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The Different Timing of Exposure to Drought Stress Differentially Affects Phenology and Growth in Goat Willow

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ABSTRACT

Periods of drought can substantially alter the phenology of forest tree species, influencing growth dynamics as well as the timing of budburst and leaf senescence. This study investigated whether the plant response varies depending on when during the growing season drought stress occurs.

Female individual of goat willow (*Salix caprea* L.) was propagated by hardwood cuttings, resulting in 151 ramets assigned to eight experimental groups. Seven groups were subjected to drought treatments at different intervals between early March and mid-July 2021, while the eighth served as a well-watered control. The same treatment schedule was repeated in 2022 with increased drought intensity. Each plant remained under drought conditions until visible stress symptoms appeared, and the total mass (container included) decreased to about half of its initial value, after which plants were rehydrated and irrigated regularly for the rest of the season. Control plants were watered continuously.

During autumn 2021, the onset of leaf senescence in the first treatment group was delayed by four days compared with the control. By the following spring (2022), this group flushed about five days earlier than the control, whereas groups 6 and 7 flushed approximately two days later. In autumn 2022, all drought-treated groups showed delayed leaf senescence, most notably those exposed later in the season—by up to 30 days on average compared to the control.

These results indicate that the timing of drought stress strongly modulates leaf phenology in *Salix caprea*, exerting both beneficial and adverse effects on height growth and flower development.

Keywords: *Salix caprea* L.; phenological changes; drought stress; flowering; growth

INTRODUCTION

Drought is a major limiting factor for the growth, reproduction, and survival of plants (Hinckley et al. 1979, Chaves et al. 2003). While forest trees possess some resilience to drought stress, such as through various water-use strategies (Morán-López et al. 2014), the increasingly frequent and intense drought periods in recent years undeniably present a significant threat to forest ecosystems (Arend et al. 2016).

Understanding the impact of drought stress and the possibilities of mitigating its negative consequences on forest trees are of great importance in the context of the increased frequency of drought episodes during the growing season. Morpho-anatomically, plants adapt to drought stress through a range of defense mechanisms such as by reducing leaf surface area, decreasing cell wall elasticity, and intensifying root growth, thereby reducing aboveground biomass production (Thomas and Gausling 2000, Arend et al. 2013). Physiologically, the adaptation mechanism to drought involves controlling stomatal opening and closure, allowing the plant to regulate transpiration and water loss (Hinckley et al. 1980, Thomas and Gausling 2000). Drought can also trigger adjustments in wood anatomical traits, such as alterations in vessel size and frequency, which affect the balance between hydraulic safety and efficiency (Balzano et al. 2025). In addition to the mentioned morpho-anatomical and physiological mechanisms, phenological processes, such as budburst and autumn leaf senescence, also exhibit responses to drought stress.

Phenological processes are predominantly determined by warm air temperature in spring, cool temperature during spring (i.e., chilling temperatures) and the photoperiod; hence, they can be called typical factors (Robson et al. 2013, Basler and Korner 2014, Kuster et al. 2014, Way and Montgomery 2015). On the other hand, a range of biotic and abiotic factors greatly influence changes in phenological processes through various stress stimuli, and they are called atypical factors (Bačurin et al. 2023). The action of stressful factors affects changes in gene expression, leading to the plant's response to stress stimuli (Ingram and Bartels 1996, Bruce et al. 2007). Epigenetic modifications are regulatory mechanisms that create a kind of “memory” and enable organisms to acclimatize to stressful factors (Bräutigam et al. 2013).

Drought stress has been shown to drive phenological changes in numerous forest tree species, such as reduced growth, shifts in the timing of spring budburst, and earlier or delayed leaf senescence in autumn. (Hinckley et al. 1979, Xie et al. 2015, Čehulić et al. 2019, Wu et al. 2022). Undoubtedly, a delayed (so-called “carry-over”) effect of drought stress on spring leafing has been established in various species of forest trees. The “carry-over” effect manifests through later or earlier budburst in the following spring. Such an effect has been recorded in pedunculate oak and beech (Yonekura et al. 2004, Kuster et al. 2014). Similarly, a negative impact of drought stress has been recorded during the initiation of generative organs in the species *Prunus armeniaca* L. (Bartolini et al. 2020).

The onset of autumn leaf senescence is modified under drought stress conditions; however, some studies report an earlier onset, while others report a delayed one. An earlier onset of leaf senescence, as well as earlier leaf shedding, was observed with extremely low nutrient remobilization caused by drought stress in *Populus tremuloides* Michx. (Killingbeck 2004, Estiarte and Peñuelas 2015). Čehulić et al. (2019) observed interannual phenological changes, noting a delay in budburst during one year and its advancement in the following season. Vander Mijnsbrugge et al. (2016) and Leuzinger et al. (2005) reported a delay in the onset of leaf senescence in *Quercus petraea* (Matt.) Liebl. during autumn. It is also important to note that shifts in leaf phenology, particularly early spring phenology, impact drought. Early leaf-out can reduce soil water reserves, which, in combination with climatic conditions, may result in less water being available during the summer (Denham et al. 2023). In contrast, autumn phenology may be affected by a drought during summer (Lukasová et al. 2020).

However, most studies have investigated the effects of a single drought event on leaf phenology, with stress typically imposed at a specific point during the growing season (Spieß et al. 2012, Kuster et al. 2014, Vander Mijnsbrugge et al. 2016). Only one study has raised the question of variations in the effects of drought stress on leaf phenology, considering different times of stress induction within the growing season (Čehulić et al. 2019).

Goat willow (*Salix caprea* L.), due to its ability for simple autovegetative reproduction, is the model species of this study. By using the same genotype in the study, intergenotypic variations are avoided, i.e., the focus is on monitoring phenotypic reactions that are predominantly a result of environmental factors.

The main objective of this study was to investigate potential differences in the effects of drought stress when it occurs at different stages of the growing season. In addition, the study aimed to evaluate the influence of such stress on height growth, leaf phenology in spring and autumn, and flower development in the goat willow clone.

MATERIALS AND METHODS

Plant Material and Experimental Design

A mature female goat willow individual was cloned using the hardwood cuttings method in early 2020. A total of 151 two-year-old ramets were cultivated in the greenhouse of the Croatian Forest Research Institute (45.6688° N, 15.6438° E) and divided into eight groups, consisting of seven experimental groups with 19 ramets each, and one control group with 18 ramets. The substrate used for planting the ramets in this experiment was Durpeta Profi 1 peat, characterized by a pH (H_2O) of 5.5–6.5 and nutrient content of N 80–140 $mg \cdot L^{-1}$, P_2O_5 100–160 $mg \cdot L^{-1}$, and K_2O 110–180 $mg \cdot L^{-1}$. The plants were cultivated in 3-L polyvinyl chloride (PVC) pots.

