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Effects of drought stress on spring bud development in poplar and willow clones

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Abstract: Understanding spring bud phenology is important for predicting tree responses to changing climates and selecting the clones appropriate for various regions. This study assessed how summer drought affects bud development in six poplar and three willow clones.

Experimental plants were grown in pots under three levels of water deficiency – 75%, 50%, and 25% moisture by volume compared to the control (light, moderate, and severe stress) throughout the growing season, preceded by monitoring of spring phenology. Bud assessments of one-year-old plants started nine months after initiating the drought treatment. Bud development was observed for 45 days using a six-score scale, which measures developmental stages ranging from dormancy (0) to full leaf expansion (6).

Under control watering, poplar buds developed faster than willow buds. Additionally, drought had no significant effect on willow buds, so we focused on the impact of drought on poplar bud development, which was affected by moderate and severe stress. Plant survivability in both poplars and willows decreased under severe drought, whereas moderate and light stress had no impact. In terms of bud development, poplar clones ‘Gulliver’, ‘Novoberlinska-3’, and ‘Slava Ukrainy’ coped with stress faster than others, while ‘Volosystoplidna’ was the most drought-sensitive.

The studied tree clones can survive under 50% of water shortage in the pots, but such severe drought conditions delayed bud development. We suppose that the most actively growing clones might be more sensitive to water shortage. Contrastingly, less productive clones were more drought-tolerant, as their bud development “recovered” faster. Therefore, such clones, even being less productive, could survive severe drought compared to clones, that are highly productive under optimal conditions. Our findings provide insights into clone selection for drought-prone environments. They also advance the understanding of woody plant responses to a changing climate.

Keywords: drought stress, spring bud phenology, bud burst, *Populus*, *Salix*

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Introduction

The application of fast-growing poplars (*Populus* spp.) and willows (*Salix* spp.) as bioenergy trees can contribute to reducing the use of fossil fuels and mitigate the increasing anthropogenic carbon dioxide emissions (Djomo et al., 2011). Poplars and willows are typically characterized by rapid growth, high biomass production, and easy vegetative propagation (Kutsokon et al., 2020), making them economically attractive bioenergy crops. Short-rotation plantations of fast-growing trees are cultivated worldwide, and advanced silvicultural techniques, appropriate planting material, water regime, soil features, and favourable climate conditions are crucial for achieving the high productivity of fast-growing trees (Bonosi et al., 2013; Kutsokon et al., 2015; Pleguezuelo et al., 2015). Studies of tree spring phenology are necessary to determine the length of the growing season, the optimal planting period, and seasonal works. Phenological observations are widely recognized for their practical significance in forest management (Harrington et al., 2016).

A longer growing season may provide a competitive advantage by promoting earlier plant growth initiation and biomass production in poplars and willows (Ceulemans et al., 1992; Pellis et al., 2004; Verwijst, 2012). However, genotypes from northern areas and high elevations generally have shorter growing seasons (Pellis et al., 2004). Early bud burst (BB) increases the risks of damage by spring frosts (Sierota et al., 2017) and defoliation by insects and fungi (Puchałka et al., 2017). On the other hand, early-flushing trees are better adapted to summer drought by extending the growing season, which allows trees to reach maximum photosynthetic capacity earlier and during the optimal growing period of late spring and early summer (Costa-e-Silva et al., 2015). In contrast, late-flushing trees, being more resistant to low spring temperatures as well as to insect and fungi damage, are more vulnerable to summer drought (Puchałka et al., 2017). Thus, since the timing of BB in plants influences productivity, biomass accumulation, and carbon sequestration (Menzel et al., 2006; Aitken et al., 2008), as well as resilience to climate change, it is important to select genotypes that can optimize the timing of BB to maximize growth potential while minimizing the negative impacts from late frosts, insect infestations, and drought. Identifying tree species or clones that can maintain a balance between early growth initiation and resistance to climate environmental stressors is an important research issue.

Bud development is a crucial part of tree growth and is regulated by numerous genes and transcription factors. Among these, the EBB1 (early bud break 1) transcription factor plays a key role in promoting bud break, particularly in species such as poplar, peach, and pear (Zhao et al., 2020).

