2}
Tâmpa	0.03107 x 10^{-4}	0.0173 x 10^{-6}	0.02152 x 10^{-3}
Solomon	0.01823 x 10^{-5}	0.02065 x 10^{-3}	0.02331 x 10^{-2}
P. Lupului	0.01274 x 10^{-3}	0.03142 x 10^{-3}	0.08511 x 10^{-3}
Ruia	**0.1723**	0.001622	0.001461


Kruskal-Wallis, p < 2.2e-16
****
****
ns
****
****
Start of yellowing (DOY)
280
270
260
Lempes (550-650m)
Tampa (650-750m)
Solomon (800-1000m)
P. Lupului (1000-1150m)
Ruia (1300-1450m)
Study sites (2021)


Kruskal-Wallis, p < 2.2e-16
****
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Start of yellowing (DOY)
290
280
270
260
Lempes (550-650m)
Tampa (650-750m)
Solomon (800-1000m)
P. Lupului (1000-1150m)
Ruia (1300-1450m)
Study sites (2022)

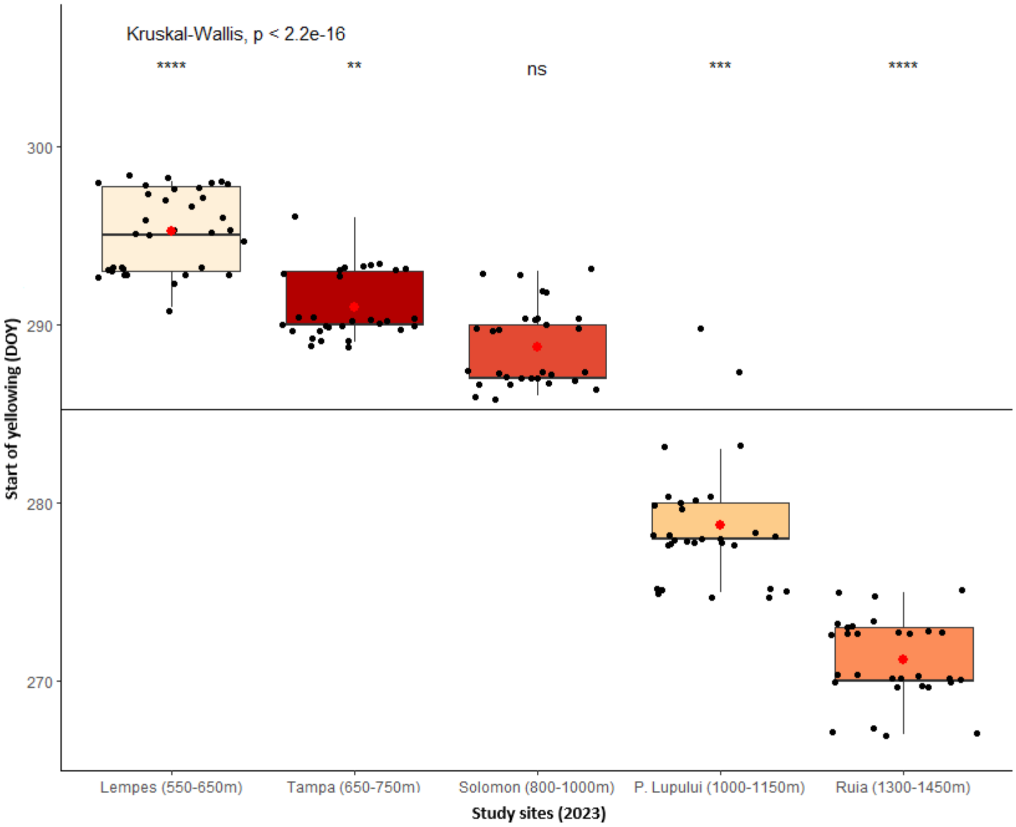


Fig. 4.5 - Intra- and inter-population variation of beech at the beginning of the leaf yellowing phenophase in the five study sites during the three years monitored (*Kruskal-Wallis* test; **** - p ≤ 0.0001, *** - p ≤ 0.001, ** - p ≤ 0.01, * - p ≤ 0.05 and ns – p > 0.05).

The phenological monitoring of the three years reveals significant differences regarding the beginning of the yellowing of the leaves at the interpopulation level, with significant differences between the individuals located in the five study sites, except those located in the Tampa and Solomon areas, in 2022 and 2023, and those located in P. Lupului and Ruia, in 2021 and 2022 (Table 4.4).

Table 4.4 – The interpopulation variation based on the differences between the averages of the five study sites during the three monitored years on the data set related to the phenological stage of the beginning of leaf yellowing (*Dunn*'s Test)

2021	Lempes	Tampa	Solomon	P. Lupului
Tampa	0.023	X	X	X
Solomon	0.0014	0	X	X
P. Lupului	0	0	0	X
Ruia	0	0	0	**0.3941**
2022	Lempes	Tampa	Solomon	P. Lupului
Tampa	0.0449	X	X	X

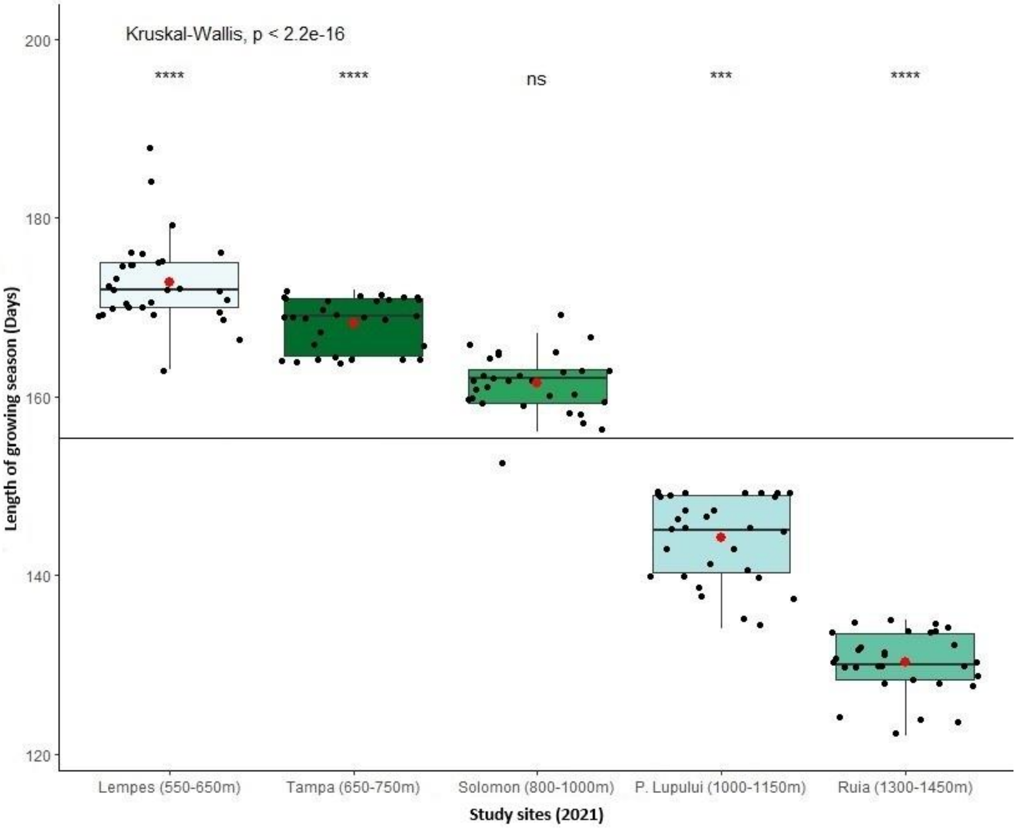
Solomon	0.0017	**0.1075**	X	X
P. Lupului	0	0	0	X
Ruia	0	0	0	**0.3297**
2023	**Lempes**	**Tampa**	**Solomon**	**P. Lupului**
Tampa	0.0012	X	X	X
Solomon	0	**0.0624**	X	X
P. Lupului	0	0	0.0008	X
Ruia	0	0	0	0.0037

