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A COMPARATIVE STUDY OF THE FLOWERING PHENOLOGY OF WILD GROWING GEOPHYTES IN TWO DIFFERENT MESOCLIMATIC AREAS IN THE CARPATHIAN BASIN

Krisztina Verbényiné Neumann[1*], Tivadar Baltazár[2], Sarolta Meinhardt[1], Orsolya Szirmai[3]

[1*]Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences. H-2100 Páter Károly street 1, Gödöllő, HUNGARY, neumann.krisztina86@gmail.com
[2]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1665/1, 613 00 Brno, Czech Republic; baltazartivadar@gmail.com
[3]Institute of Animal Sciences and Wildlife Management, Faculty of Agriculture, University of Szeged, Andrássy út 15, H-6800 Hódmezővásárhely, Hungary, szirmai.orsolya@szte.hu

*corresponding author: neumann.krisztina86@gmail.com

Abstract: Plant phenology - timing of cyclical or seasonal biological events - has proven to be a very sensitive indicator for climate change impacts. The phenology of many plant species has been advanced by warming, with earlier spring species being more sensitive. To understand better the driving factors of the changing phenology we investigated the phenology of different wild growing geophytes in the Carpathian Basin for three consecutive years. The study has been carried out as an *ex situ* experiment in two different mesoclimatic sites, one in the Gödöllő Botanical Garden of the Hungarian University of Agriculture and Life Sciences (average temperature 11,35 °C), other in the Eötvös Loránd University Botanical Garden, Füvészkert in the central part of Budapest (average temperature 13,16 °C). During the experiment 5 replicates of 5 wild growing geophytes were examined. The results show an advance of 3.64 days in Budapest, with strong variation across species. The earliest flowering species didn't bloom (*Galanthus nivalis)* or died (*Eranthis hyemalis*) by the 3rd year in the site Budapest. If global warming continues, this advance and negative effects on wild growing plant species might be more serious in the future.

Keywords: Reproductive phenology, ex situ, climate change, heat island, botanical garden, temperate zone, early-spring.

1. Introduction

Climate change rises global temperatues, thus influencing ecosystem processes (Peñuelas 2017). On average, the world has already warmed 1.1 °C, affecting natural ecosystems in Europe and everywhere on Earth (IPCC, 2022). The observed trend of warming at a global or local scale can have serious implications on living organisms. Warming will decrease suitable habitat area for current terrestrial ecosystems and change their composition (IPCC, 2022). In Europe, more than half of the vascular plant flora may become endangered by the year 2080 as a result of climatic changes (Thuiller et al. 2005). Based on current research it seems that climate change can no longer be stopped. Therefore, it is crucial to investigate possible adaptations (Li et al. 2019).

Plant phenology, the timing of seasonally recurring phenomena in plants (Schwartz 2013) has proven to be a very sensitive indicator for climate change impacts (Sparks 2002, Cleland 2007). Climate can strongly influence phenology by speeding up or delaying events such as emergence, peak activity and reproduction (Sherry et al. 2007, Wolkovich 2021). Phenology of many plant species has been already advanced by warming (Sparks et al. 2000, Fitter and Fitter 2002, Parmesan and Yohe 2003, Elzinga et al. 2007, Bertin 2008, Szabó et al. 2016, Neumann & Czóbel 2021). Changes in the plant reproductive period also have important consequences on the reproductive success of populations, and thus on their dynamics (Sherry et al. 2007). For example, changes in flowering time may disrupt plant–pollinator interactions, particularly when the pollinators are seasonal (e.g. insects), and reduce seed production of plants and food resources to the pollinators, thereby influencing the survival and success of both species (Fitter & Fitter, 2002). Many studies have reported species-specific phenological shifts in response to climate change (Forrest & Miller- Rushing, 2010; Renner & Zohner, 2018) and found that phenological timing in early-spring bloomers were more responsive to warming than mid- or late-spring bloomers (Fitter and Fitter 2002, Menzel et al., 2006; Miller-Rushing & Primack, 2008, Kubov et al 2022). Insect-pollinated plants tended to have greater advancement of flowering than wind-pollinated plants (Fitter and Fitter, 2002), annuals demonstrated greater advancement than perennials (Fitter and Fitter, 2002).