In temperate climates, bud formation is an essential adaptation for tree survival, and every year growth is synchronized with annual changes in temperature (Hänninen & Kramer 2007), which may be recognized as the important driver of bud development. The diverging timing of BB among populations from a latitudinal gradient is supposed to be the result of the plastic response of trees to changing temperatures (Guo et al., 2021). While winter chilling is crucial for dormancy release (Pletsers et al., 2015), BB depends on the accumulation of temperature units above a specific threshold (Ghelardini et al., 2014), which is generally accepted as temperatures of −5 to + 10 °C, for several weeks (Pellis et al., 2004). Longer chilling duration resulted in earlier bud burst, therefore, in warmer winters, BB may be delayed (Pletsers et al., 2015). Analyzing the timing of the start and end dates of the growing season of several poplar clones in the Kyiv region from 2012 to 2018, Ishchuk et al. (2020) defined three phenological groups regarding BB.

Another important factor of BB initiation is the photoperiodic signal; longer day length usually contributes to earlier BB, as shown in *Quercus faginea* Lam. (Sanz-Pérez et al., 2009). However, the interaction between temperature and photoperiod is also supposed to be crucial for spring bud development. Longer photoperiod may partially compensate for the insufficient chilling duration (Pletsers et al., 2015). Therefore, temperature, in combination with photoperiod and other climatic factors, triggers a number of plant hormonal responses leading to BB (Hannah, 2015; Singh et al., 2018; Signorelli et al., 2022). Although temperature and photoperiod are considered the most important factors regulating spring bud development in temperate woody plants (Singh et al., 2018), the amount of water in the environment also plays a vital role in this process (Ghelardini et al., 2014; Orlandi et al., 2020).

Drought is one of the most common key abiotic stresses for plants, impairing many cellular and other functions, which adversely affect plant growth, development, reproduction, seed and fruit production, photosynthetic capacity, transpiration rate, cambial cell production, dormancy, cold hardiness and tree phenology (Morales et al., 2013; Sritontip et al., 2013; Adams et al., 2015; Chhin, 2016; Liang et al., 2019; Inoue et al., 2020; Grossiord et al., 2020; Jensen et al., 2021; Puchałka et al., 2024).

Fast-growing poplar and especially willow trees are, in the majority, moisture-loving plants (Isebrands & Richardson, 2014). *Salix* species differ in their ecological requirements, although many of them are adapted to mesic–hydric habitats (Isebrands & Richardson, 2014). Moisture availability is an important factor controlling the native distribution and abundance of species as well as biomass production (Bonosi et al., 2013), therefore, drought tolerance is becoming an increasingly crucial feature. Nevertheless, *Populus* is a highly adaptable tree genus, including many species and hybrids that can thrive in diverse conditions and have a wide spectrum of adaptations to abiotic stressors (Chhin, 2016; Isebrands & Richardson, 2014; Kutsokon et al., 2015). Different genotypes of poplar and willow demonstrate varying drought resistance (Isebrands & Richardson, 2014). Investigating the relationships between productivity, water use efficiency, and drought tolerance in 29 genotypes of *P. deltoides* × *P. nigra* Monclus et al. (2006) found that productivity exhibited significant genotypic variability. The more productive genotypes generally showed lower drought tolerance, with a substantial decrease in biomass under drought conditions, while the less productive genotypes demonstrated a wider range of stress tolerance (Monclus et al., 2006).

Trees which adapted to water deficits to maintain their vital activities, exhibit morphological, physiological, biochemical, and molecular changes to balance water loss through transpiration and root uptake (Barchet et al., 2013; Khoma et al., 2021). Drought stress leads to stomatal closure, negatively affecting plant gas exchange and metabolism (Chen et al., 2011). As water deficit is negatively related to stomatal conductance and leaf area growth, it can reduce plant biomass productivity (Briglia et al., 2020).