Drought stress was simulated by withholding water from seven experimental groups, whereas the control group was kept well-watered for the duration of the experiment. In the first year of the experiment (2021), drought stress was induced by termination of irrigation and applied sequentially, with each of the seven groups exposed at different times between early March and mid-July, at intervals of approximately 20 days (Figure 1). Irrigation was discontinued for the first group shortly after leaf emergence (early March) to induce the first drought treatment period and each subsequent group was exposed once the majority of plants in the preceding group exhibited visible symptoms of drought stress and their mass (including container) had declined to about half of the initial value.

In the following year (2022), all the experimental groups were again subjected to drought stress by withholding irrigation. The treatment was again applied sequentially in the same order as in 2021, but it started later due to delayed leaf emergence that spring (early April), with groups exposed at average intervals of 15 days (Figure 1). In both experimental years, drought onset was defined as the day on which irrigation to the plants was suspended, whereas the end of the treatment period was determined by the rehydration of the last plant within each group, since not all plants within a group reached the stress threshold on the same day (Figure 1).

Measurements and Scoring

The mass of drought-exposed plants, including pot and substrate, was measured twice a week with a precision of 1 g to track the progression of water loss and to accurately identify the onset of drought stress symptoms, following the methodology described by Bačurin et al. (2025). Control plants were weighed at the beginning of the treatment period assigned to each group and again at the end of the

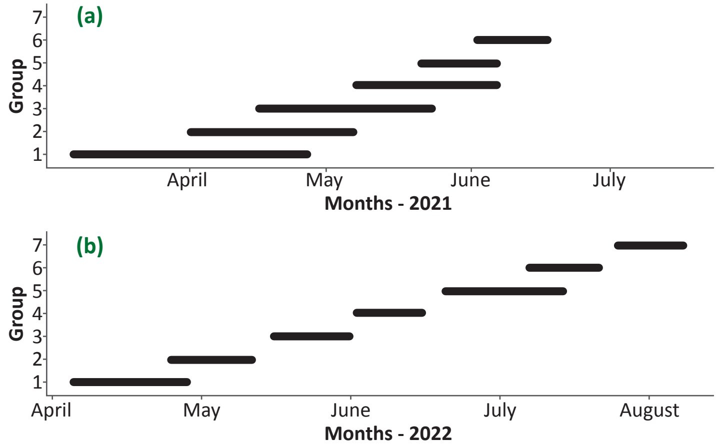

Figure 1. The duration of drought exposure for each group during the 2021 **(a)** and 2022 **(b)** growing seasons. Each row represents the treatment period for a group.

treatment. At the beginning of each drought treatment, immediately before irrigation was discontinued, the plants were first irrigated to achieve full saturation and then weighed in this fully saturated state. Because container volume and substrate amount were standardized across all ramets, the initial water content was assumed to be equal.

All plants subjected to drought treatment were regularly monitored for visible signs of water deficit. In the first group of ramets, which experienced drought stress during or immediately after leaf emergence, the earliest observable symptom was the wilting of young, recently developed leaves. In the remaining groups, where leaves were already mature and thicker, the initial response to drought appeared as yellowing, curling, or drying of the foliage. When distinct drought symptoms became evident and the total plant mass (including pot and substrate) had decreased to about half of its initial value, the plants were rehydrated and subsequently maintained under regular watering until the end of the growing season. Control ramets were consistently watered throughout the experimental period.

The intensity of drought stress was calculated as the relative reduction in plant mass within the pots. For each ramet, we determined the ratio between the mass lost up to the point when clear drought stress symptoms were observed (marking the end of the stress period) and its initial mass under fully saturated conditions was obtained, as described by Bačurin et al. (2025).

Plant heights were measured at the beginning and end of the growing season, and spring and autumn leaf phenology were monitored twice a week. Monitoring of spring phenology was conducted for all buds (flower, leaf, and flower-leaf). Leaves developed from flower-leaf buds, with this process being significantly earlier than leafing from purely leaf buds. Additionally, not all ramets possessed flower and/or flower-leaf buds. Therefore, leaf phenology was analyzed only for leaf buds. The number of ramets forming generative organs was also monitored. Leaf phenology was monitored for all plants in the experiment using an ordinal scale of 1–7: 1 – dormant bud; 2 – bud swelling; 3 – bud burst; buds open, green leaf tips are visible; 4 – folded leaf visible along the shoot; 5 – leaf unfolding, separating from each other; 6 – elongated petiole with multiple rings of leaves; larger leaves, finely serrated with a whitish edge; 7 – significantly larger leaves, serrated edges. Autumn leaf phenological stages were recorded twice per week (scored on a 0–5 ordinal scale), following the method described by Čehulić et al. (2019).

Statistical Analyses

All statistical analyses as well as visualization were carried out in the R environment (version R4.4.1; R Development Core Team 2024.). Data processing was conducted using the tidyverse (v2.0.0) and dplyr (v1.1.4) packages. The assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene tests, respectively, implemented via the rstatix package (v0.7.2). Height growth data followed a normal distribution; therefore, pairwise t-tests were used to compare each treatment group with the control. Visualization of height growth was generated using ggplot2 (v3.5.1) and ggstatsplot (v0.12.3).

For the proportion of flowering plants, Fisher's exact test was applied to assess significant differences between groups and the control.

Since phenological scores did not meet the assumption of normality (as shown by the Shapiro–Wilk test), nonparametric analyses—specifically the Kruskal–Wallis and Wilcoxon tests—were employed to detect differences among groups. Statistical significance was set at $p < 0.05$.

RESULTS

Intensity of Drought Stress Based on Ramet Mass Loss

By comparing and statistically analyzing the relative change in total mass (plant + container) measured at the end of the drought treatment compared to the initial value, we identified a difference in drought stress intensity between 2021 and 2022. In 2022, plants were rehydrated at a lower relative mass than in 2021, indicating exposure to a stronger water deficit before recovery. This difference is consistent with the smaller size and lower transpiration demand of plants in 2021, which caused them to reach higher relative mass values before rehydration. In contrast, the control group maintained a mass ratio close to 1 in both years, confirming that regularly irrigated plants did not undergo stress. Taken together, these findings indicate that drought stress in 2021 was of lower intensity, whereas in 2022 it can be considered of higher intensity (Figure 2).

The Impact of Drought on Height Growth

By measuring heights at the beginning and end of the growing season in 2021 and calculating the difference between their values, we obtained the growth for each plant within the group. Data processing revealed an average decrease in height growth in all groups after the first year of exposure to drought stress, with the decrease being statistically significant in groups 5 and 6 (Figure 3).

