Hungarian University of Agriculture and Life Sciences (MATE)

EX SITU PHENOLOGICAL EXAMINATIONS OF WILD PLANT SPECIES, AND COMPARING THE RESULTS WITH NATIONAL AND INTERNATIONAL STUDIES

Thesis of doctoral (PhD) dissertation

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1. Background and objectives

Climate change has a profound impact on terrestrial ecosystems (Peñuelas et al., 2017), and this effect extends to the biodiversity of Europe (Begum et al., 2022). By 2080, more than half of vascular plant species in our continent may become endangered due to these influences (Thuiller et al., 2005). Thus, climate change entails unforeseeable consequences for our ecosystems. In our era, one of the primary goals of ecology is to predict how species and ecosystems will respond to climate change.

Phenology is the scientific study of periodic plant and animal life cycle events and how these are influenced by biotic and abiotic factors and how these interact intraspecifically and interspecifically (Lieth 1974). The phenology of plant species is sensitive to climate change, making it a valuable indicator thereof (Sparks & Menzel, 2002; Cleland et al., 2007; Schwartz, 2013; Peñuelas et al., 2017). Phenological studies now play a prominent role in global climate change research, as the results obtained can be used to predict the timing of cyclic phenomena (Morellato et al., 2016). However, the possibilities for predicting climate change through phenological models are still quite limited (Zhao et al., 2013), necessitating further research.

Although phenological studies have been conducted since ancient times (Schwartz, 2013), their significance has grown substantially since the realization of climate change and its effects became increasingly evident in the 20th century (Chmielewski et al., 2013). The number of phenological studies has been rapidly increasing since the 1990's (Chmielewski et al., 2013; Piao et al., 2019), and according to data from the Dimensions Software platform monitoring scientific publications (Dimensions Software, 2018-), their number has more than doubled in the last four decades, particularly in the last eight years. The quantity of scientific articles on phenology exceeded 2700 by 2022.

Most globally conducted phenological studies do not track the complete annual phenological cycle of the observed specimens (Katal et al., 2022), as the latter is much more challenging to study than other plant traits requiring single-time data collection (Wolkovich et al., 2014). In general, most phenological research focuses on woody plants and economically utilized crops (Katal et al., 2022; Horbach et al., 2023). Despite estimates indicating that over 50% of the world's plant species (FitzJohn et al., 2014) and 85% of non-woody plant species in temperate ecosystems (Ellenberg, 1996) are not woody, phenological studies, especially regarding leafing-out and leaf senescence, traditionally concentrate on trees, shrubs, or crops (Chmielewski & Rötzer, 2001; Vitasse et al., 2011; Panchen et al., 2014, 2015). The responses of different life-forms' phenological patterns to environmental influences have surprisingly received limited research attention.

The Carpathian Basin is particularly sensitive to the impacts of climate change (Pongrácz et al., 2009; Gálos et al., 2011; Hlásny et al., 2014; Antofie et al., 2015); thus, it is crucial to conduct phenological studies in the region. However, there have been few phenological studies in Hungary (Walkowszky, 1998; Eppich et al., 2009; Szabó et al., 2016; Templ et al., 2017). Unfortunately, the national phenological data collection network operated by the National Meteorological Service in the second half of the 20th century has been discontinued (Hunkár et al., 2012). None of Hungary's botanical gardens has joined international phenological data collection networks, and currently, there is no organized, comparable phenological data collection in the country.

To my knowledge, no comparative *ex situ* phenological study has been conducted in Hungary, observing the complete annual cycle of wild growing species belonging to different life-forms according to the Raunkiær life-form classification over several years. The study design is also innovative in the Hungarian context.

It is generally accepted that urban climate conditions can be considered similar to the changing global climate conditions; therefore, many researchers study urbanized areas as small-scale experiments or models of global climate change (Ziska et al., 2003). This provides an opportunity for the application of the "space for time substitution" method (Pickett, 1989), in which we predict possible future impacts of climate change on phenology (Rötzer et al., 2000; White et al., 2002; Christmann et al., 2023). The two locations selected for my comparative study are both within urban areas; however, while the Budapest site is situated in a densely built, busy urban environment with multi-story buildings, the Gödöllő site is located in a sparsely built area of a four-hectare botanical garden on the university campus, adjacent to a natural forest patch and near a protected forest area. The Budapest site is thus subject to the urban heat island effect. Considering that temperature appears to be the most influential factor on plant phenology (e.g., Cleland et al., 2007; Peñuelas et al., 2009), taking into account the above, the phenological differences between the Gödöllő and Budapest sites can be partly regarded as indicative of expected phenological changes in the future.