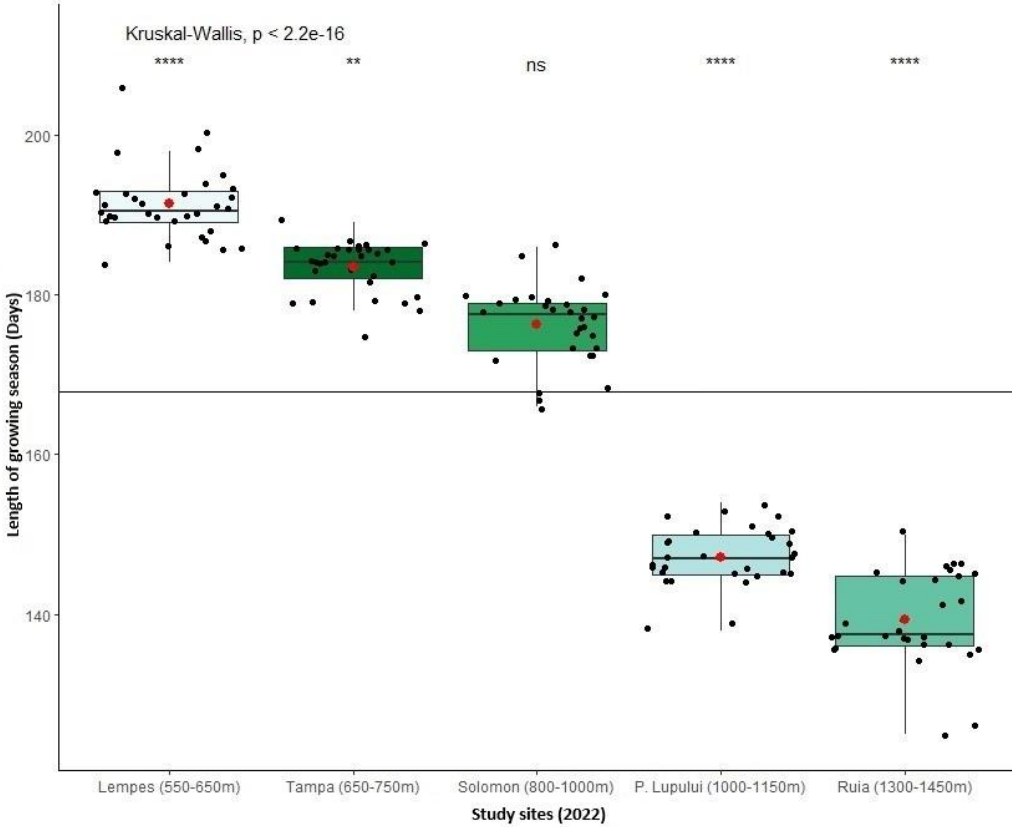
4.1.3. Length of the growing season

The length of the growing season (reported as the period between the achievement of the stage of bud burst and the yellowing of the leaves in the upper third of the crown in proportion to 50%) varied during the three years monitored (Fig. 4.6). Following the testing of normality (*Shapiro-Wilk* test) on the data set related to the length of the growing season (Table 4.5) and the application of the *Kruskal-Wallis* test, during the three years monitored in the five study sites, the intra- and interpopulation variation (Fig. 4.6) was significant in all cases, except for the Solomon site in the first two monitored years and P. Lupului in the last two years. The length of the growing season varied according to the altitudinal gradient, being inversely proportional to the increase in altitude. In 2021, a difference of 43 days was recorded between the individuals located on the limits of the altitudinal transect, 52 days in 2022, and 43 days in 2023, in the length of the growing season.

Table 4.5 - Testing for normality on the data set related to the length of the growing season during the three monitored years in the five study sites (*Shapiro Wilk* Test).

The length of the growing season			
Study sites	2021	2022	2023
Lempeș	0.01013	0.008557	0.02726
Tâmpa	0.01106×10^{-2}	0.005367	0.04114
Solomon	**0.9646**	**0.1927**	0.0141
P. Lupului	0.002449	**0.2367**	**0.3857**
Ruia	0.02563	0.02359	0.03184


Kruskal-Wallis, p < 2.2e-16
****
****
ns
***
****
Length of growing season (Days)
200
180
160
140
120
Lempes (550-650m)
Tampa (650-750m)
Solomon (800-1000m)
P. Lupului (1000-1150m)
Ruia (1300-1450m)
Study sites (2021)


Kruskal-Wallis, p < 2.2e-16
****
**
ns
****
****
Length of growing season (Days)
200
180
160
140
Lempes (550-650m)
Tampa (650-750m)
Solomon (800-1000m)
P. Lupului (1000-1150m)
Ruia (1300-1450m)
Study sites (2022)

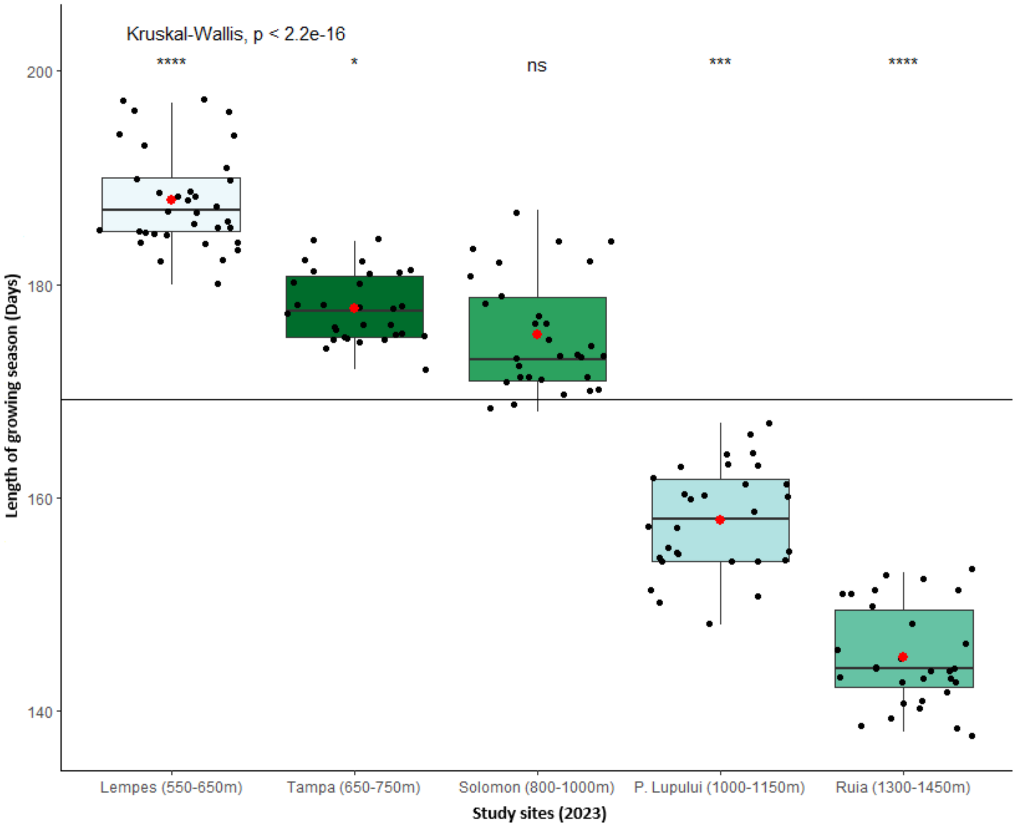


Fig. 4.6 - Intra- and inter-population variation of beech in the length of the growing season in the five study sites during the three monitored years (*Kruskal-Wallis* Test; **** - p ≤ 0.0001, *** - p ≤ 0.001, ** - p ≤ 0.01, * - p ≤ 0.05 and ns – p > 0.05).

The phenological monitoring of the three years reveals significant differences in the length of the vegetation season at the interpopulation level, with significant differences between the individuals in the five study sites, except for those in Tampa and Solomon in 2023 (Table 4.6).

Table 4.6 - The differences between the averages of the five study sites during the three monitored years on the length of the growing season data (*Dunn*'s test).