Many studies in middle and high latitudes demonstrate that the temperature is the main driving force and interannual modulator of phenological change, while other factors (e.g. photoperiod, precipitation etc.) only play a secondary role as limiting factors (Szabó et al. 2016, Wolkovich 2021). Among several environmental factors (light, moisture, temperature) that can affect bulb development, temperature has been established as playing a predominant role in controlling growth and flowering in bulbs (Khodorova and Boitel-Conti 2013). Besides that, for early spring geophytes the number of frosty days and snowmelt has an important effect on both leafing and flowering phenology, controlling spring soil temperature and moisture (Eppich et al 2009, Snopková & Hýrošová 2017, Bandoc et al. 2022).

Temperature sensitivity or phenological sensitivity, which is expressed as the date of phenological event change for per degree Celsius change of temperature (days /°C), has been widely used to characterize the plants' responses to changed temperature (Wang et. al 2015, Zhang et al. 2015). Since the temperature sensitivity of plant phenological stages determines the magnitude of phenological shifts in response to future climate warming, more attention has been paid to it, both in observational records and warming experiment studies (Wolkovich et al. 2012).

Slow-colonizing forest understorey plants are probably not able to rapidly adjust their distribution range following largescale climate change. Therefore, the acclimation potential to climate change within their actual occupied habitats will likely be key for their short- and long-term persistence (deFrenne et al. 2010). This makes it particularly important to study the responses of these species to climate change. Nevertheless, relatively few studies have addressed the phenological events of geophytes as a life form (Tarasjev 1997, Turisova et al. 2007, Eppich et al 2009, Thomson 2010, Khodorova and Boitel-Conti 2013, Szabó et al. 2016, Snopková & Hýrošová 2017, Crișan et al. 2018, Puchałka et al. 2022).

Shifts in the phenological events of geophytes in the Carpathian Basin (Central Europe) are particularly poorly documented, with a few exceptions coming from the works of Eppich 2009, Szabó et al 2016, Neumann & Czóbel 2021).

Urban climate conditions are 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). Thus the urban environment is suitable for the application of the Space for Time substitution method. Which method encompasses analyses in which contemporary spatial phenomena are used to understand and model temporal processes that are otherwise unobservable, most notably past and future events. This method is used to predict the effects of climate change on biodiversity, identifying general trends, therefore its application saves time and money compared to long-term studies. (Pickett 1989, Blous et al. 2013) Thus, it is key to examine the patterns and shifts in the patterns of flowering phenology in urban areas compared with rural ones. Cities are strongly affected by climate change.

As part of the research, we examined the phenology of 5 geophyte species wild growing in the Carpathian Basin in two areas with different mesoclimates, in an ex situ experiment. We assumed (i) that first flowering, and the end of flowering will occur earlier at the site with a higher average temperature, while (ii) the lenght of flowering will longer the in the area with a higher average temperature. Furthermore, we examined to what temperature and other climatic factors besides the average annual temperature influence the time of occurrence of the different phenophases.

2. Materials and methods

2.1. Study sites

The study was carried out in two different mesoclimatic sites, one of which located in the Gödöllő Botanical Garden of Hungarian University of Agriculture and Life Sciences (47°35’36.2"N 19°22’06.2"E, 250 m elevation) (Szirmai et al. 2014), while the other in the Eötvös Loránd University Botanical Garden in the central part of Budapest (Budapest 47°29’05.6"N 19°05’05.7"E, 114 m elevation) (Orlóci et al 2019). Within a radius of 250 m around the two botanical gardens, the following local climate zones (LCZ) are present. In Budapest: LCZ 5 - Open mid-rise 60%, LCZ 6 - Open low rise 20% LCZ 2 - Compact mid-rise 20%. In Gödöllő: LCZ A - dense trees 40%, LCZ D - low plants 50%, LCZ 6 - Open low-rise 10% (Stewart & Oke 2012, http1). The Gödöllő site is next to a natural forest fragment, part of the 4.5 hectar botanical garden. The Budapest site is located in the 3,1 hectar Füvészkert.

During the three-year experiment the average air temperature was 11.35 °C and the average annual precipitation was 475.1 mm in Gödöllő Botanical Garden, while in the Eötvös Loránd University Botanical Garden the average air temperature was 13.16 °C and an average annual precipitation was 527.4 mm. There was therefore a difference of 1.81 °C between the three-year average temperature of the two botanical gardens. We used this value to calculate the phenological sensitivity. A homogeneous patch was created for each selected species in the two sites.

