Original scientific paper
Šumarski list 150 (1–2): 7–18, 2026

Rootstock Influence on Intra-clonal Variability in Spring Leaf Phenology of Pedunculate Oak (*Quercus robur* L.)

Marko Bačurin*, Ida Katičić Bogdan, Krunoslav Sever, Saša Bogdan

University of Zagreb Faculty of Forestry and Wood Technology, Zagreb, Croatia
* Corresponding author: Marko Bačurin, e-mail: mbacurin@sumfak.unizg.hr

Abstract

This study aimed to investigate the influence of rootstock on intra-clonal variation in spring leaf phenology of pedunculate oak (*Quercus robur* L.) clones. In the context of clonal seed orchards, phenological synchrony is critical for successful pollination and seed production. While leaf phenology is largely under genetic control, increasing evidence suggests that rootstocks can influence scion phenology.

The experiment was conducted at the Brestje nursery using 43 pedunculate oak clones, each represented by three grafted ramets on genetically diverse seedling rootstocks. Phenological monitoring was carried out from 2010 to 2014, and intra-clonal differences in budburst timing (phenophase 3) were analyzed using bootstrap analysis. Additionally, the impact of a late spring frost in 2012 was assessed by comparing the timing of phenophase 4 between frost-damaged and frost-surviving ramets.

Results showed that, despite genetic uniformity, there was significant intra-clonal variability in budburst timing. In 2010, as many as 79.1% of clones exhibited a budburst range of ≥3 days between ramets, which can be considered a biologically meaningful threshold. The highest level of phenological synchrony was recorded in 2013. Notably, ramets that flushed later were less susceptible to frost damage, with the greatest observed difference between damaged and undamaged ramets being 21 days.

These findings highlight the critical role of rootstock in shaping scion phenology, particularly in the context of optimizing seed production and weather adaptability in clonal seed orchards.

Keywords: pedunculate oak, rootstock, leaf phenology, intra-clonal variation, frost resistance, clonal seed orchards

DOI:
https://doi.org/10.31298/sl.150.1-2.1

How to Cite:

Bačurin, M., I. Katičić Bogdan, K. Sever, S. Bogdan, 2026: Rootstock influence on intra-clonal variability in spring leaf phenology of pedunculate oak (*Quercus robur* L.). Šumarski list 150 (1–2): 7–18, 2026. https://doi.org/10.31298/sl.150.1-2.1

INTRODUCTION

Pedunculate oak (*Quercus robur* L.) forest stands' regeneration can be achieved naturally through shelterwood cutting. However, in many situations—particularly where natural regeneration proves insufficient—artificial methods such as seed sowing or seedling planting become necessary. To ensure effective forest regeneration in cases requiring artificial methods, it is advisable to rely on high-quality reproductive material that meets genetic and ecological standards.

To enhance the genetic quality of forest reproductive material and increase the frequency and abundance of seed production, clonal seed orchards have been established in Croatia (Vidaković 1996). These orchards are established through heterovegetative propagation (grafting) of "plus trees"—phenotypically superior individuals selected from natural forest stands.

A critical goal in clonal seed orchards' management is to ensure a sufficient number of genotypically distinct clones for seed production, i.e. to maximize effective population size (Kramer et al. 2008). To facilitate successful pollination among these clones, phenological synchrony, the uniformity of developmental timing across clones, is of vital importance (Franjić et al. 2011).

It is well known that budburst is under strong genetic control and exhibits high heritability. This has been confirmed in various tree species, including pedunculate oak (Scotti-Saintagne et al. 2004), poplar (*Populus* spp.) (Frewen et al. 2000), and birch (*Betula* spp.) (Billington and Pelham 1991). Although it is strongly influenced by the genome, the primary environmental triggers for leaf phenological events are temperature and photoperiod. These factors determine the narrow time window during which environmental conditions are optimal for plant development (Robson et al. 2013, Basler and Korner 2014, Zohner and Renner 2015).

The timing and progression of spring leaf phenology, including the phases from budburst to full leaf expansion, significantly influence the length of the growing season and act as major drivers of ecological processes in temperate forests (Polgar and Primack 2011). Trees that set buds too early in the fall or flush too late in the spring may experience a shortened growing season, reducing both competitive ability and growth potential (Frewen et al. 2000).

Optimal phenological timing allows trees to avoid late spring frosts that can damage sensitive tissues and to extend the photosynthetically active period, thereby enhancing biomass production (Lockhart 1983, Leinonen and Hänninen 2002, Gömöry and Paule 2011). While temperature and photoperiod are considered the primary drivers of phenological development, a range of additional abiotic and biotic factors—known in the literature as atypical—can also exert significant influence on phenological traits (Bačurin et al. 2023). These include drought stress (Vander Mijnsbrugge et al. 2016, Bačurin et al. 2025), nutrient availability (Bačurin et al. 2023), and, in cases of severe insect infestation, phenological shifts in spring leaf development (Haukioja et al. 1988, Kaitaniemi et al. 1997).

In addition to the previously mentioned atypical factors, several studies have indicated that grafting can also lead to rootstock-induced effects on the scion phenology (Young and Houser 1980, Durner and Goffreda 1992, Camisón et al. 2021). In forestry and horticulture, rootstocks are used to improve graft compatibility, regulate scion vigor, and enhance reproductive success (Jayawickrama et al. 1991). Studies in fruit species have shown that rootstocks can affect vegetative growth, fruit yield, as well as the phenological and physiological traits of the scion, including leaf and flower development (Wang et al. 1994; Jiménez et al. 2004, 2011; Tworkoski and Miller 2007; Neilsen et al. 2016). It has also been established that the use of specific rootstocks can improve drought tolerance (Tworkoski et al. 2016).

Although the influence of rootstocks on phenological traits is well known in fruit crops, their effects on scion phenology in forest tree species, such as pedunculate oak, remains insufficiently studied. Given the ecological and silvicultural importance of this species, understanding rootstock-scion interactions is particularly relevant in the context of establishing clonal seed orchards, since grafting is the standard methodology used for their establishment. This study aimed to assess whether different rootstock genotypes influence intra-clonal variability in budburst timing among pedunculate oak clones. Such knowledge is critical to ensuring phenological synchrony among ramets and to maximizing seed production in clonal seed orchards.

MATERIAL AND METHODS

Experimental Design and Plant Material

The data used in this study were collected from an experimental trial established at the Brestje nursery (45.84°N, 16.10°E), managed by Croatian Forests Ltd. The trial was set up using heterovegetatively propagated (grafted) pedunculate oak clones, originating from Kosovac (45°36′09.8″N 17°58′03.3″E), Petkovac (45°08′49.2″N 18°51′36.5″E), and Pleščice I (45°44′52.2″N 16°35′14.7″E) clonal seed orchards. Planting began on 13 March 2008, following a randomized complete block design with three replications. Each of the 150 clones was represented by one ramet per block, resulting in a total of 450 ramets.

Scion material for heterovegetative propagation and trial establishment was collected in the spring of 2007 from the previously mentioned clonal seed orchards and was immediately grafted onto previously prepared rootstocks. These rootstocks had been grown from acorns one year earlier, in the spring of 2006. The acorns were sampled from natural oak populations, and their genetic background is unknown. However, it is assumed that rootstocks are genetically diverse.

Phenological observations of budburst were conducted twice a week until complete leaf development was recorded for all clones. A 1–7 ordinal scale, as described by Franjić et al. (2011), was used to assess phenological phases. All observations were performed by an experienced observer, and the recorded phenological scores were used as input for subsequent statistical analyses.

Data Processing and Statistical Analysis

Although the experimental trial was established in 2008, phenological monitoring data used in this study were an-

alyzed starting from 2010, in order to avoid potential transplant shock effects that may have influenced early plant development. The dataset spans from 2010 to the end of the monitoring period in 2014. It is important to note that data from the year 2012 were excluded from this part of the analysis, which focuses on phenological synchronization, due to the occurrence of a late spring frost that year. This climatic event may have negatively affected plant development and compromised the reliability of the results.