2021	Lempes	Tampa	Solomon	P. Lupului
Tampa	0.0448	X	X	X
Solomon	0	0.0023	X	X
P. Lupului	0	0	0.0017	X
Ruia	0	0	0	0.0042
2022	Lempes	Tampa	Solomon	P. Lupului
Tampa	0.0021	X	X	X
Solomon	0	0.0134	X	X
P. Lupului	0	0	0.0006	X

Ruia	0	0	0	0.0231
2023	Lempes	Tampa	Solomon	P. Lupului
Tampa	0.0002	X	X	X
Solomon	0	**0.1793**	X	X
P. Lupului	0	0	0.0002	X
Ruia	0	0	0	0.007

4.1.4. The variation of the temperature during the three monitored years

Temperature is the meteorological trigger factor for the bud burst phenophase. When the thermal threshold of 10 °C is exceeded, the beech's growing season starts, but this condition is complementary to exceeding a specific CDD accumulation. During the three years of monitoring, bud burst occurred after accumulating at least 52 CDD in the previous seven days (Fig. 4.7).

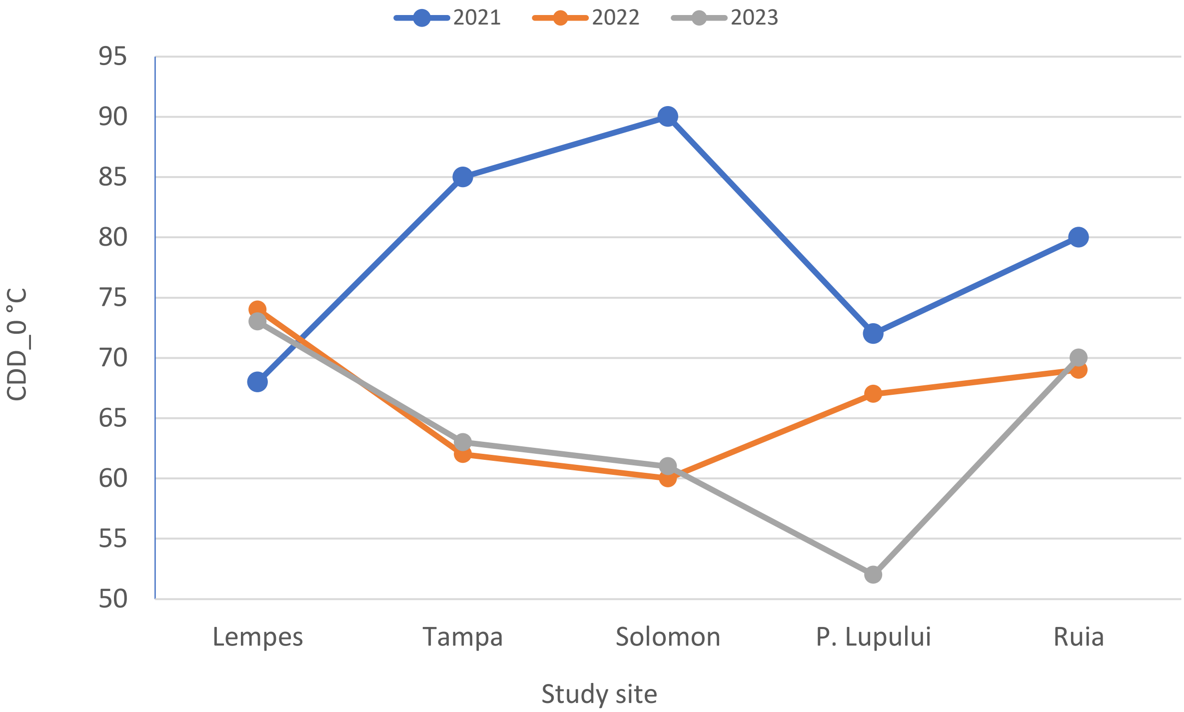


Fig. 4.7 - Cumulative CDD during the three years of monitoring the phenophase of beech bud burst, the moment associated with the start of its growing season, in the last 7 days for each of the five study sites (in order of altitudinal gradient).

Senescence is also influenced by temperature. The first phenophase of autumn phenology, leaf yellowing, is triggered by the accumulation of at least 65 ADD with a thermal threshold of 0 °C over the last seven days (Table 4.7).

Table 4.7 - Accumulation of ADD during the three years of monitoring the yellowing phenophase of beech leaves in the last 7 days for each of the five study sites (in order of altitudinal gradient).

Study site	2021	2022	2023
Lempes	72.14	81.49	76.8
Tampa	80.25	89.79	74.93
Solomon	72.87	85.47	65.83
P. Lupului	86.55	76.69	91.64
Ruia	78.97	78.35	99.96

The second phenophase of beech senescence, the fall of the leaves, is not influenced to the same extent as the first by the accumulation of certain temperatures in the last seven days or by the occurrence of frost. However, it unfolds inversely proportional to the altitudinal gradient, except for 2022 (Fig. 4.8).

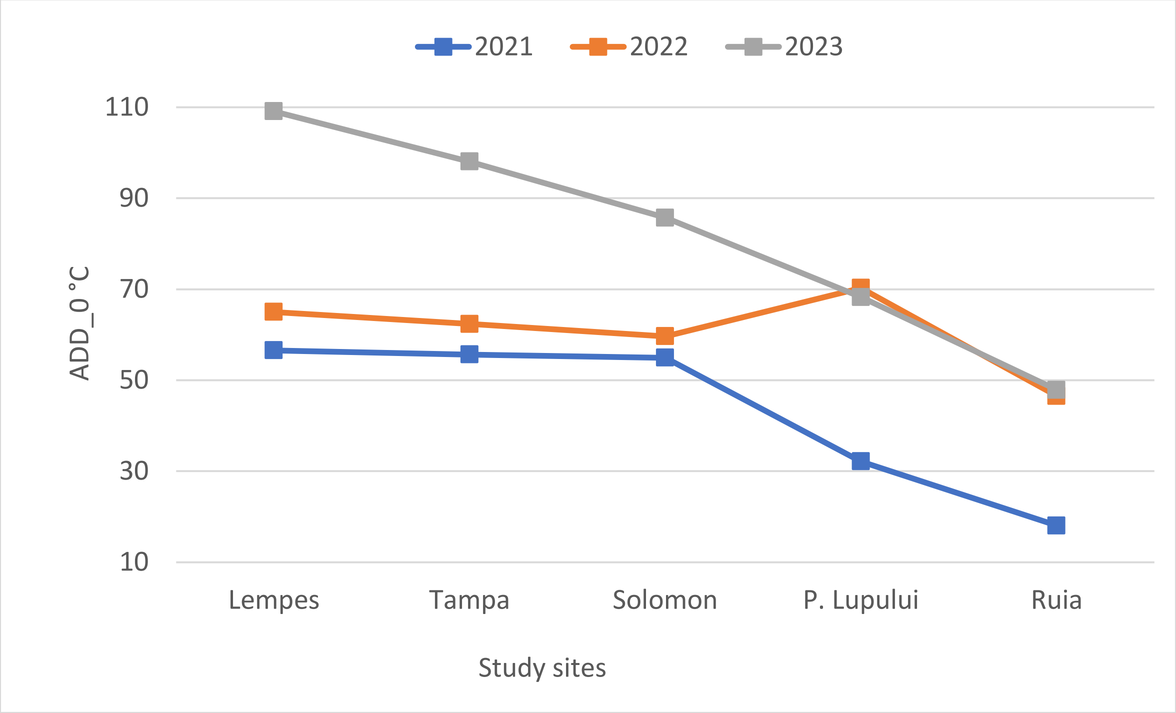


Fig. 4.8 - Accumulation of ADD during the three years of monitoring the leaf fall phenophase in beech in the last 7 days for each of the five study sites (in order of the altitudinal gradient).