In the two distinct mesoclimatic experimental sites, – to the extent possible – genetically identical specimens were planted for each species, following the same protocol to ensure the separability of the effects of meteorological parameters from other potentially confounding factors. Data collection occurred weekly, also following the same protocol, at both sites whenever possible on the same day. The experimental setup adheres to the protocol used at stations of international phenological networks. Therefore, my research can be considered a valuable contribution. With this work, I aim to draw attention to the importance of comparative phenological studies in line with international standards within the Hungarian professional community.

The main goals of the study were the following:

1. Conducting a three-year comparative phenological study on the six life forms of the Raunkiær life-form classification (microphanerophyte, chamaephyte, hemicryptophyte, geophyte, hemitherophyte, therophyte), observing five native and wild growing species per life form at two distinct mesoclimatic locations in Hungary.
2. Setting up an ex situ experiment at two different mesoclimatic locations while minimizing the impact of other climatic factors influencing the genetic similarity within each species. This involves using identical soil mixtures, uniform pot sizes, and adhering to consistent irrigation protocols.
3. Collecting data on as many phenophases as possible for the species under investigation throughout the entire calendar year.
4. For comparability with climatic parameters, installing meteorological stations at the *ex situ* experiment sites and collecting meteorological data (temperature, precipitation, relative humidity, atmospheric pressure, wind speed, solar radiation, and wind direction).
5. Recording the progression of phenology for the examined species in each phenophase and location, and determining the phenological sensitivity of the studied life forms in each phenophase.
6. Documenting annual variations in the phenological patterns of the examined species and life forms at the two distinct mesoclimatic locations.
7. Analyzing the site-specific evolution of the length of the growing season.

It is important to note that while we aimed to ensure the highest possible genetic similarity within each species during the procurement of experimental plant material, the various species did not originate from the same location. Therefore, in the phenological observations, we only considered the effect of the cultivation site, and accounting for the influence of the origin site was not the objective of the experiment.

2. Materials and methods

As the two locations for the comparative experiment, the Botanical Garden of the Szent István University (MATE) in Gödöllő (47°35'36.2"N 19°22'06.2"E, 250 m above sea level /Szirmai 2014/) and the Botanical Garden of Eötvös Loránd University, hereafter referred to as ELTE Botanical Garden (Budapest, 47°29'05.6"N 19°05'05.7"E, 114 m above sea level /Orlóci 2019/) were selected. In each location, 34 plant species participating in the experiment, with five repetitions for each life-form, were placed in identical exposure plots. The taxonomy of the examined plant species and their life-form classification follow the "New Hungarian Herb Book" (Király, 2009) in the dissertation.

The MATE Botanical Garden is situated in the Gödöllő Hills subregion (6.3.51) of the North Hungarian Mountains and Cserhát Region. The area has a moderately warm-dry climate, with an annual average temperature of 9.7-10 °C, an annual average sunlight duration of 1950 hours, 780-790 hours of sunshine in summer, and 190 hours in winter. The annual average precipitation is 540-580 mm (Dövényi et al., 2008). The measured annual average temperature at the experimental site (average for 2020, 2021, and 2022) was 11.35 °C. The ELTE Botanical Garden is located in the Pesti Loess Plain subregion (1.1.12) of the Great Hungarian Plain, Duna menti síkság (Danube Plain) subregion. The climate in this area is moderately warm and dry, with an annual average temperature of 10.2-10.6 °C. The annual average sunlight duration is 1910-1940 hours, 770-780 hours of sunshine in summer, and 180 hours in winter. The annual average precipitation is 520-550 mm (Dövényi et al., 2008). The measured annual average temperature at the ELTE Botanical Garden site (average for 2020, 2021, and 2022) was 13.16 °C.

In the experimental plots established at these two locations, five repetitions of each life-form (micro)phanerophyte, chamaephyte, hemicryptophyte, geophyte, hemicryptophyte, therophyte) and five species for each life-form (Table 1) were placed. The observation units were plastic pots of equal size, each containing at least one specimen of the respective species. I aimed for the highest possible genetic identity for each species in the selection of experimental plants. Woody species were clonally identical for each species, seeds of herbaceous species were collected from a single plant and used for further years, and other life-forms' specimens were selected from a single location per species, propagated in horticulture by vegetative means. The genetic identity of horticulturally propagated specimens were also ensured.

To ensure the survival of the experimental plants, supplementary irrigation was provided for the plants placed in the experimental pots. Based on the analysis of the soil mixture used in the experiment, it can be stated that the soil is not contaminated, has good nutrient content, with a humus content exceeding 3%, and is sandy loam (AK 34 soil), which is overall suitable for the plants.