Data were filtered to include only those clones for which all three ramets were consistently represented across all observation years included in the study. In the end, the analysis was conducted on 43 clones, each represented by three ramets.

Data processing and visualization were performed using R statistical software (version 4.4.3; R Development Core Team 2024). Data cleaning, transformation, and preparation were carried out using the packages tidyverse (version 2.0.0), dplyr (version 1.1.4), tidyr (version 1.3.1), zoo (version 1.8-13), rstatix (version 0.7.2), and lubridate (version 1.9.4).

Data visualization was conducted using ggplot2 (version 3.5.1), ggstatsplot (version 0.12.3), ggalt (version 0.4.0), and gridExtra (version 2.3). In addition, summary results were formatted and presented in tabular form using the gt (version 1.0.0) and formattable (version 0.2.1) packages.

For further analysis, the exact date when a plant entered a specific phenological phase was extracted. In cases where the entry into a phenophase was not directly observed, i.e., when the transition occurred between two monitoring dates, interpolation was used to determine the date of phase onset. These interpolated values were subsequently used in statistical analyses.

The Shapiro–Wilk test indicated that the phenological data were not normally distributed. To assess differences in phenological timing among ramets within individual clones, a bootstrap analysis was performed using the boot (version 1.3.31) package. This non-parametric resampling approach provided robust estimates of confidence intervals and test statistics based on 2,000 resamples with replacement from the original dataset. The analysis evaluated whether differences occurred among ramets of the same clone in the date of entry into phenophase 3, corresponding to budburst and marking the onset of vegetative growth—a critical stage influencing both the length of the growing season and a clone's exposure to environmental stressors such as late spring frost.

Temperature Data and Biological Threshold Determination

Daily minimum and mean air temperature data were obtained from the Croatian Meteorological and Hydrological Service and recorded at the Maksimir meteorological station (45°49'19"N 16°02'01"E), the nearest station to the experimental site. On 10 April 2012, a late spring frost occurred, causing significant damage to ramets that had already reached advanced phenological phases (≥ phenophase 4). Using an interpolation model, the exact date each ramet entered phenophase 4 (folded leaf visible) was determined. Ramets that had reached phenophase 4 or higher (≥ 4) on or before 10 April 2012, the date of the frost event, were classified as frost-damaged, while those that entered this phase afterward were classified as frost-surviving. For each clone, the difference in days between the phenophase 4 onset of damaged and surviving ramets was calculated. The analysis revealed that a delay of just three days in reaching phenophase 4 was sufficient to avoid frost injury; this difference was therefore adopted as a biological threshold for frost avoidance. This finding was then used to define a three-day or greater difference in budburst timing (phenophase 3) among ramets within a clone as biologically significant.

RESULTS

Bootstrap Analysis of Intra-clonal Variation in Spring Leaf Phenology

In 2010, the results indicated that 11.2% of clones exhibited statistically significant differences ($p < 0.05$) in phenophase dynamics between their ramets. This proportion increased in 2011 to 17.8%, suggesting greater intra-clonal variability in that year. In 2013, the proportion of clones with significant within-clone differences slightly decreased to 14.4%. However, in 2014, this percentage rose noticeably to 24.8%, representing the highest level of intra-clonal phenological divergence across the analyzed years (Figure 1).

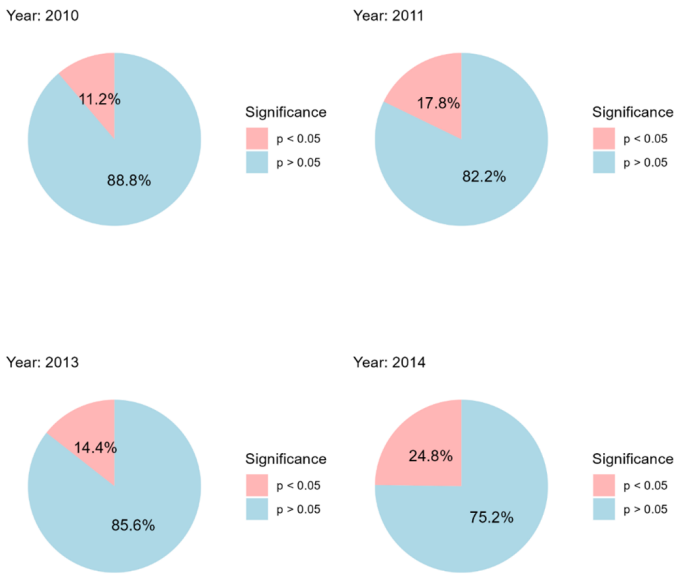

Figure 1 Proportion of clones showing significant phenological differences between their ramets across years (2010, 2011, 2013, and 2014), based on bootstrap analysis in phenophase 3. The red segments ($p < 0.05$) represent clones with statistically significant intra-clonal variation in phenophase progression, while blue segments ($p > 0.05$) indicate clones without significant differences.

Dynamics of Spring Leaf Development in Clone BJ 39

The progression of spring phenological phases was tracked in three ramets (a, b, and c) of clone BJ 39 during four years: 2010, 2011, 2013, and 2014. Clone BJ 39 was selected as a representative example to illustrate intra-clonal variation among the clones analyzed in the study. The phenological curves show year-to-year variation in both the onset and rate of development among the ramets (Figure 2).

In 2010, ramet "b" initiated spring leaf phenology slightly earlier than ramets "a" and "c", but all three ramets progressed through phenophases relatively synchronously. Bootstrap analysis for phase 3 in that year confirmed no statistically significant differences between any of the ramet pairs.

In 2011, more pronounced divergence was observed. Ramet "b" entered budburst earlier than both ramets "a" and "c", and ramet "a" lagged noticeably behind. These differences were statistically confirmed: bootstrap analysis revealed a significant difference between ramets "b" and "c" ($p < 0.001$), while differences between ramets "a" and "b", and "a" and "c", were not significant.

In 2013, all three ramets initiated spring phenology in a relatively synchronized manner; however, ramet "a" exhibited a slower progression of phenophases, indicating a generally slower developmental dynamic compared to the others.

In 2013, ramet "b" initiated spring phenology earlier than both ramets "a" and "c". Bootstrap analysis confirmed a statistically significant difference in the onset timing between ramets "b" and "c", indicating an earlier development in ramet "b".

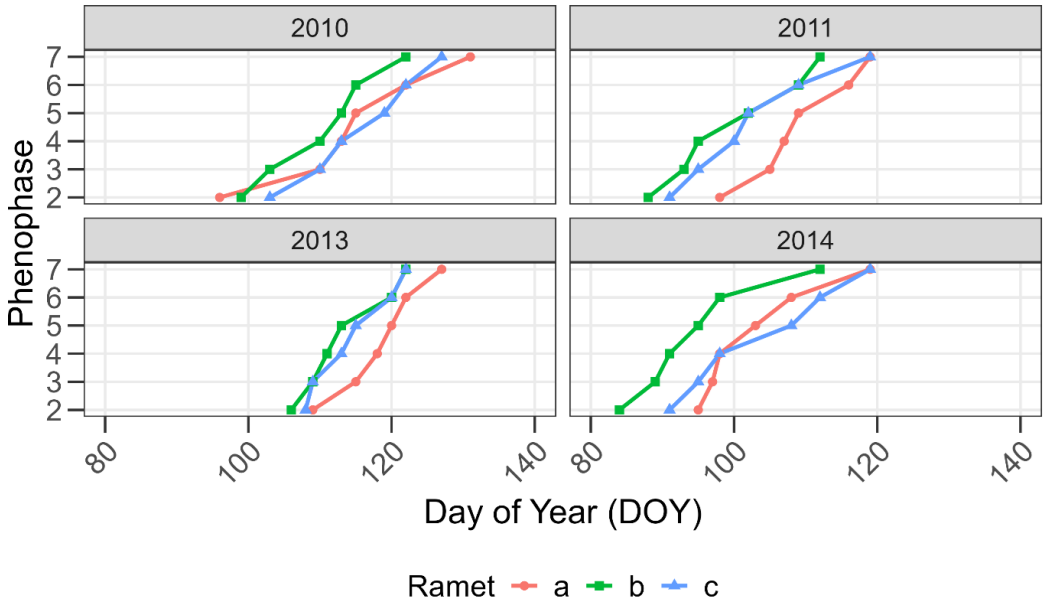

Figure 2 Phenological development of ramets "a", "b", and "c" of clone BJ 39 across four years (2010, 2011, 2013, and 2014). The y-axis represents phenophase values (phases 2–7), while the x-axis shows the day of the year (DOY). Although the general trend of development is similar, the timing and rate of progression vary between ramets and years, indicating intra-clonal variability in phenological dynamics.