Current global climate changes are leading to increasing temperatures, changing precipitation regimes, and increasing frequency, duration, and intensity of drought periods (Shukla et al., 2019). Plants can face a water shortage at any ontogenetic stage, and the severity of the negative impact depends on the phase of plant ontogenesis. The periods of organs formation and growth during spring phenology are the most sensitive to water deficit, and plants are less sensitive during dormancy (Bray, 2007). Water plays an essential role in BB in plants, and its lack can lead to changes in this process (Ghelardini et al., 2014; Orlandi et al., 2020). However, when applied in the dormancy period, limited watering may not affect the timing of bud burst while impairing other growth and physiological parameters, as shown in *P. cathayana* (Yu et al., 2018).

Studies (Signorelli et al., 2022) have shown a significant correlation between bud burst in *Vitis vinifera* L. and the presence of water in the soil. Similarly, a delay in bud burst was observed under water deficit, in contrast to well-watered trees, in longan *Dimocarpus longan* Lour. (Sritontip et al., 2013) and cork oak *Q. suber* L. (Ennajah et al., 2013). This may be due to a decrease in the amount of xylem, phloem and cortical parenchyma (Ennajah et al., 2013). In addition, young leaves have a higher photosynthetic potential than old leaves (Niinemets et al., 2005), and water deficit delays the BB date and thus reduces this potential.

Spring bud development also depends on the environmental conditions of the previous year (Čehulić et al., 2019; Sanz-Pérez & Castro-Díez, 2010). However, this part of the process is less understood in fast-growing trees. Moreover, sometimes, contrasting effects were determined. In particular, *Q. robur* experimentally exposed to drought during the spring-mid-summer period demonstrated delayed BB in the following spring. However, drought treatment during the second summer, on the contrary, advanced bud flushing (Čehulić et al., 2019). The authors supposed "that drought stress may have triggered an epigenetic response ("memory") resulting in the observed carry-over effect on bud burst date in the oaks" (Čehulić et al., 2019). In another research (Sanz-Pérez & Castro-Díez, 2010) on three Mediterranean oak species, it was observed that the lack of water in the summer period modified the percentage, the size of the buds, and the date of bud burst, moreover in different ways in evergreen and a deciduous-marcescent species.

In the face of current and projected climate changes, phenological studies provide essential insights for forest management. They contribute to optimizing the selection of climate-resilient seed sources and refining the timing of silvicultural activities such as planting and sowing to enhance tree productivity (Harrington et al., 2016). As *Populus* species contain ecotypes with considerably different phenological characteristics, they are good models for studying bud and leaf phenological traits (Pellis et al., 2004).

Our experiment was designed to study the effects of summer drought on spring bud development in poplars and willows. Its main hypothesis was that the drought treatment, applied in the previous growing season, would influence spring bud phenology in poplar and willow trees. The specific aims were to define water deficit levels that will and will not strongly affect spring bud development as well as to identify more and less drought-sensitive clones, since genotypes may exhibit varying responses to drought. This study will be important for selecting the clones planted in different regions of Ukraine. It will also advance our understanding of the possible responses of woody plants to climate change.

Materials and methods

Plant material

The planting material of fast-growing poplar and willow trees was obtained from the stock collection in the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv). Some of the most actively growing clones were chosen based on our previous experiments (Kutsokon et al., 2022). The list of fast-growing tree clones and their origin is given in Table 1.

Cuttings of 20 cm in length and up to 1 cm in diameter from six poplar and three willow clones were planted in 1 L pots filled with a soil mixture consisting of black soil, peat, and vermiculite (10:10:1.5). Each variant was initially planted in six replicates.

Table 1. Identification of the poplar and willow clones used in the current study

Species / Hybrids	Clone name
Poplars (*Populus*)	
P. deltoides Marsh. (free pollination) (Patlai & Rudenko, 1990)	‘Gulliver’
P. deltoides Marsch. × *P. balsamifera* L (Torosova et al., 2015)	‘Kanadska × balsamichna’
P. pyramidalis × *P. laurifolia* (Torosova et al., 2015)	‘Novoberlinska-3’
P. nigra cv. ‘Pyramidalis’ (Los, 2013)	‘Slava Ukrainy’
P. deltoides × *P. pyramidalis* (Starova, 1975)	‘Strilopodibna’
P. trichocarpa Torr. et Gray (Torosova et al., 2015)	‘Volosystoplidna’
Willows (*Salix*)	
S. alba × *S. fragilis* (Patlai & Rudenko, 1990)	‘Lisova pisnya’
S. alba × *S. fragilis* (Patlai & Rudenko, 1990)	‘Pechalna’
Salix sp.	‘Zhytomyrska-1’