In November 2020, meteorological stations were installed at both experimental locations. Meteorological data from the period preceding this installation were obtained from the National Meteorological Service's meteorological stations. Regarding the annual average temperatures, the temperatures measured at the Budapest site were higher in all three years. The cumulative site-specific difference in average temperatures over the three years was 1.81 °C. This value was utilized in determining the phenological sensitivity of the experimental plants (species and life-forms). Phenological sensitivity defined as the shift in phenological event date per degree of temperature change, is a commonly used metric that can be compared across studies, an important metric in phenological studies, indicating how many days a phenological event is shifted per 1 °C temperature change (Cleland et al., 2012; Wang et al., 2015). A negative value indicates an earlier shift, while a positive value indicates a later shift.

The annual average humidity was higher at the Gödöllő site in both years, despite receiving less precipitation in 2021 and 2022 compared to the Budapest site.

Table 1: Classification of plant species participating in the experiment based on life-form

Phanerophytes	**Chamaephytes**	**Hemicryptophytes**
Cornus sanguinea L.	*Dianthus plumarius* L.	*Euphorbia epithymoides* L.
Prunus spinosa L.	*Sedum album* L.	*Ajuga reptans* L.
Ligustrum vulgare L.	*Vinca minor* L.	*Inula ensifolia* L.
Cerasus fruticosa Pall.	*Thymus vulgaris* L.	*Sedum acre* L.
Cotinus coggygria Scop.	*Cerastium tomentosum* L.	*Briza media* L.
Amygdalus nana L.	*Globularia cordiflora* L.	
Rosa spinosissima L.		
Geophytes	**Hemitherophytes**	**Therophytes**
Iris pumila L.	*Daucus carota*** L.	*Hibiscus trionum* L.
Polygonatum multiflorum L.	*Dipsacus pilosus*** L.	*Solanum nigrum* L.
Convallaria majalis L.	*Dipsacus lacinatus*** L.	*Silene alba* Mill.
*Galanthus nivalis** L.	*Capsella bursa-pastoris* L.	*Portulaca oleracea* L.
*Eranthis hyemalis** L.	*Malva sylvestris*** L.	*Consolida regalis* Gray
		Papaver rhoeas L.

* Entered the experiment after the flowering period of the first year, so their flowering was only recorded from the year 2021.

** Due to their life-form, no flowering occurred in the first year.

Phenological data collection took place on a weekly basis, preferably on the same day at both locations. The examined phenophases were as follows: budburst, appearance of the first flower bud, onset of flowering (appearance of the first flower), end of flowering, fruiting, onset of leaf coloring, initiation of leaf fall or senescence, and complete leaf fall or complete senescence. Additionally, for certain life-forms, I recorded the height of specimens and, for woody species, leaf development and trunk diameter.

Data recording, storage, basic organization, and preparation were performed using the online version of Microsoft Excel 365, while statistical analyses were conducted using the R statistical environment version 4.2.2 (R Core Team, 2022) with the assistance of the RStudio script editor program (RStudio Team 2015). The relationship between the day of the year and the occurrence of phenological events at each site was determined using one-way analysis of variance (ANOVA) with Type I (sequential) sums of squares, at a significance level of 0.05% (Zar 1984).

3. Results and discussion

Regarding the examined phenological events, it can be stated in general that they turned out as expected, in line with previous research results (Chmielewski & Rötzer 2001, Fitter & Fitter 2002, Menzel et al. 2006, Wolkovich et al. 2012, 2014, Gallinat et al. 2015, Gill et al. 2015, Zhang et al. 2015, Zohner & Renner 2018, Piao et al. 2019). The appearance of buds, the onset of flowering (appearance of the first flower), and the appearance of fruits occurred on average earlier in the higher average temperature Budapest site for the six life-forms, while the state of complete senescence marking the end of the growing season occurred earlier in the lower average temperature Gödöllő site.