Dynamics of Spring Leaf Development in Clone NA 02

The progression of spring leaf phenological phases was monitored in three ramets (a, b, and c) of clone NA 02 over four growing seasons: 2010, 2011, 2013, and 2014. Clone NA 02 was selected as a representative example to illustrate intra-clonal variation among the clones analyzed in the study. Visual analysis of phenological curves revealed consistent differences in the onset and progression of development among the ramets (Figure 3).

Particular focus was placed on phenophase 3 (budburst), which marks the visible opening of buds and the initiation of leaf development. In all analyzed years, ramet "a" consistently entered this phase earlier than ramets "b" and "c", while ramet "b" was generally the latest to initiate and complete leaf development.

To assess these differences statistically, bootstrap analysis was performed specifically for phase 3. The results confirmed significant differences between ramets "a" and "b" in 2010 ($p = 0.009$) and 2011 ($p < 0.001$), as well as between ramets "a" and "c" in 2011 ($p = 0.033$). In 2013, although visual differences appeared subtle, statistical analysis revealed that significant differences existed between ramets "a" and "b" and between ramets "a" and "c", while the difference between ramets "b" and "c" was not significant. In 2014, phenological divergence reappeared, with significant differences confirmed between ramets "a" and "b" ($p < 0.001$) and "a" and "c", while again, no significant difference was observed between ramets "b" and "c".

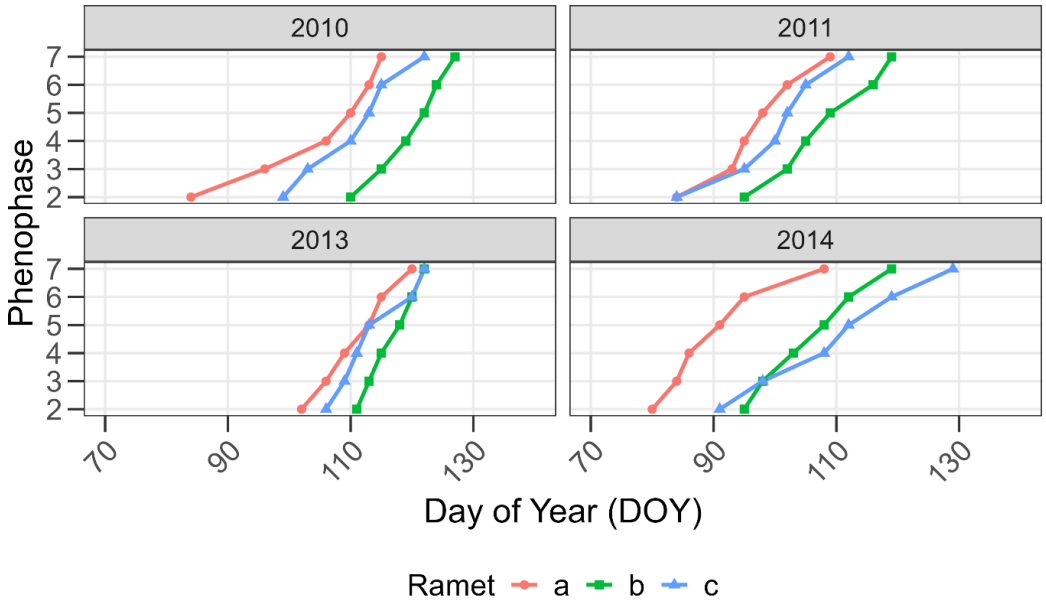

Figure 3 Phenological development of ramets "a", "b", and "c" of clone NA 02 across four years (2010, 2011, 2013, and 2014). The y-axis represents phenophase values (phases 2–7), while the x-axis shows the day of the year (DOY). Although the general trend of development is similar, the timing and rate of progression vary between ramets and years, indicating intra-clonal variability in phenological dynamics.

Temperature Data and Biological Threshold Determination

The data presented in Figure 4 illustrate the temporal dynamics of five distinct years over a three-month period from late January to late April.

The year 2010 began with temperatures slightly above freezing in early February. This was followed by a brief cooling period and then a gradual warming trend, marked by relatively low variability. The transition to spring conditions was steady and consistent, though less pronounced than in the following years.

The year 2011 started with moderate temperatures but was marked by a highly unstable March, characterized by frequent shifts between warm and cold spells. The warming trend emerged later in the season and became stable only toward the end of April.

The year 2012 featured the coldest start of all the years analyzed, with the lowest temperature recorded in early February (Figures 4 and 5). A noticeable increase in temperature followed; however, the pattern remained highly variable, with repeated alternations between warm and cold periods. Notably, on 10 April 2012, a late spring frost was recorded (Figure 5), which negatively affected ramets that were in phenophase 4 or beyond. Although meteorological station data recorded a frost event on 2 April 2012, no visible impact was observed on the ramets in the experimental trial.

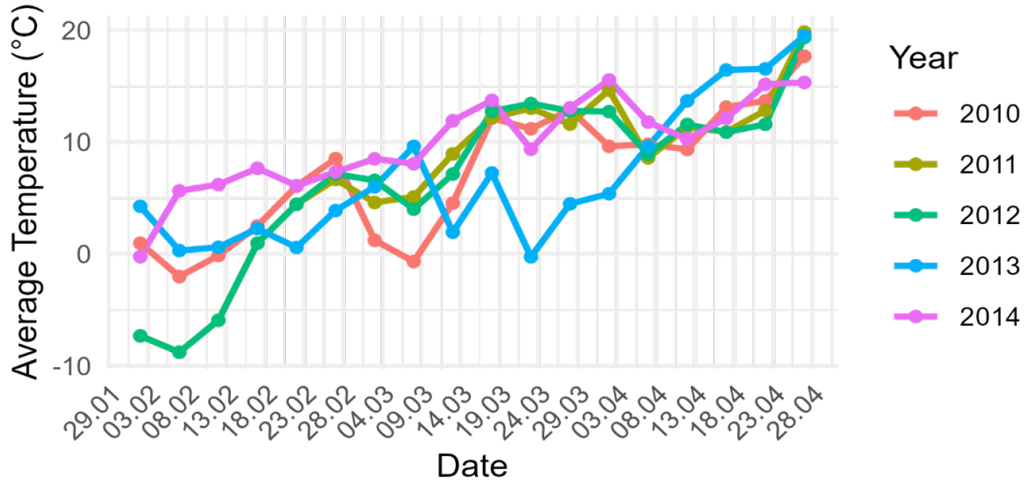

Figure 4 Five-day mean air temperatures (°C) recorded between 29 January and 28 April for five different years (2010–2014). Data were obtained from the Maksimir meteorological station and illustrate interannual variation in early spring temperature.