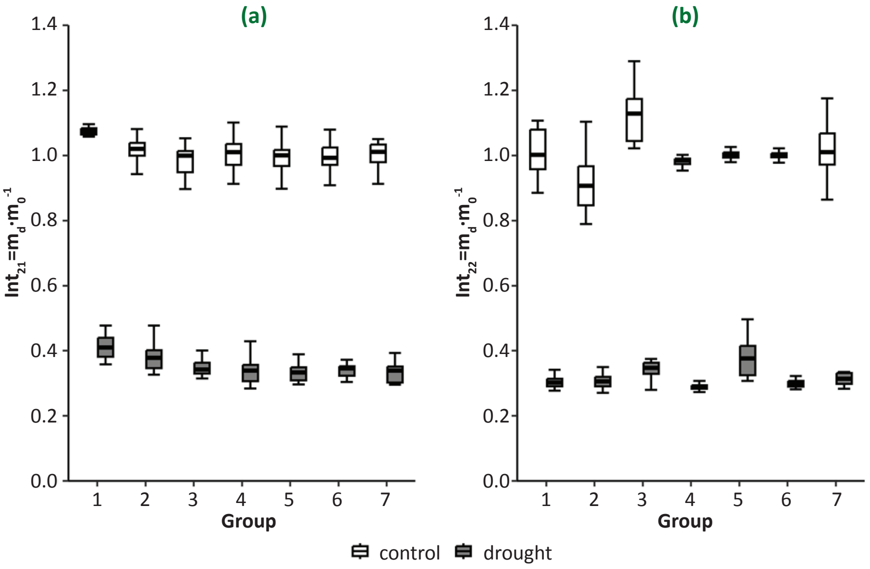

Figure 2. The plant mass ratio at the end of the drought period (upon appearance of visible symptoms, m_d) relative to the initial mass (plant + container, m_0) across groups. **(a)** Int_{21} – mass ratio in 2021; **(b)** Int_{22} – mass ratio in 2022. Control = the control group of regularly irrigated plants, whose mass ratio was recorded repeatedly at the end of each group drought treatment period (groups 1–7); Drought = mass ratio of drought-treated groups. The values are shown as pairs, e.g. 1 = mass ratio of drought group 1 and the control measured at the end of group 1 treatment; 2 = mass ratio of drought group 2 and the control measured at the end of group 2 treatment; and so on until group 7.

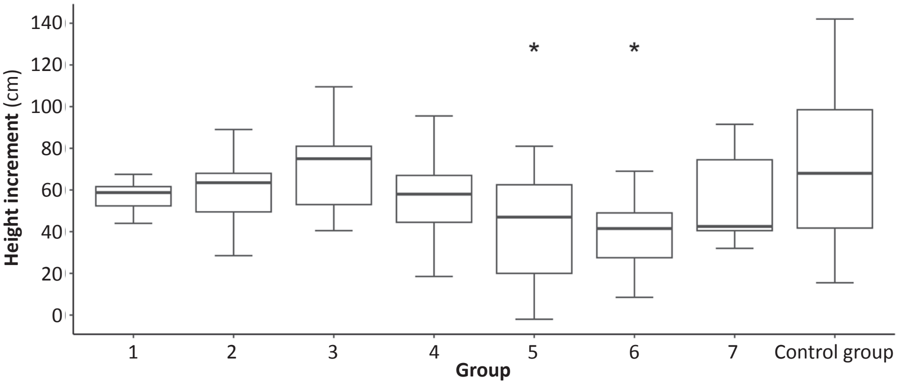

Figure 3. The distribution of height increment for groups 1–7 in 2021, exposed to drought at different times during the growing season. An asterisk highlights a statistically significant difference in annual growth between the groups and the control: *** – highly significant (p-value < 0.001); ** – very significant (p-value < 0.01); * – significant (p-value < 0.05).

By measuring plant height at the beginning and end of the 2022 growing season and by calculating the difference, we determined the height increment for each plant within its respective group. Statistical analysis revealed significant differences between the control group and groups 2, 4, and 5. Group 2 exhibited significantly lower height growth compared to the control, whereas groups 4 and 5 showed significantly greater height growth (Figure 4).

The Impact of Drought Stress on Leaf Phenology

Initial State of Leaf Phenology

The analysis of leaf phenology in the spring of 2021 revealed that there is no statistically significant difference between the other groups and the control group (Figure 5). Such a result is expected because phenological observations took place before the onset of the drought treatment.

Autumn Leaf Phenology after Drought Stress

Analyzing the impact of drought stress in the autumn of 2021, it is evident that the progression of leaf senescence is slower in the first group, exposed to drought from 8 March 2021 to 21 April 2021. A statistically significant delay compared to the control was recorded on the 300th day of 2021 (Figure 6).

Analyzing the impact of drought stress in the autumn of 2022, it is evident that there is slower progress in autumn phenology for all groups compared to the control group. When observing the relationship between groups, it is clear that the groups exposed to drought stress at the beginning of the growing season (1, 2, 3, 4, 5) show faster progress in autumn phenology compared to the groups exposed to drought stress later in the growing season (6, 7).

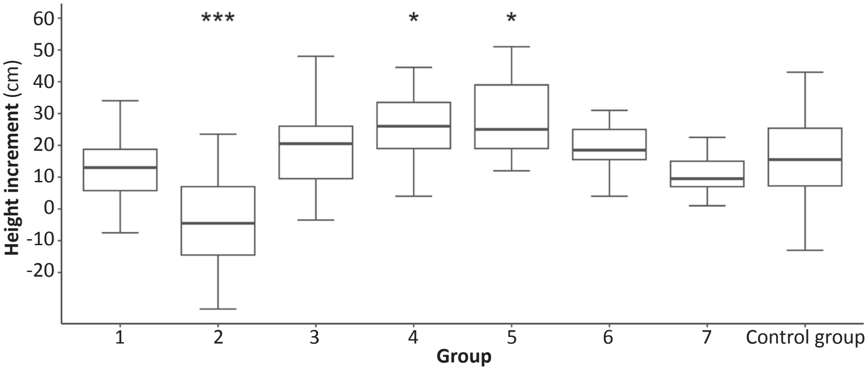

Figure 4. Distribution of height increment in 2022 for groups exposed to drought at different times during the growing season. Asterisks indicate statistically significant differences in growth compared to the control group: *** – highly significant ($p < 0.001$); ** – very significant ($p < 0.01$); * – significant ($p < 0.05$).

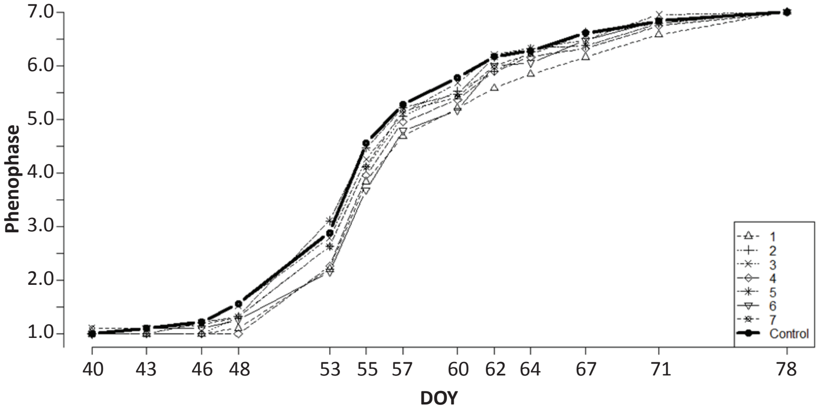

Figure 5. Spring leaf phenology of goat willow ramets in 2021 across experimental groups. The control (bold black line) is compared with drought-treated groups (symbols). The y-axis indicates the phenophase, and the x-axis represents the day of the year (DOY). Points represent mean phenological stage for each DOY.