Drought stress experimental design

During the spring rooting period, all plants were watered for 30 days with the same amount of water to field capacity. Field capacity was determined by fully saturating the soil in pots, allowing excess water to drain for 24 hours, and then measuring soil moisture with a moisture meter at multiple depths. The recorded moisture value was considered 100% field capacity and used as a reference for drought treatments. After the rooting period, differentiated watering was started. Sufficient watering, recognized as a field capacity, served as a control. The control groups (100%) were watered with 100 mL of tap water to keep them well watered, according to the visual needs of the plants, usually every other day. Experimental plants served as three water deficit variants, which were irrigated at the same time as control, but reductions in watering volume were applied for a quarter (75%, light stress), half (50%, moderate stress), and three quarters (25%, severe stress).

Plants were kept outdoors during the growing season and protected from rain by covering them with plastic wrap. The plants were arranged in a randomized design to minimize the influence of microclimatic factors. After the seasonal leaf fall in November, the plants were transferred to an unheated room with a limited natural light regime. During the dormant period and spring phenology analysis, the plants were kept indoors, with sufficient watering for all variants as needed, approximately once a week. The average temperature during storage was 5–7 °C and 7–12 °C during the bud scoring.

Assessment of spring bud development

Spring bud phenology after drought stress was assessed on one-year-old shoots, approximately nine months after experimental treatments were initiated, on February 6, 2019, and continued for 45 days. Poplar and willow plants were evaluated by the six-degree scale of spring bud development (Khoma & Kutsokon, 2019), where 0 is a dormant bud, and 6 – all leaves are fully developed (Fig. 1). Both apical and lateral buds were scored. For each plant, the phenological stage was recorded for the most developed bud per cutting. Buds were scored on eight occasions: February 6, 13, 18, 26, and March 4, 12, 19, and 23, corresponding to day of the year (DOY) 37, 44, 49, 57, 63, 71, 78, and 82).

Drought effects were estimated using two values which characterized the bud development in different terms: i) the day of reaching stage 3 was expressed for each plant as the DOY, on which at least one bud reached stage 3; ii) the stage reached by each plant on DOY 82, which was the last day of scoring. Unless otherwise stated, all data for bud developmental scores are given as median ± interquartile range (IQR).

Additionally, plant survivability on the last day of scoring (DOY 82) was calculated as the percentage of survived plants ± sp.

Data analysis

Data normality was assessed using the Shapiro-Wilk test. Due to the non-gaussian distributions of the data, nonparametric tests were applied. Median and interquartile range (first to third quartile) statistics were used to estimate bud development stages. To compare the measured parameters in the different genera (2) and watering groups (4), the Kruskal-Wallis test and post hoc pairwise comparisons were performed using Dunn’s procedure with a Bonferroni correction for multiple comparisons.

For the comparison of data for bud developmental stages between control and water-deficient variants within the same clone, the Mann-Whitney tests were performed. The differences in survivability were estimated by Fisher’s exact test. Statistical analyses were performed using OriginPro9.6 (OriginLab Corporation) and SPSS Statistics 23 (IBM) software. The significance level was set at $p<0.05$.