4.2. Phenological observations performed through remote sensing

4.2.1. Phenology prediction using aerial drone imagery

Based on the rectified aerial images obtained with the drone, for all 60 individuals in the Solomon and P. Lupului study sites, the phenophases of spring phenology and senescence were estimated as the percentage of leafy tree cover by operators with expertise who were not involved in conducting the phenological observations in the field. The value of the *Pearson* correlation coefficient between the phenological observations made in the field and those collected based on the aerial images obtained with the drone was very high (r = 0.98).

Subsequently, for each tree, from their delimited crowns (Fig. 3.3), the pixel values of the red, green, and blue visible light bands were extracted, the vegetation indices were calculated, and the mean, median, and standard error values were taken, resulting in 864 observations and 50 predictors.

All the values of the *Pearson* correlation coefficients calculated based on the phenological observations in the field and the 19 vegetation indices are illustrated in Appendix 4 (complete thesis version), in a *Pearson* correlogram between the phenological observations in the field and the vegetation indices, where the values of the correlation coefficients, the type of correlation (positive/negative) by the color scale (blue/red) and the size of the circle in which these values are mentioned are illustrated, signifying the probability of the null hypothesis.

After applying these correlations, four vegetation indices recorded values higher than 0.8: GLI (0.86), NGRGB (0.85), RGVBI (0.83), and ExG (0.81), and five obtained values higher than 0.7: MGVRI (0.73), VARI (0.73), NGRDI (0.72), GRRI (0.71), and GBDI (0.70), indicating a close connection.

Based on the relationship between vegetation indices and field phenological observations, several models have been built to predict spring phenology and senescence. To choose the most suitable one, their performance was tested on the training/calibration data set and the test data set relative to the complexity of the models (Fig. 4.9).

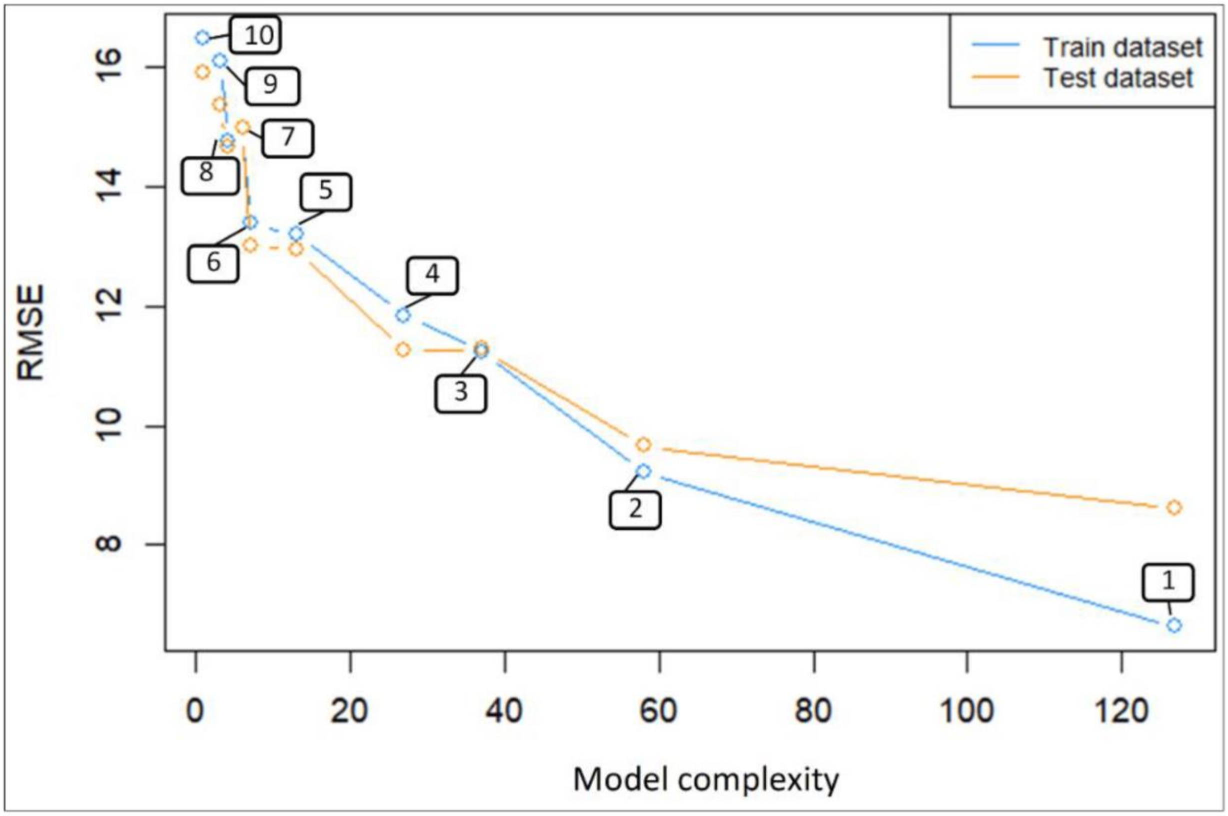


Fig. 4.9 - Complexity of the RMSE models on the calibration and test datasets. Each number corresponds to a specific model, detailed in Table 4.8.

The built models are detailed in Table 4.8, mentioning the RMSE values on the two data sets, the complexity, and their calculation formulas. The complexity of each model consists of the number of parameters involved. Although the first model had a relatively small error (6.63 on the calibration/ training data set and 8.61 on the test data set), it had the highest complexity (127), with the highest tendency to predict leaf cover percentage. Model 10 was only related to GLI, the vegetation index that was found to be most strongly correlated with field phenological observations. Still, when integrated into the model, it obtained a more significant RMSE value of 16.47 on the calibration data set and 15.91 on the test data set. Model 3 recorded close RMSE values on the two data sets (11.24 and 11.27), a relatively reduced complexity compared to the first two, of 37, showing potential in predicting new data sets. The forecast percentage of leafy tree cover compared to that estimated in the field during the phenological observations is mentioned in the two columns related to the RMSEs of the calibration and test datasets. The smaller the difference between the two, the higher the predictive capacity of the data.

Table 4.8 – The summary of the models tested on the two datasets (training & testing).

No.	Train *RMSE*	Test *RMSE*	Model complexity	Independent variable component of the linear model equation
1	6.63	8.61	127	[1]F(x)=NGRGB*GLI*ExG*RGVBI*GBDI*NGBDI*season
2	9.21	9.65	58	[1]F(x)=R+G+B+GRRI+BGRI+GBDI+RBDI+ExG+GRAY+NRRGB+NGRGB+NBRGB++NGRDI+KAW+NGBDI+GLI+MGVRI+RGVBI+VARI
3	11.24	11.27	37	[2]F(x)=NGRGB_m+NGRGB_me+NGRGB_sd+GLI_m+GLI_me+GLI_sd+ExG_m+ExG_me+ExG_sd+RGVBI_m+RGVBI_me+RGVBI_sd+GBDI_m+GBDI_me+GBDI_sd+NGBDI_m+NGBDI_me+NGBDI_sd)*season
4	11.84	11.27	27	[1]F(x)=(NGRGB+GLI+ExG+RGVBI+GBDI+NGBDI)*season*location
5	13.19	12.95	13	[1]F(x)=(NGRGB+GLI+ExG+RGVBI+GBDI+NGBDI)*season
6	13.38	13.02	7	[1]F(x)=(NGRGB+GLI+RGVBI)*season
7	14.99	15.00	6	[1]F(x)=NGRGB+GLI+ExG+RGVBI+GBDI+NGBDI
8	14.76	14.67	4	[1]F(x)=NGRGB+GLI+RGVBI+season
9	16.11	15.35	3	[1]F(x)=GLI*season
10	16.47	15.91	1	[1]F(x)=NGRGB*GLI*ExG*RGVBI*GBDI*NGBDI*season

[1] the independent variables were estimated as the mean pixel value for each crown boundary; [2] the independent variables were estimated as the mean (m), median (me), and standard error (sd) for each crown boundary.

A *random forest* algorithm was calibrated to achieve a regression model that exceeded the limits of the linear model. It had as parameters all the statistical indicators (mean, median, and standard error) of the selected vegetation indices and the time/period of the vegetation season. This model performed very well, explaining 91.1% of the variance and showing a high tendency to overlap the predicted data with the field data, with an RMSE value of only 8.12 on the test data set (Table 4.9).