2.2. Methods

To examine the effect of the different mesoclimatic environments on the phenology of geophyte species. We selected 5 different, (in the Carpathian Basin wild growing geophyte species: two early-spring bloomers *Galanthus nivalis* L., *Eranthis hyemalis* L., and three mid- and late spring bloomers *Iris pumila* L., *Convallaria majalis* L., and *Polygonatum multiflorum* L. To maximize genetic conformity, vegetatively propagated specimens from the same location were used. The specimens were grown in standardized pots with a diameter of 14 cm, and were signed individually. All selected species were pollinated by insects. 5 repetitions were used for each species at both sites.

The *ex situ* experiment were set-up between December 2019 and February 2020 The Galanthus nivalis and Eranthis hyemalis specimens were introduced in autumn 2020, so we have data from 2021. The same soil mixture and irrigation protocol was used for every specimen at both sites. The plants were watered twice a week in spring and autumn and daily in summer to keep them well hydrated.

Measurements were taken for each specimens on the same day and weekly basis at both sites for 3 consecutive years. The experiment period covers the interval between March of 2020 and December of 2022.

We used the average temperature difference (1.81 °C) between the two locations, mesured during the three years of the experiment, to calculate the phenological sensitivity. It means the difference in the appearance of a phenophase (the time interval) was divided by 1.81 to get the phenologycal sensitivity. For example in case of one day difference the phenologycal sensitivity is 1 day/1.81 °C = 0.55 day/°C.

2.3. Measurements of biotic data

The species used for the experiment all have solitary flower. We recorded the number of buds, flowers and fruits by every uniqly signed plant specimen (numbered pots), at both site on a weekly basis.

2.4 Measurements of abiotic data (environmental parameters)

The meteorological data were collected by AgroSense base weather station (Sys-Control Kft, Budapest, Hungary) installed at both sites at the end of 2020 (*Figure 1*). For the year 2020 the data (daily mean temperature and daily sum of precipitation) of the nearest station of the Hungarian Meteorological Service (Lágymányos for Budapest and Aszód for Gödöllő) were used.