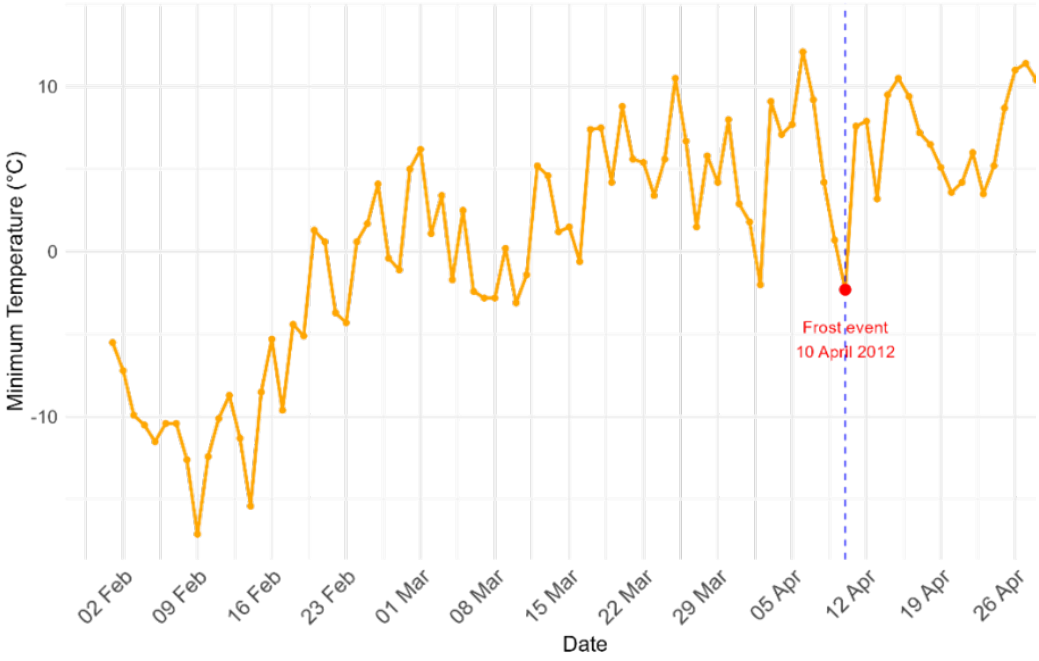

Figure 5 Daily minimum air temperatures (°C) recorded from 1 February to 30 April 2012, based on data from the Maksimir meteorological station. A significant drop in temperature was observed on 10 April 2012, indicating a late spring frost event.

The year 2013 began with temperatures near zero and exhibited considerable variability throughout March, including alternating drops and surges in temperature. Stable and warmer conditions were only recorded from mid- to late April.

The year 2014 showed a stable and gradual warming trend from the beginning of the observation period. However, noticeable cooling periods occurred in mid-March and again from early to mid-April. These cooler phases introduced a degree of instability, precisely during the period when increased budburst activity would typically be expected.

Intra-clonal Variability in Budburst Timing Across Years

The within-clone variability in the timing of budburst (phenophase 3) was assessed by calculating the range between the earliest and latest ramet transition dates for each clone (Table 1). In 2013, clones exhibited the highest degree of synchronization, with an average within-clone range of only 3.34 days and a maximum of 7 days. This was further supported by the fact that only 41.9% of clones had a range equal to or greater than three days, indicating limited intra-clonal asynchrony. In contrast, the year 2010 showed the highest variability, with a mean range of 6.31 days and a maximum of 19 days; 79.1% of clones exhibited a range of ≥3 days. Similarly, 2014 also showed considerable asynchrony (mean = 5.71 days; max = 16 days), with 72.1% of clones exceeding the 3-day threshold. The year 2011 displayed intermediate variability, with 46.5% of clones falling into this category (Figure 6). These results highlight substantial interannual differences in phenological coherence within clones, likely influenced by varying environmental conditions.

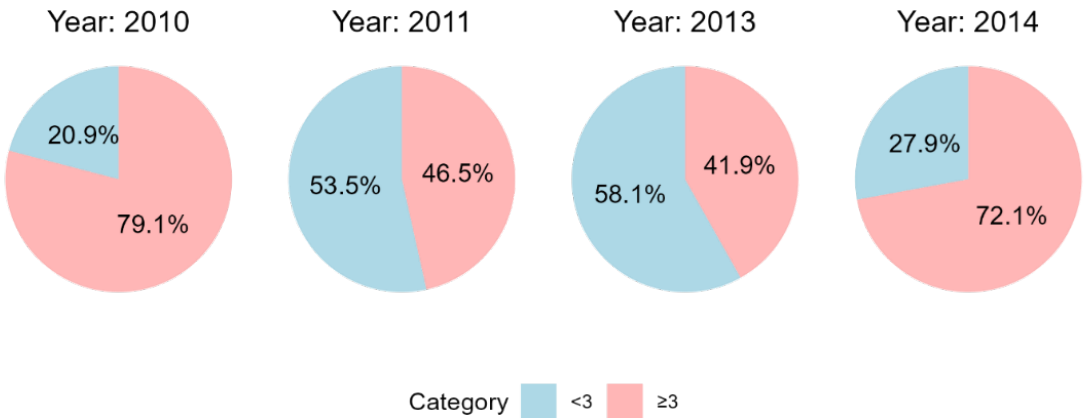

Figure 6 Distribution of clones with fewer than 3 days (<3, blue) and 3 or more days (≥3, pink) of intra-clonal variability in budburst across four years. Each pie chart shows the percentage of clones in each category for the years 2010, 2011, 2013, and 2014. The majority of clones exhibited higher within-clone variability (≥3 days) in 2010 and 2014, while in 2011 and 2013 the proportion of clones with lower variability (<3 days) was higher or more balanced.

Table 1 Intra-clonal variability in the timing of budburst (phenophase 3) across four study years (2010, 2011, 2013, and 2014) for each clone. Values represent the range (in days) between the earliest (MIN) and latest ramet (MAX) to reach phase 3 within the same clone, indicating the degree of intra-clonal synchrony. A greater range suggests lower synchrony (i.e., greater asynchrony) among ramets. A difference of three days or more was considered potentially significant.