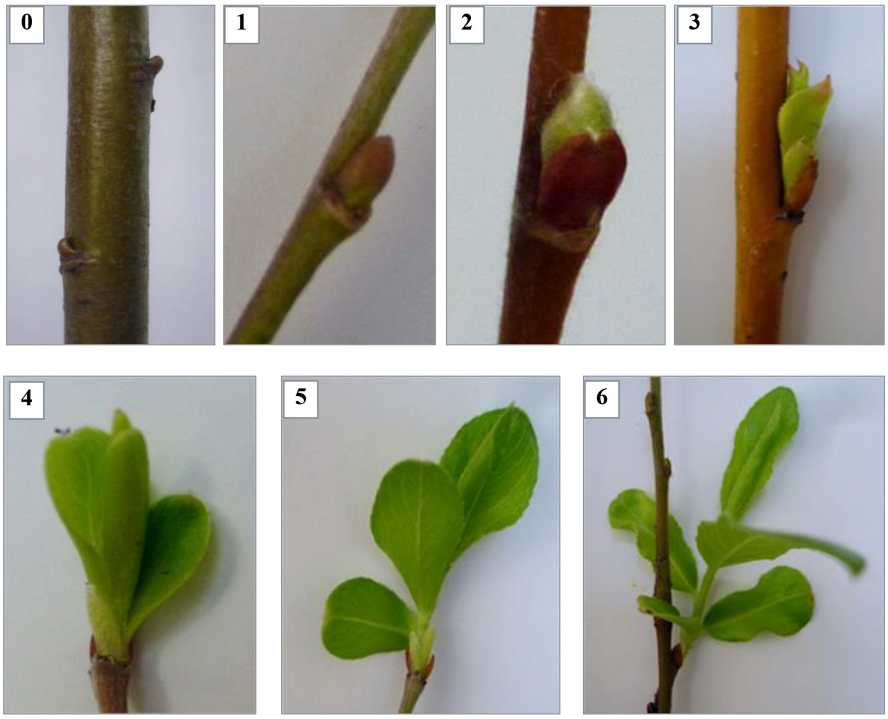

Fig. 1. Spring phenology stages in willow shoots. Discrete stages are according to Weih, 2009; Welc et al., 2017; Khoma & Kutsokon, 2019; Adler et al., 2021; with personal recommendations of Dr. Ann-Christin Rönnberg-Wästljung and Dr. Almir Karacic). (0) score 0 – dormant bud, no sign of bud swelling, bud tip is tightly pressed to the shoot. (1) score 1 – the buds are slightly swollen but closed; score 1.5 if the tip protrudes approximately 1 mm but the bud scales are closed. (2) score 2 – bud scales begin to open; the length of shoot tips approximately equals the length of covering scales. (3) score 3 – leaves are elongated and twisted, they are longer than the bud scales. (4) score 4 – the leaves are half-opened, the bud scales fall off; score 4.5 if most, but not all, leaves are opened. (5) score 5 – the leaves are completely opened. (6) score 6 – leaves are fully open, shoot increment ≥ 1 cm

Results

Differences between *Populus* and *Salix*

The percentage of surviving plants, both poplars and willows, in the control, light, and moderate drought stress groups were defined within 100–83%, which were not statistically different. However, after the severe drought stress, the survivability of *Populus* and *Salix* was greatly reduced (61 and 44%, respectively, $p < 0.05$ in Fisher's test) (Fig. 2).

One to three plants have died during the year in some variants, primarily those after severe drought stress (Table 2). Thus, 3 to 6 plants of each variant

Table 2. Median and range (Min and Max) of the score of the bud development stage on DOY 82. The effects of differential watering on trees applied in the previous growing season were studied. 100% – control plants were watered normally, 75%, 50%, and 25% – corresponding watering % of the control volume. Plants survived – a number of plants that survived on DOY 82 out of 6 initially planted. Differences between control and water-deficient variants within each clone were analyzed in Mann-Whitney tests, * $p < 0.05$. ND – sufficient data is not available