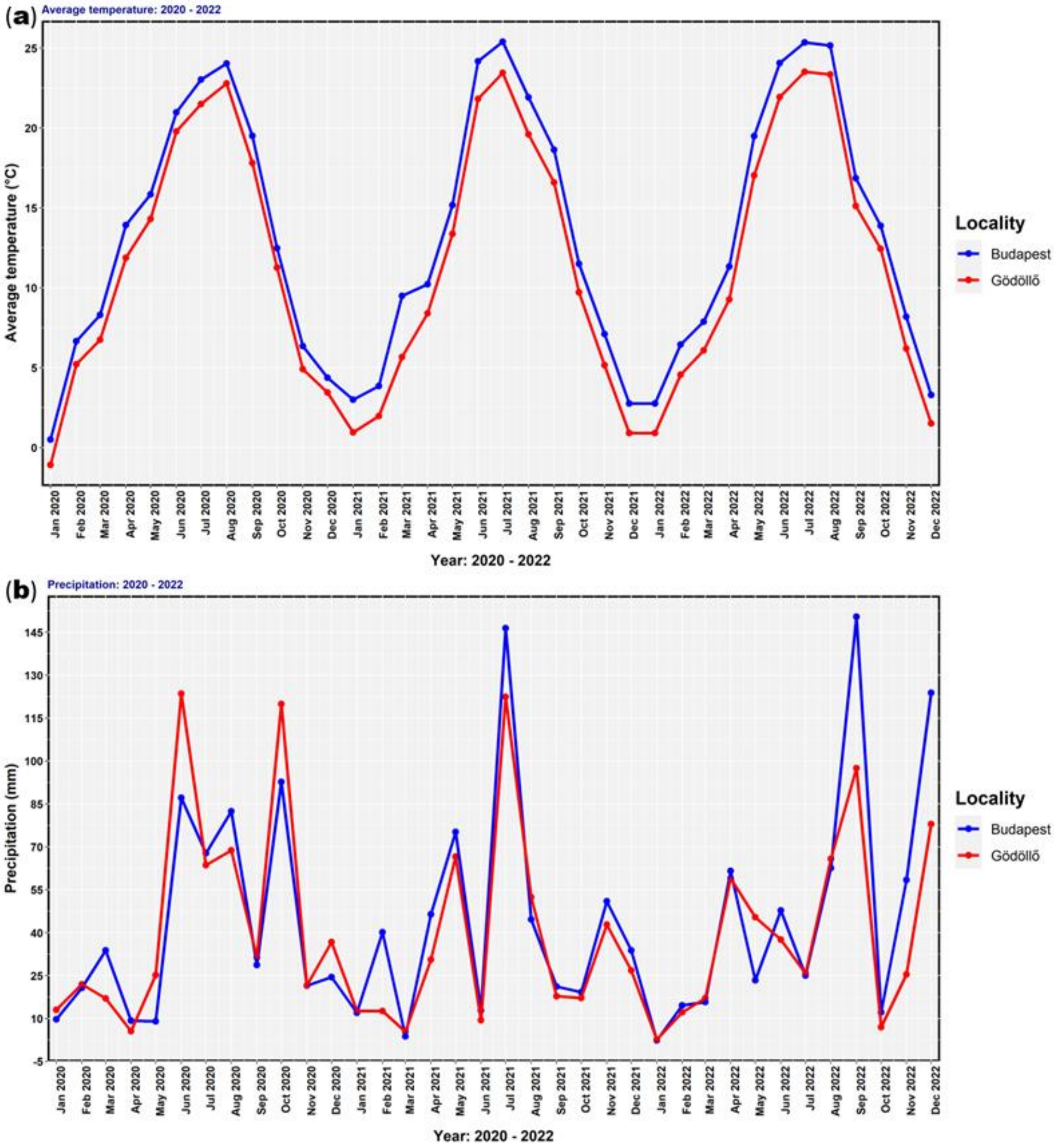

Figure 1: Climatic conditions of the sites during the duration of the experiment (March 2020 – Dec. 2022): monthly variation of mean air temperature (a) and sum of precipitation (b)

2.5. Statistical analysis

Data recording and basic data compilation was carried out in Microsoft Excel 365 online version and all statistical analyses were performed using freely available software R, version 4.2.2. (R Core Team R 2022), together with RStudio script editor (RStudio Team 2015). For advanced data processing, the additional packages "tidyverse" (Wickham et al. 2019), "dplyr" (Wickham et al. 2023) and "scales"

(Wickham et al. 2022)were also used.Package “ggplot2” (Wickham et al. 2016) was used for creating advanced statistical graphs.

3. Results

The relatively short flowering period of the species, and occasional non-flowering or mortality, resulted in relatively low element counts.

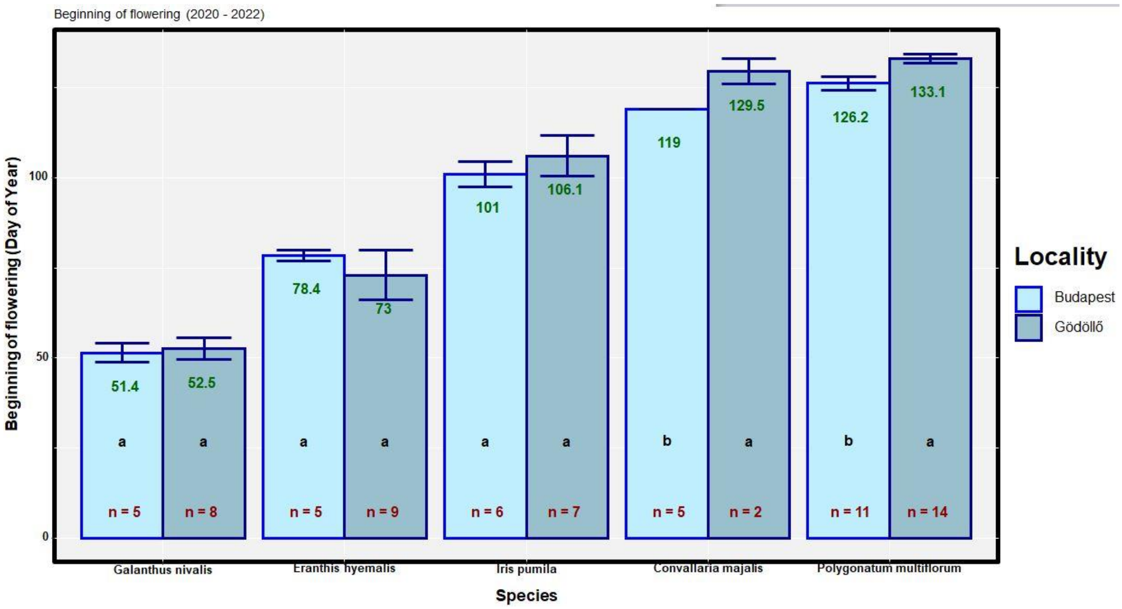

Figure 2: Date of beginning of flowering (first flower) at both sites. The letters **a** and **b** are for the mean values (green numbers), differing letters indicate a statistical significance difference at 5% significance level. The letter **n** is for the sample size. Standard deviation is shown on the top of the columns.