For woody species, budburst in five out of the seven studied species occurred earlier at the higher average temperature Budapest site in all three years. The overall phenological sensitivity of budburst for the examined shrub species was -3.87 days/°C, a value consistent with previous research results (Menzel & Fabian 1999, Chmielewski & Rötzer 2001, Menzel et al. 2006). In terms of the onset of flowering, the phenological sensitivity of woody species was -6.24 days/°C, consistent with previous research results (e.g., Chmielewski & Rötzer 2001, Schieber et al. 2009, Morin et al. 2010, Chitu et al. 2020, Vander et al. 2022a, 2022b). Among the examined life-forms, woody species showed the most significant advancement in the onset of flowering, making their phenological sensitivity the highest. The phenological sensitivity of the onset of leaf coloring and the end of leaf fall was similar, 3.73 days/°C and 3.96 days/°C, respectively. This aligns with previous research, as higher temperatures have been shown to lead to a delay in autumn phenophases (e.g., Menzel & Fabian 1999, Menzel et al. 2006, Schieber et al. 2009, Ibañez et al. 2010, Gill et al. 2015, Zhang et al. 2015, Vander et al. 2022a, 2022b, Xing et al. 2022), although their phenological sensitivity is smaller compared to spring phenophases (Menzel et al. 2006, Piao et al. 2019). By subtracting the timing of budburst from the timing of complete leaf fall, I calculated the length of the growing season. The average length of the growing season in Gödöllő was 243.81 days, while in Budapest, it was 257.65 days. As expected, the growing season was longer in the higher average temperature Budapest site. The difference averaged 13.84 days, resulting in a phenological sensitivity of the growing season length of 7.65 days/°C. Chmielewski and Rötzer (2001) found a phenological sensitivity of 5 days/1°C for woody species between 1969 and 1998.

An interesting phenomenon is that although autumn phenophases occurred later at the higher average temperature Budapest site, over the years they still showed a significant advancement at both sites, leading to an overall shortening of the growing season. Additionally, despite the fact that budburst occurred earlier at the higher average temperature site in all three years, it unexpectedly shifted to later at both sites over the years. In several studies, it has been observed that the effect of changing spring and summer temperatures counteracts the impact of autumn temperatures on senescence. Therefore, despite higher autumn temperatures, autumn phenophases tend to occur earlier (Liu et al. 2019, Chen et al. 2020).

In general, concerning the examined life-forms, it can be stated that herbaceous life-forms exhibit greater interspecific and intraspecific variation than woody plants in terms of phenology (Horbach et al. 2023), a trend that was also evident in my experiment. For geophytes, the overall phenological sensitivity of the onset of flowering is -2.01 days/°C, which is in line with previous research results (Renner et al. 2021). Regarding the end of flowering (last date when flowers were observable for the species), there was no clear trend observed. For *Galanthus nivalis*, *Eranthis hyemalis*, and *Polygonatum multiflorum*, the end of flowering occurred later at the lower average temperature Gödöllő site, while for Iris pumila and *Convallaria majalis*, it occurred later at the higher average temperature Budapest site. Regarding senescence, complete withdrawal (itt ez megint nem jó: die back???) occurred earlier for the two early-flowering, cold-adapted species (*Galanthus nivalis*, *Eranthis hyemalis*) at the higher average temperature Budapest site, while for the later, spring species (*Convallaria majalis*, *Polygonatum* *multiflorum*), complete senescence occurred earlier at the lower average temperature Gödöllő site.

For therophytes, the onset of flowering occurred earlier for all species with sufficient data for comparison at the higher average temperature Budapest site. The phenological sensitivity of flowering was -5.38 days/°C, a significantly higher value compared to values determined in previous studies (Renner et al. 2021). Although the phenological sensitivity of flowering in therophytes is the third highest among the examined life-forms after phanerophytes and chamaephytes, and the second highest among herbaceous species, the conclusion by Fitter and Fitter (2002) that the advancement of flowering in annual species is more pronounced than in perennials holds true only when compared to hemicryptophytes and geophytes. Despite the fact that the responses of species were diverse in terms of the onset of senescence and the attainment of complete senescence, with differences in direction and magnitude, overall, both phenophases occurred earlier at the lower average temperature Gödöllő site.

For hemicryptophytes, the onset of flowering varied among species, showing opposing trends and significantly different degrees of advancement. Due to these opposing trends, the phenological sensitivity of this phenophase was very low, at -0.47 days/°C. For autumn senescence, there was also opposing and significantly different shifts among species.

For chamaephytes, few phenophases were observable, and only reproductive phenological events could be recorded. The onset of flowering occurred earlier for all five cases at the Budapest site. The difference was significant for four species. The phenological sensitivity of the onset of flowering was -5.86 days/°C, the highest value after woody species.

Considering all examined life-forms, the average phenological sensitivity of the onset of flowering (-4.12 days/°C) aligns with previous research results (Menzel et al. 2006, Renner et al. 2021).

Neil and Wu's (2006) research focusing on the impact of urban environments on phenology suggests that temperature appears to be the most influential factor affecting plant phenology. From this perspective, their findings can be applied to our experiment. Similar to the results of Fitter and Fitter (2002), Neil and Wu (2006) also concluded that urban environments have a stronger impact on the phenology of insect-pollinated, early spring-flowering, annual, short-lived, and herbaceous species. These findings were only partially confirmed by my experiment.