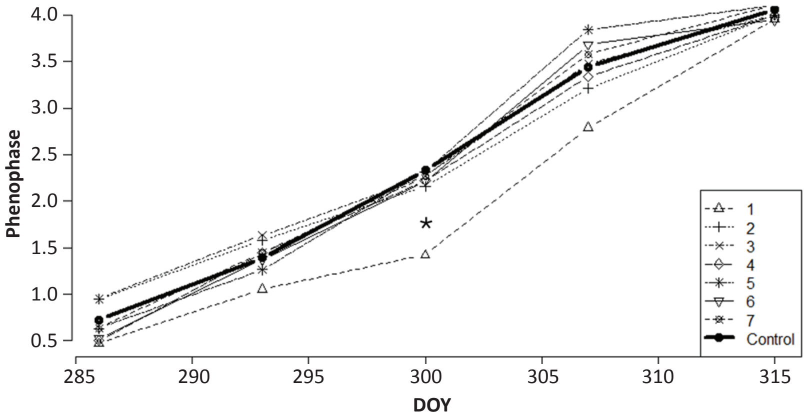

Figure 6. Autumn leaf phenology of goat willow ramets in 2021, across experimental groups. The control (bold black line) is compared with drought-treated groups (symbols). The y-axis indicates the phenophase, and the x-axis represents the day of the year (DOY). Points represent mean phenological stage for DOY. Asterisks marks dates with significant group–control differences (p < 0.05).

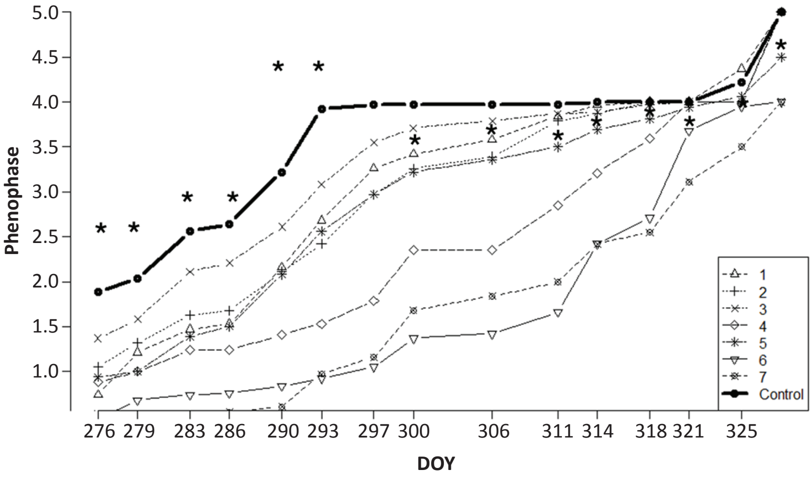

Figure 7. Autumn leaf phenology of goat willow ramets in 2022, across experimental groups. The control (bold black line) is compared with drought-treated groups (symbols). The y-axis indicates the phenophase, and the x-axis represents the day of the year (DOY). Points represent mean phenological stage for DOY. Asterisks marks dates with significant group– control differences (p < 0.05).

Spring Leaf Phenology after Drought Stress

An analysis of the impact of drought stress in the spring of 2022 reveals an earlier onset and a faster rate of leaf emergence in the first group. In contrast, delayed leaf emergence and a statistically significant slowdown were observed in groups 6 and 7 (Figure 8).

The Impact of Drought on the Formation of Female Flowers

In 2021, there was no statistically significant difference in the number of flowering ramets between the treatment groups and the control group. In 2022, the results of Fisher's exact test showed that group 5 (p-value = 0.000547) and group 6 (p-value = 0.006275) had a statistically lower number of ramets that developed flowers compared to the control group.

It was found that in the second year of the study, there was a decrease in the number of plants that developed flowers compared to the previous year (Figure 9). Although the number of flowering ramets also decreased in the control group, the reduction was more pronounced in groups 5, 6, and 7, which had been exposed to drought stress in the previous year (May 21–June 7, June 2–11, and June 30–July 9, respectively). In group 5, only 11% of ramets developed flowers, whereas 74% had developed flowers the year before the drought. In group 6, after the drought stress, 21% of ramets developed flowers, while 58% had developed flowers the year before the drought. In group 7,42% of ramets developed flowers in 2022, compared to 84% in the previous year.

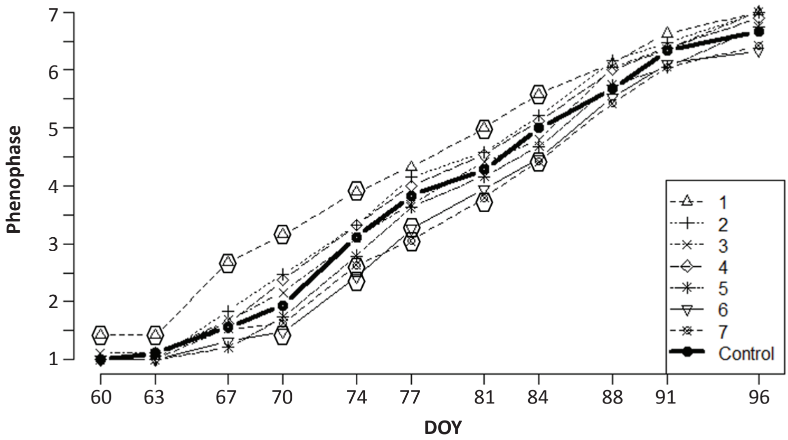

Figure 8. Spring leaf phenology of goat willow ramets in 2022 across experimental groups. The control (bold black line) is compared with drought-treated groups (symbols). The y-axis indicates the phenophase, and the x-axis represents the day of year (DOY). Points represent mean phenological stage for each DOY. Groups marked with a hexagon symbol indicate a statistically significant difference between the group and the control on a specific day of the year ($p < 0.05$).