Clones	Median (Min–Max) / (Plants survived)			
	100%	75%	50%	25%
		Poplars		
'Gulliver'	6.0(6.0)/(6)	6.0(6.0)/(5)	6.0(6.0)/(6)	6.0(6.0)/(3)
'Kanadska × balsamichna'	6.0(6.0)/(5)	6.0(5.0–6.0)/(5)	5.0(4.0–6.0)/(5)	**4.5(4.0–6.0)***/(4)
'Novoberlinska-3'	6.0(6.0)/(5)	6.0(6.0)/(5)	6.0(6.0)/(5)	6.0(6.0)/(3)
'Slava Ukrainy'	6.0(6.0)/(5)	6.0(6.0)/(6)	6.0(6.0)/(6)	6.0(5.0–6.0)/(4)
'Strilopodibna'	6.0(5.0–6.0)/(6)	6.0(5.0–6.0)/(4)	5.5(5.0–6.0)/(4)	**3.5(3.0–4.0)***/(4)
'Volosystoplidna'	6.0(6.0)/(6)	6.0(3.0–6.0)/(6)	**4.5(1.0–6.0)***/(6)	**3.0(3.0–6.0)***/(4)
		Willows		
'Lisova pisnya'	6.0(5.0–6.0)/(6)	5.5(5.0–6.0)/(6)	5.5(5.0–6.0)/(4)	5.0(5.0–6.0)/(3)
'Pechalna'	6.0(5.0–6.0)/(6)	6.0(4.0–6.0)/(5)	5.5(4.0–6.0)/(6)	5.0(2.0–6.0)/(4)
'Zhytomyrska-1'	3.0(2.0–4.0)/(6)	2.0(2.0–3.0)/(4)	2.0(2.0–3.0)/(5)	ND(2.0)/(1)

were finally analyzed for bud development. In a willow clone ‘Zhytomyrska-1’, only one plant survived after severe drought stress (25% of watering from the control), so this sample was excluded from the bud phenological analysis.

Under normal watering and light drought stress, the bud developmental scores differed significantly between poplars and willows (Fig. 3) for both parameters – the day of reaching stage 3 and the stage on the last day of scoring (DOY = 82). After normal watering, stage 3 of the bud phenology started in poplars on DOY 63±19 (median±IQR), whereas in willows – on DOY 78±11 (Fig. 3A). Water deficiency decelerated BB in poplars, significantly increasing the time for reaching stage 3 in trees after moderate (50%) and severe (25%) drought stress up to DOY 71±15. At the same time, willows unexpectedly demonstrated insignificant BB variations on DOY 75–78 under all drought stress and control regimes (Fig. 3A).

Spring bud development stage on DOY 82 in poplars was significantly delayed under moderate and severe drought stress compared to the control, as it was determined in the Kruskal-Wallis test with post hoc using Dunn’s-Bonferroni tests. However, no effects were observed in the willows after drought treatment, but in all willow variants scores were lower than in poplar control and stressed plants (Fig. 3B).

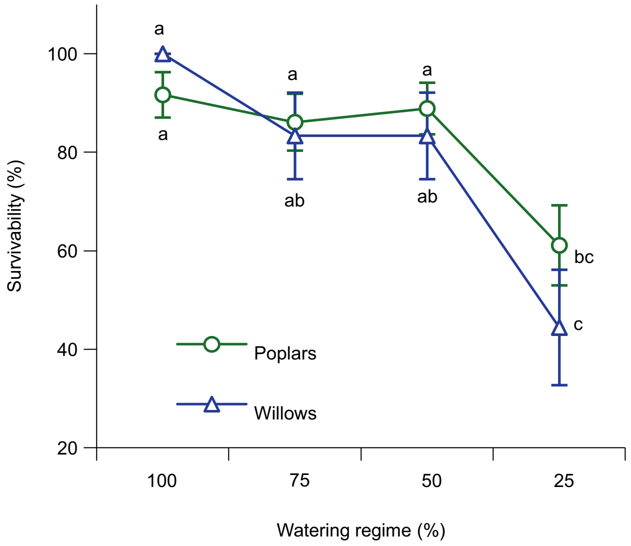

Fig. 2. Survivability percentage ± SD of poplars and willows after differential watering applied in the previous growing season: 100% – control plants were watered normally, 75%, 50%, and 25% – corresponding watering % from the control volume. a, b, c – different letters indicate the significant differences between groups after Fisher’s exact tests ($p < 0.05$). Number of poplar plants in each watering group is n = 36, and willow – n = 18

Thus, we can assume that, in general, buds in the control poplars developed significantly faster compared to willows. After treatment in the previous year with moderate (50%) and severe (25%) drought stress, poplar plants demonstrated delayed spring bud development, but no effects were observed in willows. Severe drought stress significantly reduced the survivability in both poplars and willows.