Clone	2010				2011				2013				2014			
	mean	min	max	range	mean	min	max	range	mean	min	max	range	mean	min	max	range
BJ04	99.00	99	99	0	93.67	93	95	2	108.67	108	109	1	84.67	84	86	2
BJ05	109.33	103	115	12	106.33	105	109	4	115.67	113	117	4	99.67	98	103	5
BJ07	115.67	114	117	3	101.33	100	102	2	113.00	113	113	0	98.00	98	98	0
BJ18	114.00	110	118	8	109.33	105	114	9	116.33	115	117	2	106.00	98	112	14
BJ21	106.33	103	110	7	100.00	98	102	4	111.67	111	113	2	93.67	91	95	4
BJ25	100.33	99	103	4	94.67	93	98	5	108.33	108	109	1	82.00	80	84	4
BJ28	116.67	116	117	1	112.00	112	112	0	118.67	118	120	2	99.67	98	103	5
BJ29	96.67	92	99	7	94.33	93	95	2	108.67	106	111	5	88.00	84	91	7
BJ34	99.33	96	103	7	96.67	93	102	9	109.33	106	111	5	87.00	84	91	7
BJ39	107.67	103	110	7	97.67	93	105	12	111.00	109	115	6	93.67	89	97	8
BJ41	98.00	92	103	11	94.33	93	95	2	107.67	106	109	3	85.67	84	89	5
BJ46	118.00	115	122	7	113.67	113	113	0	123.00	122	125	3	110.67	108	112	4
BJ 50	108.67	106	110	4	93.67	93	95	2	108.67	108	109	1	84.67	82	86	4
NA01	101.67	96	110	14	91.33	88	95	7	107.67	106	111	5	83.67	80	91	11
NA02	104.67	96	115	19	96.67	93	102	9	109.33	106	113	7	93.33	84	98	14
NA04	92.00	92	92	0	88.00	88	88	0	103.67	102	106	4	80.00	80	80	0
NA05	99.33	96	103	7	93.67	91	97	6	107.67	106	109	3	84.33	80	89	9
NA08	97.00	96	99	3	92.67	92	92	0	106.67	106	108	2	84.67	84	86	2
NA09	93.33	92	96	4	92.33	91	95	4	106.00	106	106	0	81.33	80	84	4
NA12	93.67	89	96	7	91.00	91	91	0	105.33	104	106	2	81.00	79	84	5
NA15	98.00	96	99	3	93.00	93	93	0	107.00	106	109	3	83.33	82	84	2
NA16	95.67	92	103	11	93.00	91	95	4	106.67	106	108	2	82.67	80	84	4
NA17	93.33	89	99	10	94.67	93	98	5	108.33	106	113	7	87.67	82	97	15
NA19	98.33	96	103	7	92.33	91	93	2	109.00	109	109	0	84.67	84	86	2
NA20	93.33	92	96	4	91.67	91	93	2	106.00	106	106	0	81.67	77	84	7
NA23	93.00	91	96	5	90.00	88	91	3	108.33	106	113	7	84.67	79	95	16
NA28	99.33	96	103	7	92.33	91	93	2	107.67	106	109	3	84.00	84	84	0
NA29	95.67	92	99	7	93.00	91	95	4	107.67	106	109	3	84.00	84	84	0
NA38	93.33	89	99	10	88.67	88	90	2	104.67	104	106	2	77.00	77	77	0
NA40	99.00	99	99	0	92.33	91	93	2	107.33	106	108	2	84.00	82	86	4
VK04	93.33	92	96	4	90.00	88	91	3	105.33	104	106	2	78.00	77	80	3
VK09	122.00	122	122	0	114.67	112	116	4	121.33	120	122	2	114.00	112	115	3
VK11	115.67	115	118	3	106.33	105	107	2	116.00	115	118	3	103.00	98	108	10
VK16	111.67	110	115	5	110.00	109	112	3	117.33	117	118	1	102.33	102	103	1
VK18	98.00	96	99	3	93.67	93	95	2	108.33	108	109	1	82.67	80	84	4
VK20	104.00	99	110	11	93.00	93	93	0	109.00	109	109	0	85.67	84	89	5
VK29	92.00	92	92	0	91.33	88	95	7	106.00	106	106	0	80.33	79	82	3
VK31	106.00	106	106	0	109.00	109	109	1	116.67	116	117	1	100.00	98	103	5
VK34	113.67	113	115	2	112.00	112	112	0	117.67	117	118	1	102.33	102	103	1
VK38	92.00	92	92	0	86.67	86	88	2	103.67	102	106	4	78.67	77	80	3
VK40	116.33	113	119	6	107.67	102	112	10	115.33	111	118	7	103.00	98	108	10
VK43	93.33	92	96	4	91.00	91	91	0	105.33	104	106	2	77.00	77	77	0
VK56	101.67	92	110	18	96.67	93	102	9	109.67	109	111	2	90.00	86	95	9

Frost Avoidance as an Outcome of Intra-clonal Phenological Variability

Table 2 presents the timing of phenophase 4 in ramets of oak clones that experienced frost damage in spring 2012, compared to ramets of the same clones that survived without visible damage. In all cases, frost-damaged ramets reached phenophase 4 earlier than the frost-surviving ramets of the same clone. This finding indicates that early budburst resulted in an earlier onset of phenophase 4, which significantly increased susceptibility to the late spring frost that occurred on 10 April 2012.

Table 2 Comparison of phenophase 4 between frost-damaged and frost-surviving ramets across clones in which at least one ramet experienced frost damage. The table presents the dates on which each ramet reached phenophase 4, along with the difference in days between damaged and surviving ramets within the same clone. Ramets within each clone are labeled as "a"" b", and "c".

Clone	Frost-damaged Ramet	Frost-survived Ramet	Phenophase 4 (damaged ramet)	Phenophase 4 (survived ramet)	Difference (days)
BJ 25	b	a	2012-04-10	2012-04-13	3.0
BJ 25	c	a	2012-04-10	2012-04-13	3.0
BJ 29	b	a	2012-04-10	2012-04-13	3.0
BJ 29	b	c	2012-04-10	2012-04-13	3.0
BJ 41	b	a	2012-04-06	2012-04-13	6.5
BJ 41	c	a	2012-04-06	2012-04-13	6.5
BJ 50	c	a	2012-04-03	2012-04-17	14.0
BJ 50	c	b	2012-04-03	2012-04-13	10.0
NA 02	a	b	2012-04-06	2012-04-27	20.5
NA 02	c	b	2012-04-10	2012-04-27	17.0
NA 08	a	c	2012-04-06	2012-04-24	17.5
NA 08	b	c	2012-04-10	2012-04-24	14.0
NA 16	b	a	2012-04-06	2012-04-13	6.5
NA 16	c	a	2012-04-10	2012-04-13	3.0
NA 19	b	a	2012-04-10	2012-04-13	3.0
NA 19	c	a	2012-04-10	2012-04-13	3.0
NA 20	a	c	2012-03-30	2012-04-20	21.0
NA 20	b	c	2012-04-10	2012-04-20	10.0
NA 23	b	a	2012-03-30	2012-04-20	21.0
NA 23	c	a	2012-04-10	2012-04-20	10.0

The differences in phenophase timing between damaged and surviving ramets varied considerably across clones, ranging from 3.0 to 21.0 days. Minimal differences (3.0–6.5 days) were observed in clones BJ 25, BJ 29, BJ 41, NA 16, and NA 19, suggesting that although these clones initiated budburst early, the phenological gap between damaged and surviving ramets was relatively small—yet still sufficient to prevent frost damage in the surviving ramets. In contrast, clones BJ 50, NA 02, NA 08, NA 20, and NA 23 exhibited much larger differences, ranging from 10.0 to 21.0 days.

The most extreme case was observed in clone NA 20, where phenophase 4 in damaged ramets occurred on 30 March, while the surviving ramets reached the same phase on 20 April, resulting in a 21-day delay. These findings clearly demonstrate that ramets with earlier budburst were more susceptible to frost damage, and that a delay of three or more days in reaching phenophase 4 was sufficient to prevent such damage.

Figure 7 illustrates the relationship between spring leaf phenology and minimum daily temperature in three ramets of the BJ 25 clone during the spring of 2012. On 10 April 2012, a late spring frost occurred, with minimum temperatures dropping to –2.3°C. At that time, two ramets ("b" and "c") had already reached phenophase 4 or higher. After the frost event, both exhibited a temporary shift from phase 4 back to phase 2, indicating frost damage to the developing buds and partial loss of newly expanded tissues. Because leaves at advanced developmental stages were destroyed, the trees subsequently initiated renewed leaf growth and were later recorded at lower phenological phases. In contrast, ramet "a" (green line) exhibited slower development, reaching only phase 3 (budburst) at the time of the frost, and showed no signs of damage thereafter. This pattern, shown in Figure 7, illustrates how earlier budburst increased frost exposure risk and led to a phenological setback in the more advanced ramets.