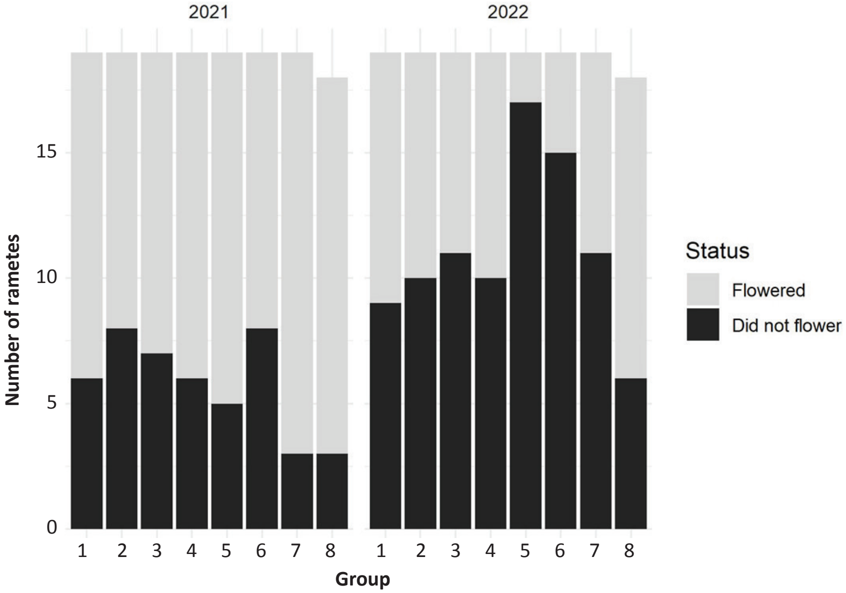

Figure 9. The number of flowering and non-flowering ramets across groups in 2021 and 2022. Bars represent the total number of ramets per group, subdivided by flowering status: black segments indicate ramets that did not flower, while light gray segments indicate ramets that flowered. The figure highlights group- and year-specific variation in flowering incidence.

DISCUSSION

Heights

An average decrease in height growth was observed in all groups after the first year of exposure to drought stress, with a statistically significant decrease in groups 5 and 6 (Figure 3). The minimal growth observed in groups 5 and 6 may be related to the interaction of drought stress with elevated temperatures during June/July, although this remains a possible explanation rather than a confirmed effect. High temperatures are to be expected during this period of the year, even though they were not directly measured in the greenhouse. Similar interactions between drought and high air temperatures have been reported to negatively affect growth in *Pinus edulis* Engelm (Adams et al. 2015).

In the following year of the experiment (2022), plants were again exposed to drought stress, but of higher intensity. Significantly, the lowest height growth was recorded in plants from the second group (Figure 4). Budburst date, or the onset of the growing season, is a key determinant of above-ground biomass production in the genus *Salix* (Weih 2009). Willows complete most of their annual height growth shortly after vegetative activity begins (Labrecque et al. 1993), which makes them particularly sensitive to water deficit at this stage. Indeed, species classified as early-season growers, such as willows, *Betula papyrifera* Marsh., and *Pinus strobus* L., have shown reduced height growth under spring drought conditions (Canham et al. 1999, Van Kampen et al. 2022). Drought reduces carbon assimilation through stomatal closure and lower leaf biomass, while increasing allocation to roots at the expense of above-ground growth (Cowan 1982, Cornelissen et al. 1996, Wikberg and Ögren 2004). Consequently, our finding that drought stress in spring significantly reduced height growth (e.g., group 2 in 2022) is consistent with the general understanding that drought during the period of intensive shoot elongation has the strongest negative impact (Foster et al. 2014).

A significantly higher growth was recorded in groups 4 and 5 compared to the control in 2022. On average, groups 3 and 6 also showed higher growth compared to the control, although these differences were not statistically significant (Figure 4). It can be assumed that the higher height growth is actually a result of the combined effect of drought stress in 2021 and 2022. Namely, this is due to its impact on leaf phenology (prolongation of vegetative activity) and the increase in non-structural carbohydrates (NSC) as a result of compensatory growth. It is important to consider that the plants were subjected to drought stress in 2021, which may have resulted in an increase in NSC. The increase in NSC content in plant tissues is a common physiological response to drought stress, contributing to defense against future drought periods (O'Brien et al. 2014). It has been proven that plants exposed to drought have significantly higher NSC levels than plants not subjected to drought the previous year (Tomasella et al. 2019). Although higher growth was not recorded in 2021 as it was in 2022, it can be assumed that the reason lies in the lower intensity of drought stress that year. Also, although the plants in groups 4 and 5 likely increased assimilation after drought stress, this did not reflect in their current height growth due to the pattern of height growth in willows (i.e., culmination of height growth at the beginning of the growing season). Therefore, it seems convincing that after drought stress in 2021, plants in groups 4 and 5 stored an increased amount of NSC and utilized it during the period of intensive height growth in 2022. In addition to the response to the 2021 drought, plants have the ability of so-called compensatory growth, as a response to recovery in the same year. The concept of compensatory growth involves increasing assimilation above values that were present before the drought stress (Arend et al. 2016, Gessler et al. 2020). Therefore, we can highlight three possible reasons for the higher growth in groups 4 and 5, namely: higher NSC levels as a result of recovery from the 2021 drought; the absence of drought stress during the period of intensive height growth in 2022; prolonged growing season in 2022 due to drought-induced delay in leaf senescence (Figure 7). More intensive height growth after the second year of exposure to drought stress may be a result of a "carry-over" effect on leaf phenology and growing season length (Figures 7 and 8), as well as a likely increase in NSC. Generally, the growth response to drought stress depends on the interaction between the timing of drought stress induction and the growth phenology specific to each individual tree species (Kozlowski 1964, Kannenberg et al. 2019, Van Kampen et al. 2022).

Autumn Phenology

It is evident that the first group of willow ramets delayed the onset of leaf senescence in 2021 (Figure 6), and the progression of the process was slower compared to the other groups. By delaying the onset of autumn leaf senescence, the plants attempt to compensate for assimilation and growth that were hindered during the period of drought. This delay, following induced drought stress, has been observed in pedunculate oak (Čehulić et al. 2019) and also in beech (Arend et al. 2016). The delay of autumn leaf senescence commonly occurs when the assimilation process during the growing season is disrupted by some stressful factors (Keskitalo et al. 2005, Mariën et al, 2019). The lack of response in the other groups of willow ramets can be at least partially explained by the lower intensity of induced drought stress in the first year of the experiment or by the higher genotype sensitivity to drought stress at the beginning of the growing season.

After the repetition of drought stress in 2022, it is evident that all groups delayed the onset of autumn leaf senescence (Figure 7). Although all groups showed a delayed onset of autumn leaf senescence, groups 6 and 7 had the greatest temporal gap compared to the control, and the process itself occurred slower in the ramets of those groups. Drought stimulates the plant to try to compensate for the previous loss of photosynthetic activity (post-drought stimulation) by delaying autumn leaf senescence and increasing the intensity of photosynthesis, a phenomenon observed in beech (Arend et al. 2016). Groups 6 and 7 were exposed to drought stress in July and August when high air temperatures prevailed. Plants exposed to the interaction of abiotic factors such as drought and high air temperature must balance between preventing additional water loss and protecting against leaf overheating (Zandalinas et al. 2018). Stomata respond to high temperature and drought by closing, leading to a decrease in CO2 levels in chloroplasts, which reduces the intensity of photosynthesis (Rennenberg et al. 2006). Likewise, negative interaction effects have been recorded through increased production of reactive oxygen species in *Populus yunnanensis* Dode, and it is known that ROS (reactive oxygen species) can cause significant cell damage and inhibit photosynthesis (Baxter et al. 2014).