Table 4.9 – Performance of the *random forest* algorithm on the two datasets (training and testing)

Error type	Test data	Setul de date de testare
MSE	23.11	159.39
RMSE	3.28	8.12

Fig. 4.10 shows the phenological data obtained through field observations and the values the *random forest* model predicted. The red trend line denotes the phenology dataset obtained from the field observations (60 individuals, of which 48 served as the model training dataset and the other 12 as the test dataset), the dark blue line denotes the model-predicted phenology values based on the calibration dataset, and the turquoise line represents the model-predicted phenology values based on the test data set.

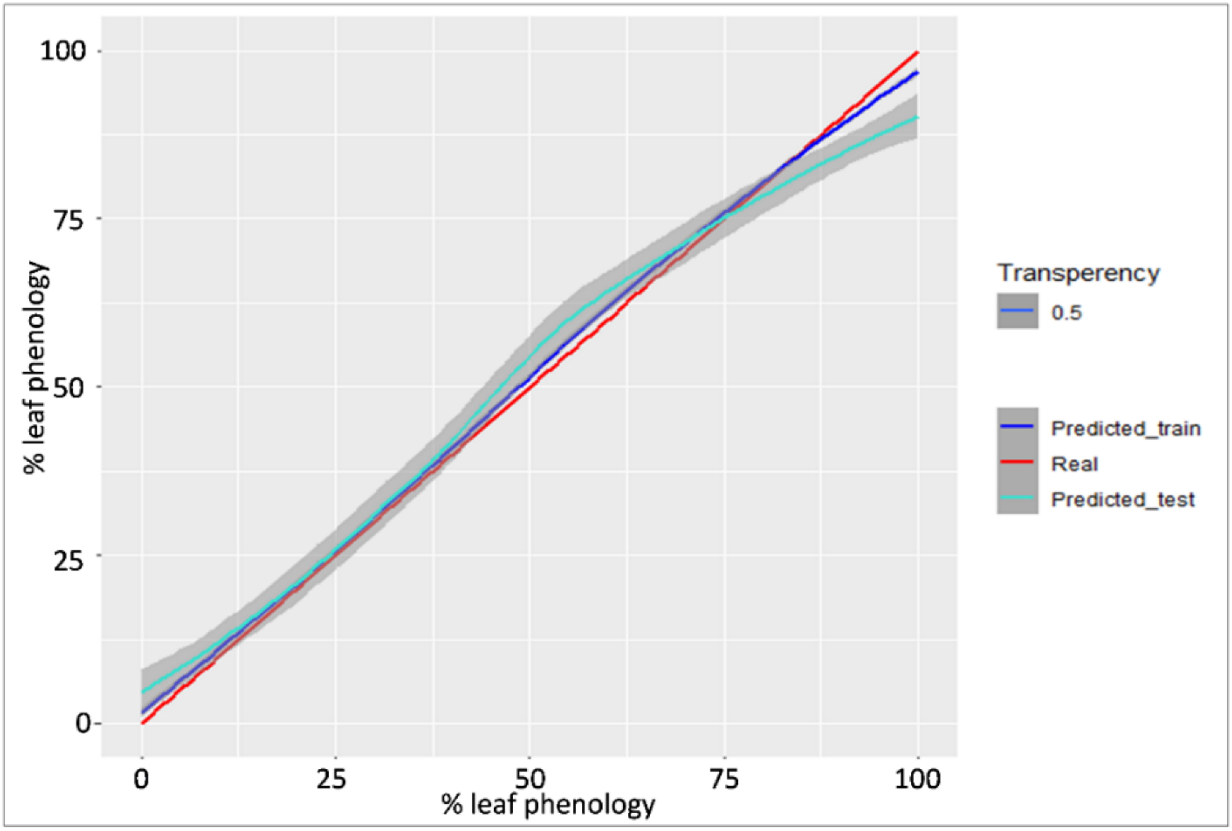


Fig. 4.10 - Comparison between the data set obtained through field phenological observations (Real) and the two predicted calibration (Predicted_train) and test (Predicted_test) data sets.

4.2.2. Phenology prediction using Copernicus biophysical parameters

Based on field observations, average values of the phenological stages were calculated for each of the five study sites, both for spring and autumn phenology. The time series of data extracted from the *Copernicus* platform for the five selected biophysical parameters (Table 3.3), reported as mean values for each study area, obtained a good correlation with the phenological data based on field observations (Fig. 4.11). The *Fraction of Vegetation Cover* (FCover) index, which is based on the proportion of the green color of the vegetation of the trees in the field, proved to be the most relevant, registering a high correlation coefficient value (r = 0.91). The LAI (*Leaf Area Index*), DMP (*Dry Matter Productivity*), and FAPAR (*Fraction of Absorbed Photosynthetically Active Radiation*) indices also obtained high correlation coefficient values, with r = 0.88, r = 0.84 and r = 0.83, respectively. In this case, the NDVI (*Normalized Difference Vegetation Index*) obtained a correlation coefficient of only 0.51, signifying a less close relationship with the field phenological data observations.

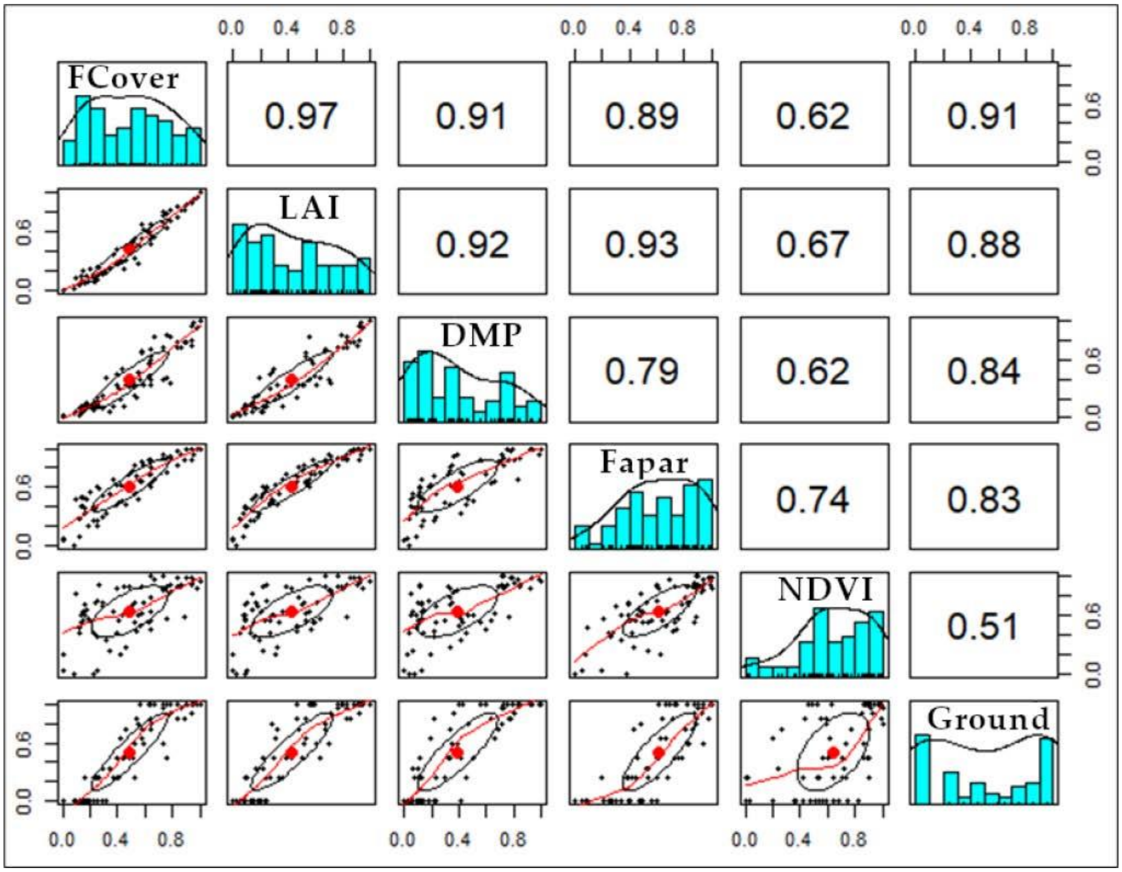


Fig. 4.11 - Correlogram of phenological data obtained through field observations and *Copernicus* biophysical parameters, reported to average values for each study site (last column; Ground - phenological data obtained in the field).