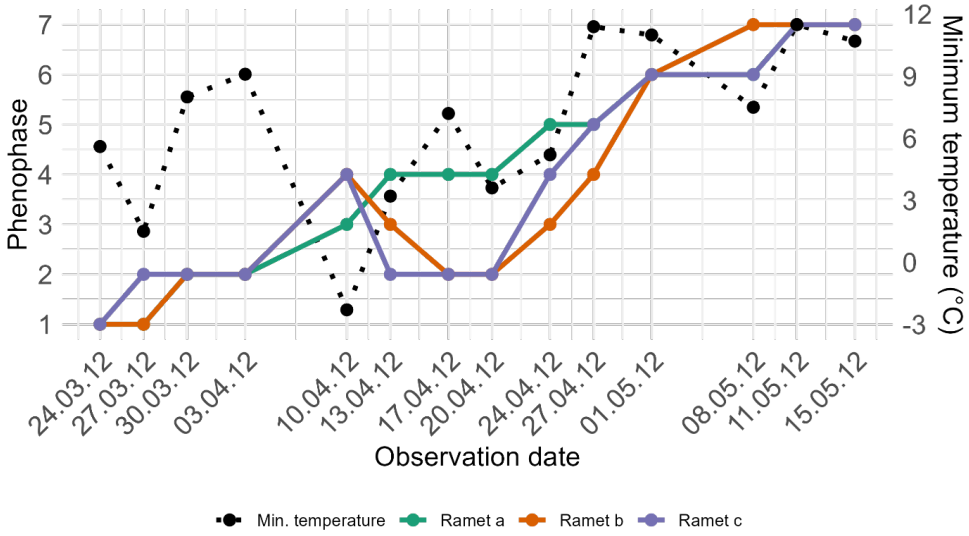

Figure 7 The relationship between spring leaf phenology and minimum daily temperature in three ramets of the BJ 25 clone during the spring of 2012. The graph shows the phenological development stages of three ramets ("a," "b," and "c") of the BJ 25 clone over time, plotted against daily minimum temperature (black line, right y-axis). A late frost event occurred around 10 April, when minimum temperatures dropped below 0°C. Ramets that had already reached or exceeded phenophase 4 by this date (notably ramets "b" and "c") were damaged by frost, highlighting the impact of early budburst on vulnerability to late spring frosts.

DISCUSSION

Intra-clonal Variability in Spring Leaf Phenology

The bootstrap analysis of intra-clonal variability in spring leaf phenology provided an additional statistical perspective on the degree of synchronization among genetically identical ramets across multiple years. While the majority of clones did not exhibit statistically significant differences among ramets, several clones showed clear intra-clonal asynchrony, indicating that temporal variation in budburst can occur even within a single genetic background.

These findings complement the descriptive analysis (Table 1, Figure 6), which captures the range of phenological variation and highlights biologically meaningful differences which may not reach statistical significance. For example, in clone NA 02, graphical analyses of phenological phase dynamics across three ramets over four years consistently revealed a lack of synchronization (Figure 3). The bootstrap test detected statistically significant differences between ramets "a" and "b" in all years except 2013, and further inspection of phenophase 3 onset showed that ramet "a" consistently flushed about seven days earlier than ramet "b", indicating a difference that is both consistent and biologically relevant.

Taken together, these results show that the bootstrap and descriptive analyses address different but complementary aspects of intra-clonal variability, providing both statistical and biological perspectives on phenological divergence among ramets.

Similarly, if we observe clone BJ 39 (Figure 2), we can see that although all three ramets belong to the same clone, the data clearly demonstrate variability in the timing and dynamics of spring phenophase progression. Throughout all observed years, the ramets of clone BJ 39 exhibited overall consistent phenological patterns, yet with notable differences among individual ramets. Ramet "b" consistently emerged as the earliest to initiate spring development, regularly reaching budburst and leaf expansion stages ahead of the others. In contrast, ramet "a" showed a clear tendency toward delayed phenological onset, with a slower progression through developmental stages in certain years. This phenological asynchronization among ramets of the same clone may be partly explained by a potential interaction between the rootstock and scion, which could influence the onset of phenological activity.

Such interactions have not been frequently reported in forest tree species; however, there are studies on certain fruit crops that provide relevant insights. For example, Wang et al. (1994) demonstrated that rootstocks significantly influence the spring phenology of 'Hayward' kiwifruit, as reflected by notable differences in the timing, synchrony, and magnitude of budburst, with direct implications for subsequent flowering intensity.

Other researchers suggest that shifts in flushing phenology and vegetative growth at the start of the growing season may result from a phenological mismatch between the rootstock and the scion. For instance, the results of the study by Clearwater et al. (2007) clearly indicate that the rootstock can affect the phenology of the scion through differences in the timing of spring root pressure development. High-vigour rootstocks developed positive root pressure before or during scion budburst, enabling uninterrupted shoot growth without signs of water stress. In contrast, low-vigour rootstocks developed root pressure only after shoot growth had already commenced, during which scions exhibited more pronounced water stress and reduced growth. Recent findings by Camisón et al. (2021) show that in chestnut (*Castanea sativa* Mill.), rootstock origin significantly affects scion budburst. Scions grafted onto drought-adapted (xe-

ric) rootstocks flushed earlier than those on humid-origin rootstocks, likely due to differences in hormone signaling and water transport. The study also found that grafting itself can delay budbreak, suggesting that both rootstock genotype and grafting effects can alter phenological timing and contribute to asynchronization among genetically identical ramets. The study demonstrated that in apple trees with upright-narrow (UN) scions, bud break occurred earlier on M.7 and M.9 rootstocks (3.7 days) compared to MM.111 and seedling rootstocks (8.8 days), confirming that rootstock can influence the budbreak phenology of apple scions. Although Tworkoski et al. (2016) did not directly monitor leaf-out phenology, their results indicate a connection between rootstock response to drought stress and its influence on scion physiology. They demonstrated that ABA concentrations in scion leaves varied depending on the rootstock, suggesting that distinct patterns of root-derived hormonal signaling can modulate scion responses. This variability in rootstock reaction to environmental conditions highlights the importance of rootstock as a factor in the plant's overall adaptation to stress.

Intra-annual Variation in Budburst: the Roles of Temperature Sensitivity and Rootstock–scion Interactions

When examining the range of budburst within the same clone, it becomes evident that this range varies notably from year to year (Table 2). Such variability can be attributed primarily to differences in weather conditions, particularly temperature fluctuations between seasons. A comparison between spring temperature trends (Figure 4) and budburst phenology reveals a strong association between thermal conditions and both the timing and synchrony of budburst across oak clones. Although the exact physiological mechanisms regulating leaf emergence are not fully understood for most plant species, it is well established that temperature plays a key role in leaf development (Linkosalo et al. 2006). This sensitivity to temperature results in considerable interannual variability in the onset of spring phenology (Polgar and Primack 2011).

In 2010, an initially warm early season was followed by a cold spell, with stable temperatures above 10°C occurring only from mid- to late April. That year recorded the highest intra-clonal variability in budburst timing, with a mean range of 6.31 days and a maximum of 19 days. Moreover, 2010 had the largest proportion of clones (79.1%) exhibiting a spread of three or more days between ramets, indicating a pronounced lack of synchrony in budburst among genetically identical individuals. This asynchrony likely resulted from insufficient thermal accumulation early in the season and from differences in the timing at which individual ramets reached their required cumulative temperature thresholds.

Since it is well known that flushing in deciduous forest trees is regulated by genetic mechanisms that define how individuals respond to temperature cues (Derory et al. 2006, Vitasse et al. 2010), several studies have demonstrated that the temperature thresholds required to trigger budburst are genetically controlled (Kramer 1995, Rousi and Pusenius 2005, Körner and Basler 2010, Basler and Körner 2012). The genetic response to rising temperatures may explain the significant variation in budburst timing observed among clones across years (Table 1).

However, the question arises as to why such differences also occur between ramets of the same clone. Given that environmental conditions were uniform across the experimental plot, it is reasonable to assume that this variability arises from interactions between the rootstock and scion. As previously noted, different genotypes can have distinct temperature thresholds (Vitasse et al. 2009, 2010). Since rootstocks were grown from seed and thus genetically different, they may possess varying sensitivities to temperature cues, leading some to trigger budburst in the scion earlier, while others may delay it.