Groups 4, 5, and 6 demonstrate that manipulating drought stress can even have a positive effect because prolonging growing season indirectly affects greater height growth. However, with the extension of the growing season, the risk of early autumn frosts also increases. From the leaves affected by frost, the plant cannot recycle nutrients, causing a deficiency of nutrients needed for growth at the beginning of the next growing season (Fracheboud et al. 2009).

Spring Phenology

In this study, goat willow ramets exposed to drought at different times during the growing season (in 2021) showed shifts towards earlier or delayed budburst in spring 2022 (Figure 8). Groups exposed to drought stress from March to early June (groups 1, 2, 3, and 4) on average leafed out earlier than the control group. Groups exposed to drought stress from early June to mid-July (groups 5, 6, and 7) leafed out later than the control group. It can be clearly seen that different times of drought stress induction affect shifts in spring phenology in different directions compared to the control group. It is known that drought stress caused delayed budburst in pedunculate oak (*Quercus robur* L.) (Vander Mijnsbrugge et al. 2016), while some authors report earlier budburst in *Quercus ilex* L. and *Quercus coccifera* L. (Sanz-Pérez and Castro-Díez 2010), whereas in pedunculate oak, drought stress caused delayed budburst in one year, followed by early budburst the next year (Čehulić et al. 2019). Čehulić et al. (2019) hypothesize that the different time periods during which plants are exposed to water deficit during the growing season probably cause a “carry-over” effect, resulting in different leaf-out times. Therefore, it can be assumed that drought stress caused an epigenetic (memory) imprint, and the timing of stress induction conditioned different budburst times. Through genetic or biochemical reactions to stressful factors, the plant acquires a so-called stress imprint, which determines the plant's response to overcoming future stresses (Bruce et al. 2007). It is now known that plants, to cope with acute stress, have developed mechanisms that recognize undesirable conditions and respond to them through DNA methylation, histone modification, or chromatin remodeling without changing the nucleotide sequence (Madlung 2004, Bruce et al. 2007). Additionally, it is important to note the role of plant hormones, such as abscisic acid (ABA), which participate in the expression of specific genes that can be linked to drought stress (Chaves et al. 2003).

When observing the spring leaf phenology of the first group in 2022, it can be noticed that it leafed out earliest compared to all groups (Figure 8). An interesting fact is that the same group experienced delayed onset of autumn senescence the year before (Figure 6). From the above, we can assume that in addition to the epigenetic impression, the phenology of the following growing season may also be influenced by the amount of reserves the plant stores the previous year. Since it extended its growing season in the autumn of 2021, it had a longer period in which assimilation was possible, and as a result of good nutritional status, responded with the earliest budburst. Therefore, we can assume that spring phenology is influenced by the concentration of NSCs the plant accumulates in the previous year's autumn. Amico Roxas et al. (2021) have demonstrated that low concentrations of NSCs in the autumn affect the delayed onset of spring phenology in several species of deciduous Mediterranean trees. Accordingly, higher levels of NSCs in the autumn could potentially influence earlier budburst, which should definitely be further investigated.

Flowering Analysis

By tracking the number of flowers, it was determined that there was a decrease in the number of flowering ramets in all groups during the second vegetative period, including the control group that was not exposed to drought (Figure 9). It can be assumed that the general reduction in the number of flowering ramets occurred due to a decrease in starch reserves since the ramets originated from hardwood cuttings. It is known that during rooting, there is a gradual decrease in carbohydrate reserves in the aboveground part of the cutting, as carbohydrates are allocated towards root, leaf, and callus development (Okoro and Grace 1976). Although it is not sufficiently understood how carbohydrate deficiency affects the formation of flower buds and flowering in forest tree species, it has been found that low starch levels negatively affect the differentiation of flower buds in fruit crops. For example, Goldschmidt and Golomb (1982) discovered a positive correlation between starch levels and the number of flower buds in citrus.

Despite the overall decrease in the number of flowering ramets, in groups 5 and 6, which were exposed to drought stress in June/July, there was a significantly more intense decrease in the number of flowering ramets compared to the control (Figure 9). This suggests that flowering is negatively affected by drought stress, especially if induced in late spring or early summer. It is known that drought stress during the month of July slows down the process of flower bud differentiation and prevents subsequent recovery, compromising the regularity of flower differentiation processes in species such as *Prunus armeniaca* L., and negatively impacting fruiting in the following growing season (Bartolini et al. 2020). The initiation of female flower primordia in willows typically occurs after the completion of apical growth (Junttila 1976), i.e., in the early spring period, as is the case with some other tree species, e.g., oaks (Tantray et al. 2017). Therefore, the probable reason for the more intense decrease in the number of flowering ramets in these groups is that drought stress disrupted the initiation process of flower primordia.

CONCLUSIONS

This study demonstrates that the timing of drought stress induction plays a crucial role in shaping the phenological and growth responses of goat willow (*Salix caprea* L.). Drought stress applied at different stages of the growing season resulted in distinct effects on height growth, leaf phenology, and flower formation. Early-season droughts negatively impacted height growth due to overlap with the period of intensive vegetative development, while late-season droughts led to prolonged leaf retention and, in some cases, increased height growth, likely due to physiological compensation and accumulated non-structural carbohydrates (NSCs) Spring and autumn phenological shifts, both advances and delays, indicate the presence of “carry-over” effects, potentially linked to epigenetic memory or changes in resource allocation. Notably, groups exposed to summer droughts showed a marked reduction in flowering, suggesting that drought timing also influences reproductive capacity.

Overall, the findings underline the importance of considering drought timing—not merely its presence—when evaluating the resilience of forest tree species to climate change. The precise mechanisms behind these phenological and developmental responses, especially the interplay between NSCs, hormonal signals, and epigenetic regulation, warrant further investigation to better inform adaptive forest management strategies under increasing climate variability.

Author Contributions

SB and MB conceived and designed the research, SB and MB developed the methodology and validated the results, MB, SB, and IČ carried out the filed measurements, MB performed the formal analysis, MB and SB provided resources, MB and SB handled data curation, MB wrote the original draft, IKB, SB, and IČ reviewed and edited the manuscript, MB prepared visualization, SB provided supervision.

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Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

Adams HD, Collins AD, Briggs SP, Vennetier M, Dickman LT, Sevanto SA, Garcia-Forner N, Powers HH, McDowell NG, 2015. Experimental drought and heat can delay phenological development and reduce foliar and shoot growth in semiarid trees. *Glob Change Biol* 21(11): 4210-4220. https://doi.org/10.1111/gcb.13030.