In summary, it can be stated that different species (Root et al. 2003; König et al. 2018) and functional groups react differently to climate change (Lavorel & Garnier 2002, Ibañez et al. 2020), a pattern that was also evident in my experiment.

4. Conclusions and recommendations

The majority of phenological studies do not track the complete annual phenological cycle of the studied specimens (Katal et al. 2022). Most studies target specific phenophases, usually focusing on the onset of flowering, or at most, examining the reproductive phenological events (e.g., Menzel et al. 2006, Sherry et al. 2007). In this regard, the present work is groundbreaking, as I followed the development of almost all measurable phenophases of 33 species belonging to six life-forms over three growing seasons. I conducted this study in two different mesoclimatic locations, enabling meaningful comparisons.

It is generally accepted that urban climate conditions can be considered analogous to changing global climate conditions. Therefore, many researchers study urbanized areas as small-scale experiments or models of global climate change (Ziska et al. 2003). This provides an opportunity for the application of the space-for-time substitution method (Pickett 1989), wherein possible future effects of climate change on phenology are predicted (Rötzer et al. 2000, White et al. 2002, Christmann et al. 2023). According to Park et al. (2023), models exploring phenological responses to urbanization that focus solely on temperature interaction are likely overly simplistic. However, it is worth emphasizing that based on research, temperature seems to be the most influential factor on plant phenology (Cleland et al. 2007, Peñuelas et al. 2009, Chuine 2010, Szabó et al. 2016).

Several researchers (Zhang et al. 2004, Neil & Wu 2006, Jochner & Menzel 2015, Lahr et al. 2018, Christmann et al. 2023) suggest that the urban environment, while not a perfect model, is suitable for studying future responses of plants to climate change due to its elevated temperature and CO_2 concentration, as well as increasingly severe droughts. In my case, both locations are situated within urban areas; however, while the Budapest site is in a densely built, busy urban environment with multi-story buildings, the Gödöllő site is located in the sparsely built area of the university campus, a four-hectare botanical garden in the immediate vicinity of a natural forest patch within a protected landscape area. Since both locations have street lighting, the presence of artificial light does not distort the effects of temperature and other factors. Considering these factors, the phenological differences between the Gödöllő and Budapest sites can be partly considered indicative of future phenological changes. For more precise predictions, additional calculations and longer-term experiments are necessary. In future experiments, it is crucial to ensure international standard ex situ experiments at each site for comparability.

To facilitate similar future research, it is recommended to install permanent meteorological stations at the MATE Botanical Garden and the ELTE Botanical Garden, providing reliable, continuous data monitoring for multiple meteorological parameters in accordance with standards. Over the three years of the experiment, valuable insights were gained regarding the applicability of life-forms and species for such research. Most phenophases were successfully recorded in phanerophytes. The maintenance of woody plants proved to be the simplest, requiring minimal or no weeding, aside from irrigation. Due to their long lifespan, they are suitable for participation in long-term experiments, allowing the examination of genetically identical specimens across multiple locations using clonal species. Since most phenological research has been conducted with woody plants, data from experiments with woody plants are easily comparable. Well-established international protocols (e.g., IPG gardens) further facilitate comparability. The only drawback of applying woody plants in phenological experiments is that specimens planted in their seedling stage do not produce or produce very few flowers in the first one or two years, although vegetative phenophases are well observable during this period. Overall, the use of phanerophytes proves to be the best decision in terms of invested energy and return.

Geophytes are the second most suitable life-form, with their perennial nature (they are perenniel plants ami eredetileg van, az nem jó, vh fogalmazd át légyszi. köszi), and many phenophases were successfully recorded in the experiment. However, their care requires more attention than that of woody plants, involving weeding in addition to regular irrigation. Despite these efforts, several specimens perished, and surviving ones did not tolerate direct sunlight well. For *Eranthis hyemalis* and *Galanthus nivalis*, the death and deterioration of specimens greatly hindered or made impossible data collection and comparability. Hemicryptophytes also required irrigation and weeding, and their perennials allowed for multi-year observations. Besides reproductive phenophases, senescence was also observable in their case. The specimens of *Sedum acre* did not thrive at either location, dying in both locations after flowering each year. Of the *Ajuga reptans* specimens, only one survived until the end of the third year at the Budapest site. I recommend the application of this life-form, especially for *Euphorbia epithymoides* and *Inula ensifolia*, where senescence is well observable. *Briza media* thrived at both locations, flowering and producing seeds in all three years, but only partially showed signs of autumn senescence (partial leaf discoloration). Additionally, by the third year, it outgrew the pots visibly; after being planted outdoors, it began to develop rapidly. Therefore, if the experiment had continued for another year, it is uncertain whether it would have flowered or if the differences between locations and/or years would have been caused by meteorological parameters.