This is particularly plausible in years such as 2010 and 2014, when warm and cold periods alternated over a longer duration, and warming progressed slowly. In such years, it is likely that some rootstocks reached their temperature thresholds earlier, especially those with a faster physiological response to warming. Consequently, greater intra-clonal variability was observed. By contrast, in years when the early season was dominated by low temperatures followed by a rapid warming event, such as in 2011 or 2013, intra-clonal differences in budburst were smaller. The more synchronized thermal signal may have aligned the temperature response thresholds across ramets, minimizing budburst variation.

Interaction Between Intra-clonal Phenological Variability and Frost Susceptibility

Our findings suggest that variability in phenological timing among ramets within the same clone—referred to as intra-clonal phenological asynchronization—may enhance a clone's ability to cope with climatic stressors, particularly late spring frosts. Slower developing ramets are less likely to be affected by low temperatures, thereby increasing the overall frost resilience of the clone. An early flushing strategy can be advantageous by enabling rapid early-season growth and a longer growing period; however, it also increases the risk of frost damage (Vitasse and Rebetez 2018). Foliage loss caused by late spring frost represents a major stress factor for deciduous trees, as it disrupts nutrient allocation, compromises growth and reproduction, and impairs canopy development (Vitasse et al. 2014). Although oaks exhibit a strong ability to regenerate foliage, this compensatory response is energetically costly and shortens the growing season, ultimately diminishing their net annual productivity (Vitasse et al. 2014, Baumgarten et al. 2023). In addition, it is important to highlight that the newly flushed leaves produced during refoliation often coincide with elevated levels of powdery mildew inoculum, and since environmental conditions during this period are generally favorable for fungal development, trees become highly susceptible to severe foliar infections (Marçais et al. 2009).

While ramets in phenophase 3—defined as budburst (widely spaced bud scales and visible green leaf tips)—showed no visible damage, those in phenophase 4 (folded leaf visible) exhibited significant injury, as clearly demonstrated by clone BJ 25 (Figure 7). All ramets that had reached phenophase 4 on the day of the frost event displayed clear symptoms of frost damage, highlighting the increased susceptibility of more advanced phenological phases. This supports previous research indicating that early-flushing genotypes are more vulnerable to spring frost (Utkina and Rubtsov 2017).

In the establishment of clonal seed orchards intended for the production of high-quality forest reproductive material, heterovegetative propagation is commonly applied. Therefore, beyond the selection of the scion, it is essential to consider the phenotypic and physiological traits of the rootstock to prevent phenological asynchrony between graft components. This approach aligns with strategies already utilized in agronomy for frost mitigation. A relevant example comes from fruit production, where Durner and Goffreda (1992) demonstrated that the choice of rootstock in peach significantly affects both the timing and rate of flower bud development. Their study showed that certain rootstocks can delay blooming, thereby reducing the risk of frost damage and enhancing yield. Importantly, they emphasized that even a delay of just one to two days in specific bud development stages can markedly reduce frost injury. These findings underscore the biological importance of fine-scale phenological control, the principles that are equally applicable when designing clonal forestry systems where frost sensitivity is a limiting factor.

These findings directly support the biological threshold applied in our research, where a difference of three days in budburst among ramets was considered meaningful. This underlines that even slight shifts in developmental timing—if strategically managed—can play a critical role in minimizing frost damage and ensuring the stability and productivity of clonal seed orchards. In addition, by selecting rootstocks, it may be possible to increase the phenological synchrony of clones in plantations, thereby contributing to an increase in their effective population size and overall productivity.

CONCLUSION

This study confirms that rootstocks can significantly influence intra-clonal variation in the spring phenology of pedunculate oak, despite the genetic uniformity of ramets. Grafting onto different rootstocks resulted in notable differences in budburst timing within clones, with up to 79.1% of clones in 2010 and 72.1% in 2014 exhibiting a budburst range of three or more days between ramets, indicating pronounced phenological asynchrony.

Importantly, even small delays in budburst (≥3 days) were associated with successful frost avoidance, suggesting that such intra-clonal asynchrony may enhance the ability of some ramets to escape frost events. Beyond their scientific implications, these findings provide practical guidance for the management of clonal seed orchards and nursery production. When establishing or renewing seed orchards, particular attention should be given to the selection of rootstocks with compatible phenological behavior to minimize asynchrony among ramets and enhance cross-pollination efficiency. In nurseries, monitoring and recording the phenological traits of potential rootstocks could serve as a useful criterion for selecting grafting material. Such an approach could improve both the stability of seed yields and the overall adaptability of orchard trees to variable environmental conditions, thereby supporting long-term, sustainable seed production.

REFERENCES

- Bačurin, M., S. Bogdan, I. Katičić Bogdan, K. Sever, 2023: Leaf phenological responses of juvenile beech and oak provenances to elevated phosphorus. Forests 14 (4): 834. https://doi.org/10.3390/f14040834
- Bačurin, M., I. Čehulić, I. Katičić Bogdan, S. Bogdan, 2025: The different timing of exposure to drought stress differentially affects phenology and growth in goat willow. SEEFOR – South-east European forestry 16 (2): 155–166. https://doi.org/10.15177/seefor.25-17
- Basler, D., C. Korner, 2014: Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. Tree Physiology 34 (4): 377–388. https://doi.org/10.1093/treephys/tpu021
- Basler, D., C. Korner, 2012: Photoperiod sensitivity of bud burst in 14 temperate forest tree species. Agricultural and Forest Meteorology 165: 73–81. https://doi.org/10.1016/j.agrformet.2012.06.001
- Baumgarten, F., A. Gessler, Y. Vitasse, 2023: No risk—no fun: Penalty and recovery from spring frost damage in deciduous temperate trees. Functional Ecology 37 (3): 648–663. https://doi.org/10.1111/1365-2435.14243
- Billington, H.L., J. Pelham, 1991: Genetic variation in the date of budburst in Scottish birch populations: implications for climate change. Functional Ecology 5 (3): 403–409. https://doi.org/10.2307/2389812
- Camisón, Á., M.Á. Martín, V. Flors, P. Sánchez-Bel, G. Pinto, M. Vivas, V. Rolo, A. Solla, 2021: Exploring the use of scions and rootstocks from xeric areas to improve drought tolerance in *Castanea sativa* Miller. Environmental and Experimental Botany 187: 104467. https://doi.org/10.1016/j.envexpbot.2021.104467
- Clearwater, M., P. Blattmann, Z. Luo, R Lowe, 2007: Control of scion vigour by kiwifruit rootstocks is correlated with spring root pressure phenology. Journal of Experimental Botany 58 (7): 1741–1751. https://doi.org/10.1093/jxb/erm029
- Derory, J., P. Léger, V. Garcia, J. Schaeffer, M.T. Hauser, F. Salin, C. Luschnig, C. Plomion, J. Glössl, A. Kremer, 2006: Transcriptome analysis of bud burst in sessile oak (*Quercus petraea*). New Phytologist 170 (4): 723–738. https://doi.org/10.1111/j.1469-8137.2006.01721.x
- Durner, E.F., J.C. Goffreda, 1992: Rootstock-induced differences in flower bud phenology in peach. Journal of the American Society for Horticultural Science 117 (5): 690–697. https://doi.org/10.21273/JASHS.117.5.690
- Franjić, J., K. Sever, S. Bogdan, Ž. Škvorc, D. Krstonošić, I. Alešković, 2011: Fenološka neujednačenost kao ograničavajući čimbenik uspješnoga oprašivanja u klonskim sjemenskim plantažama hrasta lužnjaka (*Quercus robur* L.). Croatian Journal of Forest Engineering 32 (1): 141–154. https://hrcak.srce.hr/68072
- Frewen, B.E., T.H.H. Chen, G.T. Howe, J. Davis, A. Rohde, W. Boerjan, H.D. Bradshaw, 2000: Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics 154 (2): 837–845. https://doi.org/10.1093/genetics/154.2.837
- Gömöry, D., L. Paule, 2011: Trade-off between height growth and spring flushing in common beech (*Fagus sylvatica* L.). Annals of Forest Science 68 (5): 975–984. https://doi.org/10.1007/s13595-011-0103-1
- Haukioja, E., E. Pakarinen, P. Niemelä, L. Iso-Iivari, 1988: Crowding-triggered phenotypic responses alleviate consequences of crowding in *Epirrita autumnata* (Lep., Geometridae). Oecologia 75: 549–558. https://doi.org/10.1007/BF00776419
- Jayawickrama, K.J.S., J.B. Jett, S.E. McKeand, 1991: Rootstock effects in grafted conifers: A review. New Forest 5: 157–173. https://doi.org/10.1007/BF00029306
- Jiménez, S., A. Garín, Y. Gogorcena, J.A. Betrán, M.A. Moreno, 2004: Flower and foliar analysis for prognosis of sweet cherry nutrition: Influence of different rootstocks. Journal of Plant Nutrition 27 (4): 701–712. https://doi.org/10.1081/PLN-120030376
- Jiménez, S., J. Pinochet, J. Romero, Y. Gogorcena, M.A. Moreno, J.L. Espada, 2011: Performance of peach and plum based rootstocks of different vigour on a late peach cultivar in replant and calcareous conditions. Scientia Horticulturae 129 (1): 58–63. https://doi.org/10.1016/j.scienta.2011.03.006
- Kaitaniemi, P., K. Ruohomäki, E. Haukioja, 1997: Consequences of defoliation on phenological interaction between *Epirrita autumnata* and its host plant, mountain birch. Functional Ecology 11 (2): 199–208. https://doi.org/10.1046/j.1365-2435.1997.00063.x