Amico Roxas A, Orozco J, Guzmán-Delgado P, Zwieniecki MA, 2021. Spring phenology is affected by fall non-structural carbohydrate concentration and winter sugar redistribution in three Mediterranean nut tree species. *Tree Physiol* 41(8): 1425-1438. https://doi.org/10.1093/treephys/tpab014.

Arend M, Brem A, Kuster TM, Günthardt-Goerg MS, 2013. Seasonal photosynthetic responses of European oaks to drought and elevated daytime temperature: Photosynthetic responses to drought and elevated temperature. *Plant Biol* 15(1): 169-176. https://doi.org/10.1111/j.1438-8677.2012.00625.x.

Arend M, Sever K, Pflug E, Gessler A, Schaub M, 2016. Seasonal photosynthetic response of European beech to severe summer drought: Limitation, recovery and post-drought stimulation. *Agr Forest Meteorol* 220(4): 83-89. https://doi.org/10.1016/j.agrformet.2016.01.011.

Bačurin M, Bogdan S, Katičić Bogdan I, Sever K, 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, Katičić Bogdan I, Sever K, Bogdan S, 2025. The effects of drought timing on height growth and leaf phenology in pedunculate oak (*Quercus robur* L.). *Forests* 16(3): 397. https://doi.org/10.3390/f16030397.

Balzano A, Amitrano C, Arena C, Pannico A, Caputo R, Merela M, Cirillo C, De Micco V, 2025. Does pre-acclimation enhance the tolerance of *Quercus ilex* and *Arbutus unedo* seedlings to drought? *Plants* 14(3): 388. https://doi.org/10.3390/plants14030388.

Bartolini S, Lo Piccolo E, Remorini D, 2020. Different summer and autumn water deficit affect the floral differentiation and flower bud growth in apricot (*Prunus armeniaca* L.). *Agronomy* 10(6): 914. https://doi.org/10.3390/agronomy10060914.

Basler D, Korner C, 2014. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiol* 34(4): 377-388. https://doi.org/10.1093/treephys/tpu021.

Baxter A, Mittler R, Suzuki N, 2014. ROS as key players in plant stress signalling. *J ExpBot* 65(5): 1229-1240. https://doi.org/10.1093/jxb/ert375.

Bräutigam K, Vining KJ, Lafon-Placette C, Fossdal CG, Mirouze M, Marcos JG, Fluch S, Fraga MF, Guevara MÁ, Abarca D, Johnsen Ø, Maury S, Strauss SH, Campbell MM, Rohde A, Díaz-Sala C, Cervera MT, 2013. Epigenetic regulation of adaptive responses of forest tree species to the environment. *Ecol Evol* 3(2): 399-415. https://doi.org/10.1002/ece3.461.

Bruce TJA, Matthes MC, Napier JA, Pickett JA, 2007. Stressful "memories" of plants: Evidence and possible mechanisms. *Plant Sci* 173(3): 603-608. https://doi.org/10.1016/j.plantsci.2007.09.002.

Canham CD, Kobe RK, Latty EF, Chazdon RL, 1999. Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. *Oecologia* 121(1): 1-11. https://doi.org/10.1007/s004420050900.

Chaves MM, Maroco JP, Pereira JS, 2003. Understanding plant responses to drought — from genes to the whole plant. *Funct Plant Biol* 30(3): 239. https://doi.org/10.1071/FP02076.

Cornelissen JHC, Diez PC, Hunt R, 1996. Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. *J Ecol* 84(5): 755. https://doi.org/10.2307/2261337.

Cowan IR, 1982. Regulation of water use in relation to carbon gain in higher plants. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology II. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 589-613. https://doi.org/10.1007/978-3-642-68150-9_18.

Čehulić I, Sever K, Katičić Bogdan I, Jazbec A, Škvorc Ž, Bogdan S, 2019. Drought impact on leaf phenology and spring frost susceptibility in a *Quercus robur* L. provenance trial. *Forests* 10(1): 50. https://doi.org/10.3390/f10010050.

Denham SO, Barnes ML, Chang Q, Korolev M, Wood JD, Oishi AC, Shay KO, Stoy PC, Chen J, Novick KA, 2023. The rate of canopy development modulates the link between the timing of spring leaf emergence and summer moisture. *J Geophys Res - Biogeo* 128(4): e2022JG007217. https://doi.org/10.1029/2022JG007217.

Estiarte M, Peñuelas J, 2015. Alteration of the phenology of leaf senescence and fall in winter deciduous species by climate change: effects on nutrient proficiency. *Glob Change Biol* 21(3): 1005-1017. https://doi.org/10.1111/gcb.12804.

Foster TE, Schmalzer PA, Fox GA, 2014. Timing matters: the seasonal effect of drought on tree growth. *The Journal of the Torrey Botanical Society* 141(3): 225-241. https://doi.org/10.3159/TORREY-D-13-00060.1.

Fracheboud Y, Luquez V, Björkén L, Sjödin A, Tuominen H, Jansson S, 2009. The control of autumn senescence in European aspen. *Plant Physiol* 149(4): 1982-1991. https://doi.org/10.1104/pp.108.133249.

Gessler A, Bottero A, Marshall J, Arend M, 2020. The way back: recovery of trees from drought and its implication for acclimation. *New Phytol* 228(6): 1704-1709. https://doi.org/10.1111/nph.16703.

Goldschmidt EE, Golomb A, 1982. The carbohydrate balance of alternate-bearing citrus trees and the significance of reserves for flowering and fruiting[1]. *J Amer Soc Hort Sci* 107(2): 206-208. https://doi.org/10.21273/JASHS.107.2.206.

Hinckley TM, Dougherty PM, Lassoie JP, Roberts JE, Teskey RO, 1979. A severe drought: Impact on tree growth, phenology, net photosynthetic rate and water relations. *American Midland Naturalist* 102(2): 307. https://doi.org/10.2307/2424658.

Hinckley TM, Duhme F, Hinckley AR, Richter H, 1980. Water relations of drought hardy shrubs: osmotic potential and stomatal reactivity. *Plant Cell Environ* 3(2): 131-140. https://doi.org/10.1111/1365-3040.ep11580919.

Ingram J, Bartels D, 1996. The molecular basis of dehydration tolerance in plants. *Annu Rev Plant* 47(1): 377-403. https://doi.org/10.1146/annurev.arplant.47.1.377.

Junttila O, 1976. Apical growth cessation and shoot tip abscission in *Salix*. *Physiol Plantarum* 38(4): 278-286. https://doi.org/10.1111/j.1399-3054.1976.tb04004.x.

Kannenberg SA, Maxwell JT, Pederson N, D'Orangeville L, Ficklin DL, Phillips RP, 2019. Drought legacies are dependent on water table depth, wood anatomy and drought timing across the eastern US. *Ecol Lett* 22(1): 119-127. https://doi.org/10.1111/ele.13173.