Chamaephytes also required irrigation and weeding, with their perennials making them suitable for multi-year observations. Apart from the fact that the specimens of *Dianthus plumarius* perished in both locations over the years, with only one surviving until the end of the experiment in Gödöllő, the species of this life-form performed well. They flowered in all three years at both locations, and species other than *Dianthus plumarius* visibly thrived, spreading, etc. The disadvantage of this life-form is that the species were only suitable for observing flowering. The application of therophytes and hemitherophytes, apart from irrigation and weeding, logically required annual seed harvesting and sowing. Thus, in addition to energy and time investment, there is another disadvantage compared to perennial species: the strict genetic identity cannot be maintained. Moreover, it is highly likely that hemitherophytes will not flower in the first year. In the experiment, it was observed that although theoretically all vegetative and reproductive phenophases can be observed on them, in reality, many specimens perished, and surviving specimens were much smaller and weaker than those occurring in nature. Based on the cost-benefit ratio, the most recommended application is the use of phanerophytes, followed by the application of hemicryptophytes and geophytes, the application of chamaephytes, and finally, the application of therophytes and hemicryptophytes.

The Carpathian Basin is particularly sensitive to the impacts of climate change (Pongrácz et al., 2009; Gálos et al., 2011; Hlásny et al., 2014; Antofie et al., 2015). Therefore, it is of paramount importance to conduct phenological studies in the region. Phenological investigations now hold a prominent position in global climate change research, enabling the prediction of the timing of cyclic phenomena based on the results obtained (Morellato et al., 2016). However, the application of these findings in conservation planning is still in its infancy (Morellato et al., 2016). Based on the above, I propose the establishment of long-term ex situ experiments for phenological observations at numerous locations across the country. Utilizing the results in conservation efforts, such as in the planning of ecological restoration projects or the development of conservation management plans, could significantly contribute to nature conservation.

Given that our country is not represented by any botanical garden connected to international phenological networks (IPG, PhenObs, etc.), and the phenological observation network operated by the National Meteorological Service ceased more than twenty years ago, there is an urgent need to establish one or more stations that comply with international standards, thus enabling comparisons and long-term phenological observations. Since operating an independent network created exclusively for this purpose is quite costly, and the stations of international networks are also situated in botanical gardens, I propose the connection of the two university botanical gardens serving as the location for my experiment, namely, the MATE Botanical Garden and the ELTE Botanical Garden, to the IPG network. As the dataset with the broadest and longest history at our disposal pertains to woody plants, forming the basis for potential comparisons, and my experiment has demonstrated that the care and long-term maintenance of woody plants, provided by the representatives of this life-form, yield the most abundant and usable data, I suggest initially connecting to the IPG network focusing on woody plants. However, considering that climate change increasingly threatens European biodiversity, understanding the responses of herbaceous species to climate change is of fundamental importance. For this purpose, I propose connecting to the PhenObs network, which focuses on wild herbaceous plants.

Additionally, it is recommended to establish a simple observational network in schools based on the principles of citizen science. These observation points could simultaneously contribute to education, environmental education, and mindset formation, while providing valuable phenological data from various regions of the country. Following a centrally developed protocol, schools could observe a few easily maintainable, resilient, native perennial species (based on our experience, preferably woody plants such as *Prunus spinosa*, *Cotinus coggygria*, and *Cornus sanguinea*), recording their budburst, flowering onset and end, and the beginning and end of leaf coloration. All of these phenophases are expected to occur during the school teaching period, and the species can be easily maintained and observed in the schoolyard. The results could reveal phenological differences across various regions of the country (see Walkovszky 1998), as well as differences observed over the years (see Walkovszky 1998). Establishing such a network is particularly important considering that there has been no national phenological observation network for more than 20 years.