Due to the linearity between field phenological data and biophysical parameters extracted from the *Copernicus* platform, several linear models were tested. The best-fitting algorithm, which recorded the lowest value of error (RMSE) of 7.84% and the highest coefficient of determination R^2 of 0.94 was model 2, with all five *Copernicus* biophysical parameters as variables and location as the independent population separation variable (Table 4.10). In the other models, location was not used as a parameter. Although they recorded a higher RMSE value, they proved more effective in predicting beech leaf phenology outside the monitored study areas. The best balance between the RMSE value and that of R^2, is recorded in the case of model 3, which had as variables the first four most effective *Copernicus* biophysical parameters and their interaction with the two monitored seasons (spring and autumn).

Table 4.10 – Description of the models used and RMSE and R^2 results obtained after their testing

No.	*RMSE*	R^2	Model complexity	Linear model formula
1	11.65	0.87	11	F(x)=(FCover+LAI+FAPAR+DMP+NDVI)*season
2	7.84	0.94	9	F(x)=FCover+LAI+FAPAR+DMP+NDVI+location
3	11.89	0.85	9	F(x)=(FCover+LAI+FAPAR+DMP)*season
4	12.32	0.85	7	F(x)=(FCover+LAI+FAPAR)*season
5	12.57	0.85	5	F(x)=FCover+LAI+FAPAR+DMP+NDVI
6	12.99	0.84	5	F(x)=(FCover+LAI)*season
7	13.00	0.84	3	F(x)=FCover*season
8	13.11	0.83	1	F(x)=FCover

4.3. Association analysis of phenological, genetic, and architectural data obtained using terrestrial laser scanning

4.3.1. Competition intensity

The size of the competition index value of a target beech individual is influenced by the distance between it and its competitors. As this distance increases, the value of the *Hegyi* competition index decreases, and there is an inversely proportional relationship between the two (according to the 6th order polynomial function: $y = 3E\text{-}06x^6 - 0{,}0002x^5 + 0{,}0042x^4 - 0{,}0489x^3 + 0{,}309x^2 - 1{,}0275x + 1{,}5836$). The obtained results reveal that the effect exerted by a competitor on a target tree located at a distance greater than 4 m decreases significantly (Fig. 4.12). Together with the distance, the diameter of the competing trees influences the value of the competition index, and there is a directly proportional relationship between them.

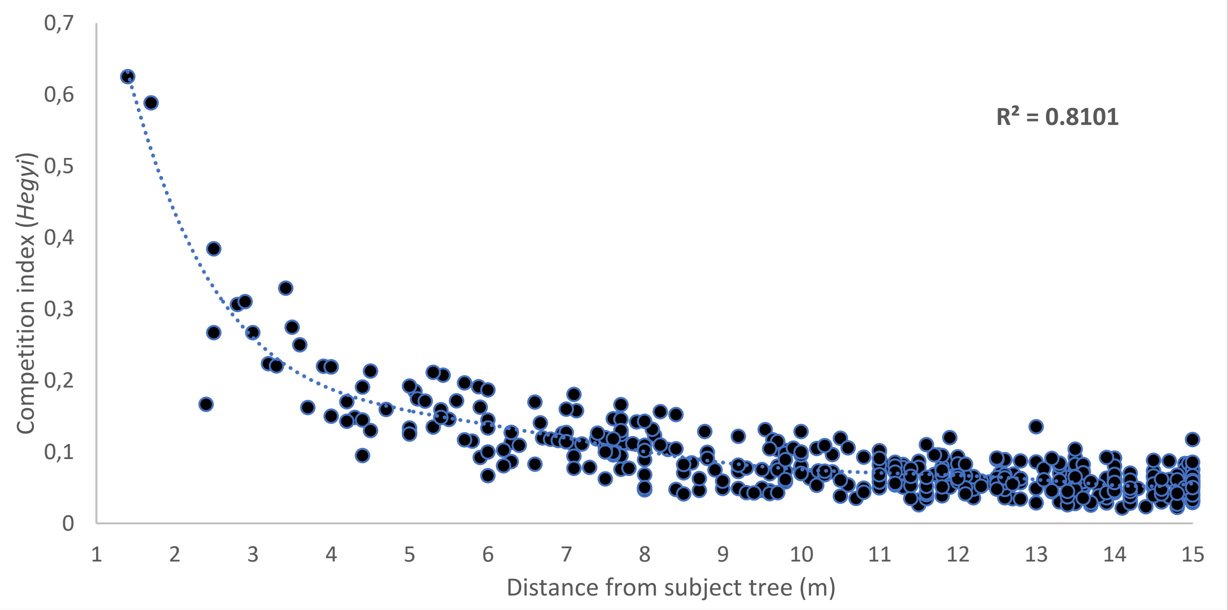


Fig. 4.12 – Competition index values according to *Hegyi* (1974) for competitor trees (*i*) of sampled dominant target trees (*j*)

4.3.2. Effects of competition on phenology and crown and stem architectures of beech individuals

The results obtained reveal a significant and positive correlation between the competition exercised by the competitors of the 55 selected individuals (quantified through the *Hegyi* competition index) and their slenderness (r = 0.32) (Table 4.11). Thus, an increase in the competition implies a decrease in biomass indicators: total volume (r = -0.38), trunk volume (r = -0.39), branch volume (r = -0.36), tree height (r = -0.38), branch length (r = -0.30), diameter measured at the height of 1.30 m (r = -0.39) and crown diameter (r = -0.34).

Table 4.11 – Effects of competition *Hegyi* index on beech phenology and crown and stem architectures

Correlation between competition and …	*Pearson* correlation value
Bud burst	0.08
Length of the growing season	-0.09
Slenderness	**0.32***
Forking	0.13

Total volume	**-0.38****
Trunk volume	**-0.39****
Branch volume	**-0.36****
Tree height	**-0.38****
Trunk length	**-0.34***
Branch length	**-0.30***
Number of branches	-0.12
Maximum branch order	-0.16
DBH	**-0.39****
Crown diameter (average)	**-0.34***

* - r > 0.266, ** - r > 0.363 and *** - r > 0.432

4.3.3. Relationship between slenderness, phenology, and crown and stem architectures of beech individuals

The results obtained reveal a significant and positive correlation between the slenderness of the analyzed beech individuals and the competition exercised by their competitors (Table 4.12). The high slenderness of beech individuals implies a highly significant decrease in their trunk volume (r = -0.62), volume (r= -0.47), branch length (r= -0.41), and crown diameter (r= -0.58). The start of the growing season (r= -0.23) and the length of it are not significantly influenced by the slenderness of the specimens analyzed. Genetic diversity (quantified at the individual level by heterozygosity) does not affect tree slenderness (r = -0.16).

Table 4.12 – Effects of slenderness on beech phenology and crown and stem

Correlation between competition and ...	*Pearson* correlation value
Bud burst	-0.23
Length of the growing season	0.24
Forking	0.24
Competition	**0.32***
Heterozygosity	-0.16
Trunk volume	**-0.62*****
Branch volume	**-0.47*****

Trunk length	-0.10
Branch length	**-0.41****
Number of branches	-0.14
Maximum branch order	-0.26
Crown diameter (average)	**-0.58*****

* - r > 0.266, ** - r > 0.363 and *** - r > 0.432

4.3.4. Relationship between altitude and phenology and crown and stem architectures of beech individuals

The influence of altitude on the start and length of the growing season of the analyzed beech individuals is confirmed by the highly significant values of the correlation coefficients obtained from the data analysis of 0.90 and -0.91, respectively (table 4.13). Thus, an increase in altitude implies a late start of the beech in the growing season and a shorter length. At the same time, the results show that an increase in altitude will cause a decrease in tree height (r = -0.61) and trunk length (r = -0.44).