- Körner, C., D. Basler, 2010: Phenology under global warming. Science 327: 1461–1462. https://doi.org/10.1126/science.1186473
- Kramer, A.T., J.L. Ison, M.V. Ashley, H.F. Howe, 2008: The paradox of forest fragmentation genetics. Conservation Biology 22 (4): 878–885. https://doi.org/10.1111/j.1523-1739.2008.00944.x
- Kramer, K.,1995: Phenotypic plasticity of the phenology of seven European tree species in relation to climatic warming. Plant, Cell, and Environment 18 (2): 93–104. https://doi.org/10.1111/j.1365-3040.1995.tb00356.x
- Leinonen, I., H. Hänninen, 2002: Adaptation of the timing of bud burst of Norway spruce to temperate and boreal climates. Silva Fennica 36 (3): 695–701. https://doi.org/10.14214/sf.534
- Linkosalo, T., R. Hakkinen, H. Hanninen, 2006: Models of the spring phenology of boreal and temperate trees: Is there something missing? Tree Physiology 26 (9): 1165–1172. https://doi.org/10.1093/treephys/26.9.1165
- Lockhart, J.A., 1983: Optimum growth initiation time for shoot buds of deciduous plants in a temperate climate. Oecologia 60: 34–37. https://doi.org/10.1007/BF00379317
- Marçais, B., M. Kavkova, M.L. Desprez-Loustau, 2009: Phenotypic variation in the phenology of ascospore production between European populations of oak powdery mildew. Annals of Forest Science 66: 814–814. https://doi.org/10.1051/forest/2009077
- Neilsen, D., G.H. Neilsen, T. Forge, G.A. Lang, 2016: Dwarfing rootstocks and training systems affect initial growth, cropping and nutrition in 'Skeena' sweet cherry. Acta Horticulturae 1130: 199–206. https://doi.org/10.17660/ActaHortic.2016.1130.29
- Polgar, C.A., R.B. Primack, 2011: Leaf-out phenology of temperate woody plants: from trees to ecosystems. New Phytologist 191 (4): 926–941. https://doi.org/10.1111/j.1469-8137.2011.03803.x
- Robson, T.M., E. Rasztovits, P.J. Aphalo, R. Alía, I. Aranda, 2013: Flushing phenology and fitness of European beech (*Fagus sylvatica* L.) provenances from a trial in La Rioja, Spain, segregate according to their climate of origin. Agricultural and Forest Meteorology 180: 76–85. https://doi.org/10.1016/j.agrformet.2013.05.008
- Rousi, M., J. Pusenius, 2005: Variations in phenology and growth of european white birch (*Betula pendula*) clones. Tree Physiology 25 (2): 201–210. https://doi.org/10.1093/treephys/25.2.201
- Scotti-Saintagne, C., C. Bodénès, T. Barreneche, E. Bertocchi, C. Plomion, A. Kremer (2004): Detection of quantitative trait loci controlling bud burst and height growth in *Quercus robur* L. Theoretical and Applied Genetics 109: 1648–1659. https://doi.org/10.1007/s00122-004-1789-3
- Tworkoski, T., G. Fazio, D.M. Glenn, 2016: Apple rootstock resistance to drought. Scientia Horticulturae 204: 70–78. https://doi.org/10.1016/j.scienta.2016.01.047
- Tworkoski, T., S. Miller, 2007: Rootstock effect on growth of apple scions with different growth habits. Scientia Horticulturae 111: 335–343. https://doi.org/10.1016/j.scienta.2006.10.034
- Utkina, I.A., V.V. Rubtsov, 2017: Studies of phenological forms of pedunculate oak. Contemporary Problems of Ecology 10 (7): 804–811. https://doi.org/10.1134/S1995425517070101
- Vander Mijnsbrugge, K., A. Turcsán, J. Maes, N. Duchêne, S. Meeus, K. Steppe, M. Steenackers, 2016: Repeated summer drought and re-watering during the first growing year of oak (*Quercus petraea*) delay autumn senescence and bud burst in the following spring. Frontiers in Plant Science 7: 419. https://doi.org/10.3389/fpls.2016.00419
- Vidaković, M. 1996: Establishment of clonal seed orchard of pedunculate oak. In (Klepac, D., ed.): Pedunculate oak (*Quercus robur* L.) in Croatia. Croatian Academy of Sciences and Arts – Center for Scientific Work in Vinkovci, Vinkovci – Zagreb, pp. 127–138.
- Vitasse, Y., C.C. Bresson, A. Kremer, R. Michalet, S. Delzon, 2010: Quantifying phenological plasticity to temperature in two temperate tree species. Functional Ecology 24: 1211–1218. https://doi.org/10.1111/j.1365-2435.2010.01748.x
- Vitasse, Y., S. Delzon, E. Dufrêne, J.Y. Pontailler, J.M. Louvet, A. Kremer, R. Michalet, 2009: Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? Agricultural and Forest Meteorology 149 (5): 735–744. https://doi.org/10.1016/j.agrformet.2008.10.019
- Vitasse, Y., A. Lenz, C. Körner, 2014: The interaction between freezing tolerance and phenology in temperate deciduous trees. Frontiers in Plant Science 5: 541. https://doi.org/10.3389/fpls.2014.00541
- Vitasse, Y., M. Rebetez, 2018: Unprecedented risk of spring frost damage in Switzerland and Germany in 2017. Climate Change 149: 233–246. https://doi.org/10.1007/s10584-018-2234-y
- Wang, Z.Y., K.J. Patterson, K.S. Gould, R.G. Lowe, 1994: Rootstock effects on budburst and flowering in kiwifruit. Scientia Horticulturae 57 (3): 187–199. https://doi.org/10.1016/0304-4238(94)90140-6
- Young, E., J. Houser, 1980: Influence of Siberian C rootstock on peach bloom delay, water potential, and pollen meiosis. Journal of the American Society for Horticultural Science 105 (2): 242–245. https://doi.org/10.21273/JASHS.105.2.242
- Zohner, C.M., S.S. Renner, 2015: Perception of photoperiod in individual buds of mature trees regulates leaf-out. New Phytologist 208 (4): 1023–1030. https://doi.org/10.1111/nph.13510