To better understand the factors influencing phenophases and to more reliably predict their occurrences, average temperature-calculated phenological sensitivity is not sufficient. According to Richardson et al. (2006), the timing of spring phenophases is determined by the accumulated extent of warming, known as Heating Degree Days (HDD). For a more accurate prediction of budburst, leafing-out, and flowering times in the future, it would be advisable to perform calculations in this direction. Regarding autumn phenophases, it is worthwhile to consider the accumulated extent of cooling, known as Cooling Degree Days (CDD) (Gill et al. 2015). Studies confirm that during the spring period, phenology is more sensitive to temperature increases during the daytime than at night, both at the species and community levels (Piao et al. 2015, Rossi & Isabel 2017). Taking into account that in recent decades, nighttime warming has been faster than daytime warming (Davy et al. 2017), the absence of asymmetrical warming in phenological models may lead to an underestimation of spring phenology temperature sensitivity (Piao et al. 2015). Regarding autumn phenophases, it is possible that while higher nighttime temperatures delay the onset of leaf coloring, higher daytime temperatures, due to increased evaporation, bring it forward (Wu et al. 2018, Chen et al. 2020). Eppich et al. (2009), when processing a dataset collected over about forty years at the ELTE Botanical Garden, containing the timing of budburst, flowering, and withering of the flowers, found that the average daily temperature fluctuation, heat sum, and the number of frosty days had the strongest impact on the mentioned phenophases. Based on the above, it would be advisable to perform calculations with daily temperature data (daily temperature fluctuation, HDD, CDD, daytime and nighttime temperatures) for a more precise understanding of the development of both spring and autumn phenophases. Since these are quite complex and time-consuming operations, especially considering the huge size of the experiment database (about 53,000 rows of an Excel spreadsheet), I had to acknowledge that performing these calculations does not fit within the scope of this doctoral thesis.

5. Overview of new scientific results

My new scientific results are summarised in the following points:

1. During the experiment, I examined 33 predominantly native species in terms of phenology belonging to six different life-forms according to the Raunkiær life-form classification at two distinct mesoclimatic sites over three vegetation periods. A similar *ex situ* experiment, adhering to international standards and simultaneously investigating multiple life-forms, has not been conducted in Hungary to date.

2. For the six life-forms, I determined the phenological sensitivity of the maximum recordable phenophases (budburst, appearance of buds, onset of flowering, end of flowering, appearance of fruits, onset of senescence, complete senescence).

3. I determined the spatial and temporal variations in phenological events of the six life-forms included in the experiment and the 33 species within them.

4. When studying phanerophytes, I recorded both the onset of the growing season represented by budburst and the state of complete leaf fall, senescence. Based on these, in both locations and over three years, I determined the length of the growing season, the phenological sensitivity of the growing season compared with temperature data, and the changes in the onset, end, and length of the growing season between years at the locations.

5. Among the six life-forms included in the experiment, I established a ranking based on their applicability in *ex situ* phenological experiments and the cost-benefit ratio, which could facilitate the setup of similar experiments in the future.

6. Meteorological base stations were installed in the two botanical gardens serving as the experiment's locations, namely, the MATE Botanical Garden and the ELTE Botanical Garden. Using these two stations, meteorological data were collected every ten minutes for two years, including temperature, precipitation, relative humidity, air pressure, wind speed, sunshine duration, and wind direction. Based on the data, I determined the average temperature in the two botanical gardens. The comparison of phenological data with meteorological parameters is essential for phenological research. Long-term meteorological data collection with such frequency and covering this many parameters has not been conducted in the two botanical gardens until now.

6. Publications related to the topic of the dissertation

6.1. Publications in international peer-reviewed journals

In a foreign-language, impact factor journal:

Verbényiné Neumann, K.; Baltazár, T.; Saláta, D.; Szirmai, O.; Czóbel, S. (2023): Comparative Study of the Phenology of Seven Native Deciduous Tree Species in Two Different Mesoclimatic Areas in the Carpathian Basin. *Forests* 14(5): 885. https://doi.org/10.3390/f14050885

Ordonez, J. S., Deák, B., Valkó, O., Szász, V., **Verbényiné Neumann K.**, Elhouda, Z. N., & Csergő, A. M. (2023). A long-term demographic study of *Salvia nemorosa* L. to determine the effects of landscape structure on the mechanisms of population persistence. *Palaearctic grasslands* 57 pp. 26-27.