Table 4.13 – Effects of altitude on beech phenology, genetic diversity, and crown and stem architectures

Correlation between competition and ...	*Pearson* correlation value
Bud burst	**0.90*****
Length of the growing season	**-0.91*****
Competition	0.28
Forking	**0.41****
Total volume	-0.20
Trunk volume	-0.20
Branch volume	-0.19
Tree height	**-0.61*****
Trunk length	**-0.44****
Branch length	0.08
Number of branches	0.24
Maximum branch order	0.20
DBH	-0.17

Crown diameter (average)	-0.10

** - r > 0.363 and *** - r > 0.432

4.3.5. Relationship between genetic diversity and phenology and crown and stem architectures of beech individuals

A significant influence of heterozygosity on growing season length (r = 0.30) was observed, with a beech individual with greater genetic diversity exhibiting an earlier onset of bud burst (r = -0.21) and a longer growing season (table 4.14). Also, a significant value of the correlation coefficient between heterozygosity and the trunk volume of the beech individuals was obtained (r = 0.35), but also one close to the critical significance threshold with the total volume of the trees (r = 0.26). A weak negative correlation was obtained between heterozygosity and the degree of forking of the analyzed beech trees (r = -0.21).

Table 4.14 – The influence of genetic diversity on phenology, genetic variability, and architectural characteristics of the analyzed beech individuals

Corelația dintre heterozigoție și ...	Valoarea coeficientului de corelație *Pearson*
Bud burst	-0.21
Length of the growing season	**0.30***
Forking	-0.21
Total volume	0.26
Trunk volume	**0.35***
Branch volume	0.22
Tree height	0.18
Trunk length	-0.06
Branch length	0.06
Number of branches	-0.16
Maximum branch order	-0.08
DBH	**0.30***
Crown diameter (average)	0.25

* - r > 0.266

5. CONCLUSIONS. PERSONAL CONTRIBUTIONS. RESULTS DISSEMINATIONS. FUTURE RESEARCH DIRECTIONS

5.1. Conclusions

5.1.1. Conclusions following the phenological observations

● The results indicate a variation at the interpopulation level (among the five study sites) due to different site conditions and at the intrapopulation level (thirty individuals from each study site) due to microclimate conditions and different genotypes.

● Significant differences were identified between the means of the limits of the altitudinal gradient, both in the onset of spring phenology (24-28 days) and the onset of senescence (12-24 days), implying considerable differences also in the length of the growing season (43-52 days).

● In the delayed onset of vegetation growing (2021 and 2023), faster transitions from one phenophase to another were recorded, implying faster leaves unfolding. The analyzed beech individuals started into the growing season with a 3-3.5 days delay per 100 m increase in altitude.

● Within the same study sites, significant differences were identified from 3 to 12 days at the start of the bud burst, from 5 to 16 days at the start of senescence, and from 14 to 24 days in the length of the growing season, thus highlighting the precocious and late phenological behaviors of the analyzed beech individuals.

● The association of phenological and meteorological data confirms that temperature is the triggering factor of beech phenology, both spring and autumn. When the thermal threshold of 10°C is exceeded, the beech starts in the growing season, but this condition is complementary to exceeding a specific number of cumulated degree days (CDD). During the three years of monitoring, bud burst occurred after accumulating at least 52 CDD seven days before phenophase.

● The senescence of beech individuals was influenced by temperature. The first phenophase of beech autumn phenology, leaf yellowing, is triggered by accumulating at least 65 autumn-degree days (ADD) seven days before the phenophase.

● The second phenophase of beech autumn phenology, the fall of the leaves, is not influenced to the same extent as the first by the accumulation of certain temperatures or early frost.

5.1.2. Conclusions following the phenological observations performed through remote sensing

● The use of images collected using the drone allows the monitoring of the phenology of the beech trees at the individual level. Orthophoto products based on aerial images provide an overview of the tree crown, comparable to the one in the field, offering an effective alternative.

● Vegetation indices calculated based on these images effectively estimated the phenological stage, comparable to that assigned through field observations. Overall, phenological observations performed using the drone can considerably reduce the effort required to carry out field observations. The vegetation indices that proved most accurate in assigning a phenological stage were GLI, NGRGB, RGVBI, and ExG, which use the RGB model's all three bands (red, green, and blue).

● The linear and non-linear prediction models, including the *random forest* algorithm, proved accurate. However, on small samples of trees, they tended to overfit, and replication in other study areas presented the risk of not providing the same quality results. The algorithms proved high accuracy when color and texture were used as independent variables.

● Biophysical parameters extracted from the *Copernicus* platform (based on the *Sentinel* satellites) have generally shown good accuracy in assigning a phenological stage (quantified as the percentage of tree leaf cover) at the study site level. The most effective index was the *Fraction of Vegetation Cover* (FCover).

● The two remote sensing methods (drone and satellite images) used to perform beech phenological observations proved effective. The results closely correlated with those obtained following phenology observations in the field, thus representing a faster and less resource-consuming alternative (human resources, transport).

5.1.3. Conclusions following the correlation of phenological, genetic, and architectural data obtained using terrestrial laser scanning method

● The increase in altitude causes a late start and an early end of the growing season for the analyzed beech individuals. This fact directly implies a shorter length of the entire growing season. The height of the trees and, implicitly, the length of the trunk decreased simultaneously with the increase in altitude. The degree of forking of the selected trees increased in proportion to the altitude.

● The influence of competitors on target trees is directly determined by the distance between them (the most significant effects being felt at a distance of less than 4 m) and the diameter of the competitors (inversely proportional relationship). Greater competition exerted on the target trees implies a reduction of the biomass indicators of the analyzed beech individuals (tree volume, trunk volume, tree height, trunk length, diameter, branch volume, branch length, and crown diameter).

● An individual with a higher heterozygosity, implicitly a higher genetic diversity, implies a longer growing season. This means an early start in the growing season and a late onset of senescence. Also, individuals with a higher heterozygosity were associated with higher values of the biomass indicators. A negative relationship, close to the significance threshold, was identified between the genetic diversity and the degree of forking of the analyzed beech trees.

● Terrestrial laser scanning methods have shown a high potential in extracting the biomass indices values of the evaluated beech trees. However, the complementary use of conventional methods for data validation is still recommended, even if they are time and resource-consuming.

5.2. Personal contributions

Following the research that led to writing this doctoral thesis, the following original contributions can be revealed:

1. Performing a phenological study of the common beech (*Fagus sylvatica*) for three years along an altitudinal gradient with a high amplitude of elevation (900 m) and five sample sites with significant portions of rough terrain, representative of the natural range of this species in the Brasov area.

2. Carrying out phenological monitoring through field observations at a high frequency of approx. 2-3 days, especially in the case of spring phenology, to capture at a high resolution the transitions between phenophases, thus reducing the negative effect of data interpolation.

3. For the first time in Romania, beech phenological observations at individual levels in rough terrain were carried out based on the high-resolution orthorectified products obtained using a UAV (drone) device. These observations were made in parallel with field observations and the testing of 19 vegetation indices, which were calculated based on the pixel values extracted from these images to identify those with high precision.

4. Test five vegetation biophysical parameters extracted from satellite images obtained from the Copernicus platform and identify those proven effective in estimating the spring and autumn phenology of beech at the study site level.

5. Test and identify linear and non-linear models (random forest algorithm) that have proven effective in estimating common beech's spring and autumn phenology at individual and study site levels.

6. Association of phenological data with meteorological data obtained by temperature and relative humidity sensors installed inside the stands/study sites.

7. Designing the accumulation of senescence degree days, ADD, the trigger of autumn phenology, based on the cumulation of daily temperatures necessary for the beech to start in the growing season. This can be replicated in other species as well.

8. Analysis of architectural characteristics and biomass indicators of beech individuals obtained through remote sensing, more precisely terrestrial laser scanning (TLS).