Keskitalo J, Bergquist G, Gardeström P, Jansson S, 2005. A cellular timetable of autumn senescence. *Plant Physiol* 139(4): 1635-1648. https://doi.org/10.1104/pp.105.066845.

Killingbeck KT, 2004. Nutrient resorption. In: Noodén LD (ed) Plant cell death processes. Academic Press, pp. 215-226. https://doi.org/10.1016/B978-012520915-1/50017-5.

Kozlowski TT, 1964. Shoot growth in woody plants. *Bot Rev* 30(1): 335-392. https://doi.org/10.1007/BF02858538.

Kuster TM, Dobbertin M, Günthardt-Goerg MS, Schaub M, Arend M, 2014. A phenological timetable of oak growth under experimental drought and air warming. *PLoS ONE* 9(2): e89724. https://doi.org/10.1371/journal.pone.0089724.

Labrecque M, Teodorescu TI, Cogliastro A, Daigle S, 1993. Growth patterns and biomass productivity of two *Salix* species grown under short-rotation intensive culture in southern Quebec. *Biomass and Bioenergy* 4(6): 419-425. https://doi.org/10.1016/0961-9534(93)90063-A.

Leuzinger S, Zotz G, Asshoff R, Korner C, 2005. Responses of deciduous forest trees to severe drought in Central Europe. *Tree Physiol* 25(6): 641-650. https://doi.org/10.1093/treephys/25.6.641.

Lukasová V, Vido J, Škvareninová J, Bičárová S, Hlavatá H, Borsányi P, Škvarenina J, 2020. Autumn phenological response of European beech to summer drought and heat. *Water* 12(9): 2610. https://doi.org/10.3390/w12092610.

Madlung A, 2004. The effect of stress on genome regulation and structure. *Annals of Botany* 94(4): 481-495. https://doi.org/10.1093/aob/mch172.

Mariën B, Balzarolo M, Dox I, Leys S, Lorène MJ, Geron C, Portillo-Estrada M, AbdElgawad H, Asard H, Campioli M, 2019. Detecting the onset of autumn leaf senescence in deciduous forest trees of the temperate zone. *New Phytol* 224(1): 166–176. https://doi.org/10.1111/nph.15991.

Morán-López T, Poyatos R, Llorens P, Sabaté S, 2014. Effects of past growth trends and current water use strategies on Scots pine and pubescent oak drought sensitivity. *Eur J Forest Res* 133(1): 369-382. https://doi.org/10.1007/s10342-013-0768-0.

O'Brien MJ, Leuzinger S, Philipson CD, Tay J, Hector A, 2014. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nat Clim Change* 4(8): 710-714. https://doi.org/10.1038/nclimate2281.

Okoro OO, Grace J, 1976. The physiology of rooting *Populus* cuttings. I. carbohydrates and photosynthesis. *Physiol Plantarum* 36(2): 133-138. https://doi.org/10.1111/j.1399-3054.1976.tb03923.x.

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A, 2006. Physiological responses of forest trees to heat and drought. *Plant Biol* 8(5): 556-571. https://doi.org/10.1055/s-2006-924084.

Robson TM, Rasztovits E, Aphalo PJ, Alia R, Aranda I, 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. *Agr Forest Meteorol* 180(1): 76-85. https://doi.org/10.1016/j.agrformet.2013.05.008.

Sanz-Pérez V, Castro-Díez P, 2010. Summer water stress and shade alter bud size and budburst date in three mediterranean *Quercus* species. *Trees* 24(1): 89–97. https://doi.org/10.1007/s00468-009-0381-5.

Spieß N, Oufir M, Matušíková I, Stierschneider M, Kopecky D, Homolka A, Burg K, Fluch S, Hausman JF, Wilhelm E, 2012. Ecophysiological and transcriptomic responses of oak (*Quercus robur*) to long-term drought exposure and rewatering. *Environ Exp Bot* 77(1): 117-126. https://doi.org/10.1016/j.envexpbot.2011.11.010.

Tantray YR, Wani MS, Hussain A, 2017. Genus Quercus: an overview. *International Journal of Advance Research in Science and Engineering* 6: 1880-1886.

Thomas FM, Gausling T, 2000. Morphological and physiological responses of oak seedlings (*Quercus petraea* and *Q. robur*) to moderate drought. *Ann For Sci* 57(4): 325-333. https://doi.org/10.1051/forest:2000123.

Tomasella M, Nardini A, Hesse BD, Machlet A, Matyssek R, Häberle KH, 2019. Close to the edge: effects of repeated severe drought on stem hydraulics and non-structural carbohydrates in European beech saplings. *Tree Physiol* 39(5):717-728 https://doi.org/10.1093/treephys/tpy142.

Van Kampen R, Fisichelli N, Zhang YJ, Wason J, 2022. Drought timing and species growth phenology determine intra-annual recovery of tree height and diameter growth. *AoB PLANTS* 14(3): plac012. https://doi.org/10.1093/aobpla/plac012.

Vander Mijnsbrugge K, Turcsán A, Maes J, Duchêne N, Meeus S, Steppe K, Steenackers M, 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. *Front Plant Sci* 7(1): 419. https://doi.org/10.3389/fpls.2016.00419.

Way DA, Montgomery RA, 2015. Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant Cell Environ* 38(9): 1725-1736. https://doi.org/10.1111/pce.12431.

Weih M, 2009. Genetic and environmental variation in spring and autumn phenology of biomass willows (*Salix* spp.): effects on shoot growth and nitrogen economy. *Tree Physiol* 29(12): 1479-1490. https://doi.org/10.1093/treephys/tpp081.

Wikberg J, Ögren E, 2004. Interrelationships between water use and growth traits in biomass-producing willows. *Trees* 18(1): 70-76. https://doi.org/10.1007/s00468-003-0282-y.

Wu C, Peng J, Ciais P, Peñuelas J, Wang H, Beguería S, Andrew Black T, Jassal RS, Zhang X, Yuan W, Liang E, Wang X, Hua H, Liu R, Ju W, Fu YH, Ge Q, 2022. Increased drought effects on the phenology of autumn leaf senescence. *Nat Clim Change* 12(10): 943-949. https://doi.org/10.1038/s41558-022-01464-9.

Xie Y, Wang X, Silander JA, 2015. Deciduous forest responses to temperature, precipitation, and drought imply complex climate change impacts. *P Natl A Sci USA* 112(44): 13585-13590. https://doi.org/10.1073/pnas.1509991112.

Yonekura T, Yoshidome M, Watanabe M, Honda Y, Ogiwara I, Izuta T, 2004. Carry-over effects of ozone and water stress on leaf phenological characteristics and bud frost hardiness of *Fagus crenata* seedlings. *Trees* 18(5): 581-588. https://doi.org/10.1007/s00468-004-0345-8.

Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A, 2018. Plant adaptations to the combination of drought and high temperatures. *Physiol Plantarum* 162(1): 2-12. https://doi.org/10.1111/ppl.12540.