The appearanace of the first flower (*Figure 2*) occurred earlier for 4 of the 5 species in all three years at the warmer Budapest site. The difference is significant in 2 species. One species, Eranthis hyemalis, showed the opposite effect, the beginning of flowering was earlier at the Gödöllő site, but the difference is not significant. In the case of this species, the result is influenced by the fact that the individuals of the species died at the Budapest site by 2022, so they did not bloom here that year at all, while they did in Gödöllő. On average, the time of the appearanace of the first flower at the Budapest location is day of year (DOY) 95.20, while at the Gödöllő location DOY 98.84, the difference is 3.64 days. A virágzás hossza átlagosan 10.52 nap volt a budapesti helyszínen, míg 8.22 a gödöllői helyszínen, the difference is 2.3 days. Phenological sensitivity: 2.01 day/°C.

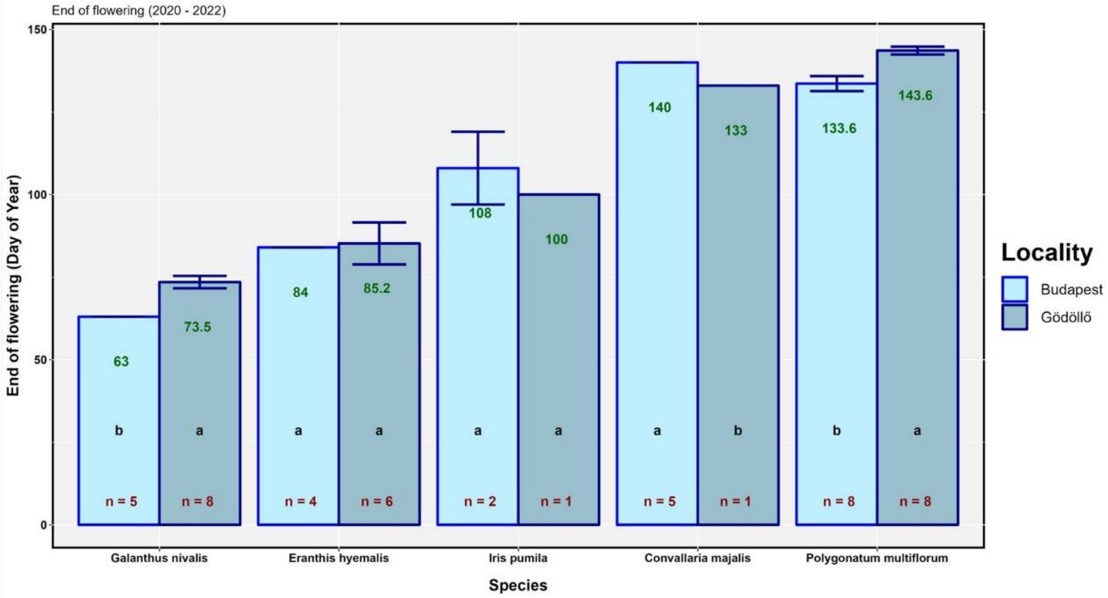

Figure 3: End of flowering. The letters **a** and **b** are for the mean values (green numbers), differing letters indicate a statistical significance difference at 5% significance level. The letter **n** is for the sample size. Standard deviation is shown on the top of the columns.

The end of flowering (*Figure 3*) occurred earlier for 3 of the 5 species in all three years at the warmer Budapest site. The difference is significant in 2 species – with earlier end of flowerint at the Budapest site, and in one species with earlier end of flowerint at the Gödöllő site.

On average, the time of the end of the flowering at the Budapest location is DOY 105.72 while at the Gödöllő location DOY 107.06, the difference is 1.34 days. Phenological sensitivity: 0.74 day/°C. On average, the lenght of flowering at the Budapest location was 10.52 days, while at the Gödöllő location 8.22 days, the difference is 2.3 days. Phenological sensitivity: 1.27 day/°C.

4. Discussion

Considering all species, the first flower appeared at the Budapest location on DOY 95.20, while at the Gödöllő location on DOY 98.84. The difference is 3.64 days which indicates a phenological sensitivity of 2.01 days/°C. On average, the end of the flowering occured at the Budapest site on DOY 105.72 while at the Gödöllő site on DOY 107.06. The difference is 1.34 days, which indicates a phenological sensitivity of 0.74 days/°C. The difference between the sensitivity of the beginning and the end of flowering amounts to a difference of 2.72 times, where the srtonger one is the sensitivity of the beginning of the flowering. It means, that the lenght of flowering was in Budapest 10,52 days long, while in Gödöllő 8,22 days long. The difference between the phenologycal sensitivity of the beginning and the end of flowering results in a longer flowering period at the site with a higher average temperature. This is in contrast to previous research (Miller-Rushing et al. 2009, Sherry et al. 2011, Bock et al. 2014, Nagahama et al. 2018), which found that higher temperatures shorten flowering time.