9. Evaluation of the genetic diversity of beech individuals, quantified using the heterozygosity index, based on six SSRs and six (EST)-SSRS markers.

10. Conduct exploratory research on the association of individual genetic diversity with architectural, biomass indices, and phenological characteristics in common beech.

11. Meteorological and phenological database from the studied sites.

12. Installation of a phenological network, which can be used in future multidisciplinary studies.

5.3. Results dissemination

The results obtained from the research carried out during this doctoral stage were disseminated in five scientific articles, four of which were indexed by ISI (at the time of submitting the thesis, the fifth one being in the review stage and published as a preprint), and participation in nine international scientific conferences and symposia.

List of scientific articles published in *Web of Science* journals:

1. **Ciocîrlan, M.I.C.**., Curtu, A.L., Radu, G.R. (2022). Predicting Leaf Phenology in Forest Tree Species Using UAVs and Satellite Images: A Case Study for European Beech (*Fagus sylvatica* L.). *Remote Sens.*, *14*, 6198. https://doi.org/10.3390/rs14246198
   Q1/ IF=5,349
2. **Ciocîrlan, M.I.C.**., Ciocîrlan, E., Chira, D., Radu, G.R., Păcurar, V.D., Beșliu, E., Zormpa, O.G., Gailing, O., Curtu, A.L. (2024). Large Differences in Bud Burst and Senescence between Low- and High-Altitude European Beech Populations along an Altitudinal Transect in the South-Eastern Carpathians. *Forests*, *15*, 468. https://doi.org/10.3390/f15030468
   Q1/ IF=2,4

3. **Ciocîrlan, M.I.C.**, Ciocîrlan, E., Radu, G.R., Chira, D., Gailing, O., Vînătoru, C., Zormpa, O.G., Vucetic, B., Budde, K.B., Florea, S., Niță, M.D., Curtu, A.L.. (2024). Exploring the association between adaptive and growth traits and within-individual genetic diversity in common beech (*Fagus sylvatica*). *Annals of Forest Research*, *67(2): 151-166.*
   Q2/ IF=1,7
4. Budde, K., Zormpa, O. G., Wilhelmi, S., Vucetic, B., Müller, M., Gailing, O., **Ciocîrlan, M.I.C.**, Ciocîrlan, E., Curtu, A. L., Targem, M. & Wildhagen, H. (2024). Differences in fine-scale spatial genetic structure of European beech populations along elevational gradients. *ResearchSquare* (Preprint)
5. Beșliu, E.; Curtu, A. L.; Budeanu, M.; Apostol, E. N.; **Ciocîrlan, M. I. C.** (2024). Exploring the effects of the assisted transfer of European beech (*Fagus sylvatica* L.) provenances in the Romanian Carpathians. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *52*(3), 13968. https://doi.org/10.15835/nbha52313968
   Q3/ IF=1,4

List of presentations given at international scientific conferences:

1. **Ciocîrlan, M.I.C.**, Radu, R.G., Curtu, A.L. (2022). Phenology monitoring in European beech (Fagus sylvatica) in the Carpathian Mountains using UAVs sensor. Conferința PHENOLOGY 2022 – „Phenology at the crossroads", 20-24 Iunie, Avignon, Franța.
2. **Ciocîrlan, M.I.C.**, Radu, R.G., Curtu, A.L. (2022). Spring and autumn phenology monitoring in European beech along an altitudinal transect in Brasov during 2021 and 2022. 10TH INTERNATIONAL SYMPOSIUM FOREST AND SUSTAINABLE DEVELOPMENT, 14-15 Octombrie, Brașov, România.
3. Targem, M.B., Zormpa, O. G., Götz, J., Schneider, T.M.T., Müller, M., Budde, K.B., Koebel, R., Hofmann, M., Steiner, W., Hertel, D., Leuschner, C., **Ciocîrlan, M.I.C.**, Curtu, A.L., Gailing, O., Wildhagen, H. (2022). Entwicklung genetischer Marker zur Analyse von Anpassungen an Trockenstress bei Trauben-Eiche und Buche. Waldklimafonds-Kongress 2022, 11-12 Octombrie, Göttingen, Germania.
4. Zormpa, O. G., Budde, K., Vucetic, B., **Ciocîrlan, M.I.C.**, Müller, M., Curtu, A. L., Gailing, O., (2022). Genetic and phenotypic variation in Romanian beech along an altitudinal gradient. Ecology and Evolution: New perspectives and societal challenges. 21-25 Noiembrie 2022, Metz, Franța.
5. **Ciocîrlan, M.I.C.**, Radu, R.G., Ciocîrlan, E., Chira, D., Chira, F., Mantale, C.Ș., Gailing, O., Curtu, A.L. (2023). Spring and autumn phenology monitoring in European beech (*Fagus sylvatica*) in the Carpathian Mountains using Uavs images and Copernicus biophysical parameters. "*Forest science for people and societal challenges".* The 90th "Marin Drăcea" INCDS Anniversary, 2-5 Octombrie, București, România.

6. **Ciocîrlan, M.I.C.**, Ciocîrlan, E., Chira, D., Chira, F., Mantale, C.Ș., Radu, R.G., Gailing, O., Curtu, A. L. (2023). Bud-burst monitoring in the European beech population (*Fagus sylvatica* L.) along an altitudinal gradient in the South-Eastern Carpathian Mountains. Second EVOLTREE Conference 2023 - "*Resilient forests for the future",* 12-15 Septembrie 2023, Brașov, România.
7. Zormpa, O. G., Wilhelmi, S., Vucetic, B., **Ciocîrlan, M.I.C.**, Müller, M., Ciocîrlan, E., Curtu, A. L., Targem, M.B., Wildhagen, H., Gailing, O., Budde, K. (2023). Fine scale spatial genetic structure in *Fagus sylvatica* along a steep elevational gradient in the Carpathian Mountains. Second EVOLTREE Conference 2023 - "*Resilient forests for the future",* 12-15 Septembrie 2023, Brașov, România.
8. **Ciocîrlan, M.I.C**., Ciocîrlan, E., Radu, R.G., Chira, D., Gailing, O., Zormpa, O. G., Niță, M.D., Curtu, A.L. (2024). Does heterozigosity and competition affects the bud burst and architectural tree characteristics? A case study for European beech. 11TH INTERNATIONAL SYMPOSIUM FOREST AND SUSTAINABLE DEVELOPMENT, 17-18 Octombrie, Brașov, România.

List of presentations given in national symposia:

1. Ciocîrlan, M.I.C., Beșliu, E., Stoica, E., (2024). "Participation in training for improving research staff in institutions and universities abroad". The symposium "Institutional performance - Institutional development and financing excellence in RDI", organized on the occasion of the completion of the Project Increasing the institutional capacity and performance of INCDS "Marin Drăcea" in RDI activity - CresPerfInst, May 25, 2024, Bucharest, Romania.

5.4. Future research directions

The continuation of research on beech behavior is particularly important for managing beech forests in the context of climate change. At the same time, the methods used in this doctoral thesis can be replicated for other species of interest in forestry.

Therefore, I believe that the presented research can be continued in the following directions:

1. Evaluation of beech phenology to obtain a more complex calibration data set to create an improved model for predicting phenological behavior.
2. Carrying out genetic analyses at a more complex level by approaching the concept of polymorphism at the level of a single nucleotide, SNP, and leading to obtaining a new index for quantifying genetic diversity, which, subsequently, can be associated with the phenological behavior of the beech and the identification of possible particularities of the specimens that showed both a phenological precocity or lateness, as well as specific architectural characteristics.

3. The inclusion of thermal inversions in the meteorological and phenological data correlation.
4. Testing of several vegetation indices that included the rate of green color in the calculation formula, a hypothesis that justifies being tested following the results obtained in this work.
5. Replication of phenological observations using remote sensing, drone, and satellite images on other tree species of interest for forest management.
6. Improving the accuracy of obtaining meteorological data up to the microclimate level by installing sensors at different heights and on different exposures of the same tree.
7. Continuation of the phenological observations of the beech to establish a long-term phenological network.

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