Demeter A., Saláta D., Tormáné Kovács E., Szirmai O., Trenyik P., Meinhardt S., Rusvai K., **Verbényiné Neumann K.,** Schermann B., Szegleti Zs., Czóbel Sz. (2021): Effects of the Invasive Tree Species Ailanthus altissima on the Floral Diversity and Soil Properties in the Pannonian Region. *Land* 10: 1155. https://doi.org/10.3390/land10111155

In a foreign language, non-impact factor journal:

Verbényiné Neumann K., Czóbel Sz. (2021: Comparative study of flowering phenology of selected plant life-forms in urban and rural environments. Preliminary results, pp. 25-36 *Columella — Journal of Agricultural and Environmental Sciences* Vol. 8. No.1 (2021) p. 65, DOI: 10.18380/SZIE.COLUM.2021.8.1.25

Verbényiné Neumann, K., Baltazár T., Meinhardt S., Szirmai O. (2023): "A Comparative Study of the Flowering Phenology of Wild Growing Geophytes in Two Different Mesoclimatic Areas in the Carpathian Basin". *Review on Agriculture and Rural Development* 12 (1-2):3-14. https://doi.org/10.14232/rard.2023.1-2.3-14

6.2. Conference proceedings with ISBN, ISSN or other certification

Full text, peer-reviewed, in English:

Verbényiné Neumann, K.; Czóbel, S. (2021): Comparative Study of Flowering Phenology of Selected Plant Life-forms Located in Urban and Rural Environments in Central Europe. Preliminary Results pp. 1-9 In: Proceedings of the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution, 15–31 March 2021, MDPI: Basel, Switzerland, doi:10.3390/BDEE2021-09453

Full text, peer-reviewed, in Hungarian:

Verbényiné Neumann, K. (2021): Különböző növényi életformák fenológiai eseményeinek összehasonlító vizsgálata eltérő mezoklimatikus környezetben pp. 294-305 In: Molnár Dániel, Molnár Dóra (eds.) „XXIV. Tavaszi Szél Konferencia 2021 Tanulmánykötet I." 773 p. Budapest 2021, Doktoranduszok Országos Szövetsége, ISBN 978-615-81991-1-7

Abstract in English or in Hungarian, based on oral presentation or poster:

Verbényiné Neumann K., Czóbel Sz. (2021): Ex situ flowering phenological study of three different plant life-forms of wild plant species under different mesoclimatic conditions/Harom kulonboző eletformat kepviselő vadon elő novenyfaj ex situ viragzasfenologiai vizsgalata elterő mezoklimatikus viszonyok között, pp. 118. In: Takács A. & Sonkoly J. (eds.) XIII. Aktuális Flóra-és Vegetációkutatás a Kárpát-medencében nemzetközi konferencia. Program és összefoglalók./13th "Advances in Research on the Flora and Vegetation of the Carpato-Pannonian Region" International Conference. Programme and Abstracts., 2021. november 11–14., Debrecen, ISBN 978-963-490-342-0

Verbényiné Neumann, K. (2021): Különböző növényi életformák fenológiai eseményeinek összehasonlító vizsgálata eltérő mezoklimatikus környezetben pp. 170 In: Molnár Dániel, Molnár Dóra (eds.) „XXIV. Tavaszi Szél Konferencia 2021 Absztraktkötet" 671 p. Budapest 2021, Doktoranduszok Országos Szövetsége, ISBN 978-615-5586-99-6

Verbényiné Neumann K.; Czóbel Sz. (2021): A fővárosi hőtöbblet hatása őshonos cserjefajok őszi fenológiájára, 12. Magyar Ökológus Kongresszus, 2021. 08. 24-26., Vác, pp. 215. In: Tinya Flóra (ed.) „12. Magyar Ökológus Kongresszus Előadások És Poszterek Összefoglalói" Abszraktkötet, 219 p. Vácrátót, 2021. augusztus 1., Magyar Ökológusok Tudományos Egyesülete (MÖTE) és Ökológiai Kutatóközpont (ÖK), Digitális kiadvány: https://mok12.ecology.hu/sites/default/files/12MOK_absztraktkotet_vegleges_0.pdf

Verbényiné Neumann K., Czóbel Sz. (2022): Őshonos cserjefajok őszi fenológiájának összehasonlítása eltérő mezoklimatikus környezetben = Comparative study of autumn phenology of autochton microphanerophytes in different mesoclimatic environments, In: Fodor Marietta , Bodor-Pesti Péter, Deák Tamás (eds.) „A Lippay János – Ormos Imre – Vas Károly (LOV) Tudományos Ülésszak tanulmányai [Proceedings of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting]", Budapest, Magyarország 2021.11.28. - 2021.11.28. (Magyar Agrár- és Élettudományi Egyetem),MATE Budai Campus, pp 784-786

Verbényiné Neumann K., Czóbel Sz. (2023): Comparative study of the flowering phenology of wild growing geophytes in two different mesoclimatic areas in the Carpathian Basin, pp. 61, In: Gyalai I., Czóbel Sz. (eds.) Book of abstracts, 20th Wellmann International Scientific Conference, 3rd April 2023 Hódmezővásárhely, Hungary, ISBN 978-963-306-924-0

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