However, it is worth examining the early bloomer species (*Galanthus nivalis* and *Eranthis hyemalis*) and the mid- and late-bloomer species (Iris pumila, *Convallaria majalis* and *Polygonatum multiflorum*) separately. Regarding the early bloomer geophytes, the first flower appeared on average on DOY 62.75 in Gödöllő and on DOY 64.9 in Budapest. It means, the flowering advanced 2.15 days at the site with the lower average temperature, in contrary to previous researches (Sparks et al. 2000, Fitter and Fitter 2002, Parmesan and Yohe 2003, Elzinga et al. 2007, Bertin 2008, Szabó et al. 2016, Neumann & Czóbel 2021). There are several reasons for this. On the one hand, there was little data available, as the early geophytes only arrived in the second year of the experiment, and on the other hand, the fact that the early geophyte specimens at the Budapest site were dead by 2022 may have put a damper on the data. If we consider only the year 2021, when both early species flowered at both sites, the onset of flowering is DOY 54 and 47.5 in Budapest, values that are consistent with the general trend of earlier flowering in areas with higher average temperatures (Sparks et al. 2000, Fitter and Fitter 2002, Parmesan and Yohe 2003, Elzinga et al. 2007, Bertin 2008, Szabó et al. 2016, Neumann & Czóbel 2021).

Fitter et al. (1995) found that early-flowering species are very variable, regarding the flowering onset dates. The early bloomers showed the end of flowering on DOY 79.35 in Gödöllő and on 73.5 in Budapest. It results in a lenght of flowering of 16.6 days in Gödöllő and 8.6 days in Budapest. It means that the flowering period was shorter at the site with the higher average temperature which is In line with the previous research (Miller-Rushing et al. 2009, Sherry et al. 2011, Bock et al. 2014, Nagahama et al. 2018). It means a phenological sensitivity of 4,42 days/°C.

Regarding the mid- and late bloomer geophytes, the first flower appeared on average on DOY 122.9 in Gödöllő and on DOY 115.4 in Budapest. The difference is 7.5 days, which indicates a phenological sensitivity of 4.14 days/°C. It is line with previous research, Fitter et al. (1995) found that high spring temperatures advanced flowering by a mean of 4 days per degree. The end of flowering occured on DOY 125.5 in Gödöllő and on DOY 127.2 in Budapest. The difference is 1,7 days, surprisingly with earlier occurance at the Gödöllő site with the lower average temperature. This indicates a phenological sensitivity of -0,94 days/°C. The lenght of flowering was in Budapest 11,8 days long, while in Gödöllő 2,6 days long. This is in contrast to previous research (Miller-Rushing et al. 2009, Sherry et al. 2011, Bock et al. 2014, Nagahama et al. 2018), which found that higher temperatures shorten flowering time.

Previous research found that ephemerals, early-spring bloomers, and insect-pollinated plants in these environments tend to be more sensitive and show a greater advancement than perennials, mid- or late-spring bloomers, and wind-pollinated plants. It is line with other reserches (Fitter and Fitter 2002, Nail and Wu 2006). To find out, a longer-term, multi-species study would be needed.

Changes in the phenology of different native geophyte species due to climate change will bring about various other changes and will challenge native conservation. De Frenne et al. (2011) found that understorey species will probably advance with increasing temperatures in the future, the effects on growth and reproductive performance are species-dependent. It is in line with our findings. de Frenne et al. (2011) conlcluded that these divergent responses of understorey plants could alter future forest understorey dynamics inculded community assembly. The phenological shift can cause several problems, some also related to the nature conservation. Zettlemoyer et al. (2019) found that warming-led non-native species were likely to flower earlier and more plastic to temperature than the natives. Phenology will alter temporal overlap between plants and pollinators. can cause mismatches between plants and their pollinators (Forrest 2015). Warming caused shortened winter periods, alongside decreasing snow cover duration, increase late spring frosts frequency (Liu et al., 2018; Zohner et al., 2020), which can negatively afect plant growing conditions in spring.

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