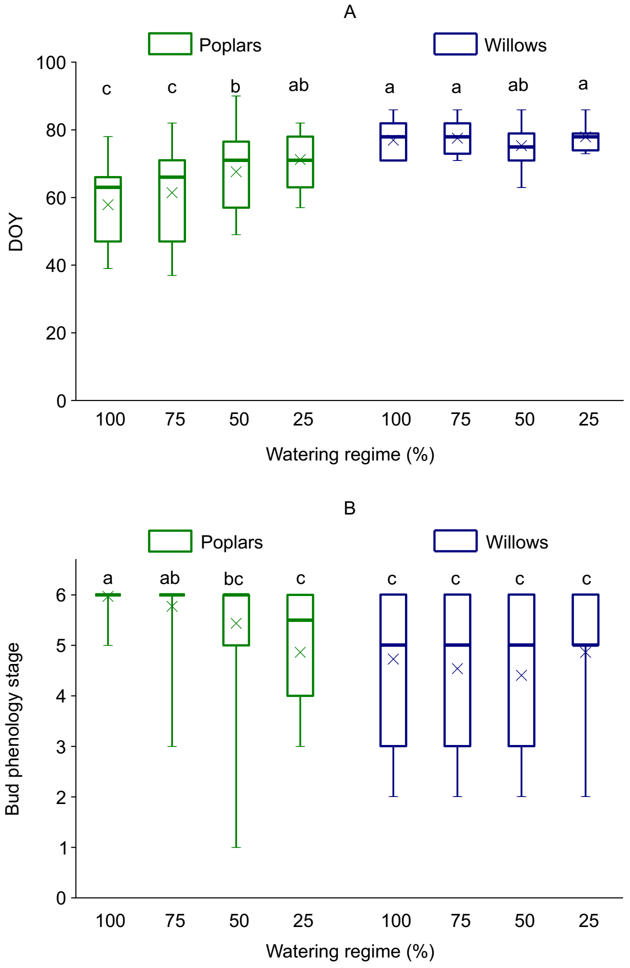

Fig. 3. Progression of spring bud development of poplars and willows after differential watering applied in the previous growing season: 100% – control plants were watered normally, 75%, 50%, and 25% – corresponding watering % from the control volume. (A) Time of entering stage 3. (B) The score of the bud stage on DOY 82. DOY – day of the year. For detailed descriptions of each bud phenology stage, see Fig. 1. On the box and whisker plots, the bold lines denote the median; the cross symbol is the mean; the box represents values within the 25–75% quartiles, and the whiskers show minimum and maximum values. In each panel, A or B, variants sharing the same letters were not statistically different in Kruskal-Wallis test with Dunn’s-Bonferroni post hoc test. Number of replicates ranged from 22 to 33 for poplars and from 7 to 18 for willows

Clonal variations in spring bud development

Bud development was affected by water deficit in all poplar clones, although some clones, such as ‘Gulliver’, ‘Novoberlinska-3’, and ‘Slava Ukrainy’, showed tolerance to severe drought stress (Fig. 4). Nevertheless, similarly to the pooled results described in the previous section, all willow clones did not differ in control and stressed conditions (Fig. 5).

The phenological time series (Fig. 4), analyzed together with the scores on DOY 82 (Table 2), demonstrated that the bud development in poplars was affected by drought in all clones at different stages. Severe stress delayed bud burst (stage 3) in six clones, and moderate stress delayed it in five clones, except ‘Strilopodibna’. At the end of the analyses, three clones (‘Gulliver’, ‘Novoberlinska-3’, ‘Slava Ukrainy’) were able to reach the stage 6 even after severe drought stress (Fig. 4, Table 2). Thus, according to the parameters of spring bud development, these clones coped with the negative consequences of drought stress in a shorter time than others.

On the last day of analysis, the most considerable delay in the bud development was found in three poplar clones (‘Kanadska × balsamichna’, ‘Strilopodibna’, ‘Volosystoplidna’) after severe drought stress. These clones could not reach the control levels of the bud stage during the time of scoring. Moreover, clone ‘Volosystoplidna’ should be recognized as the most

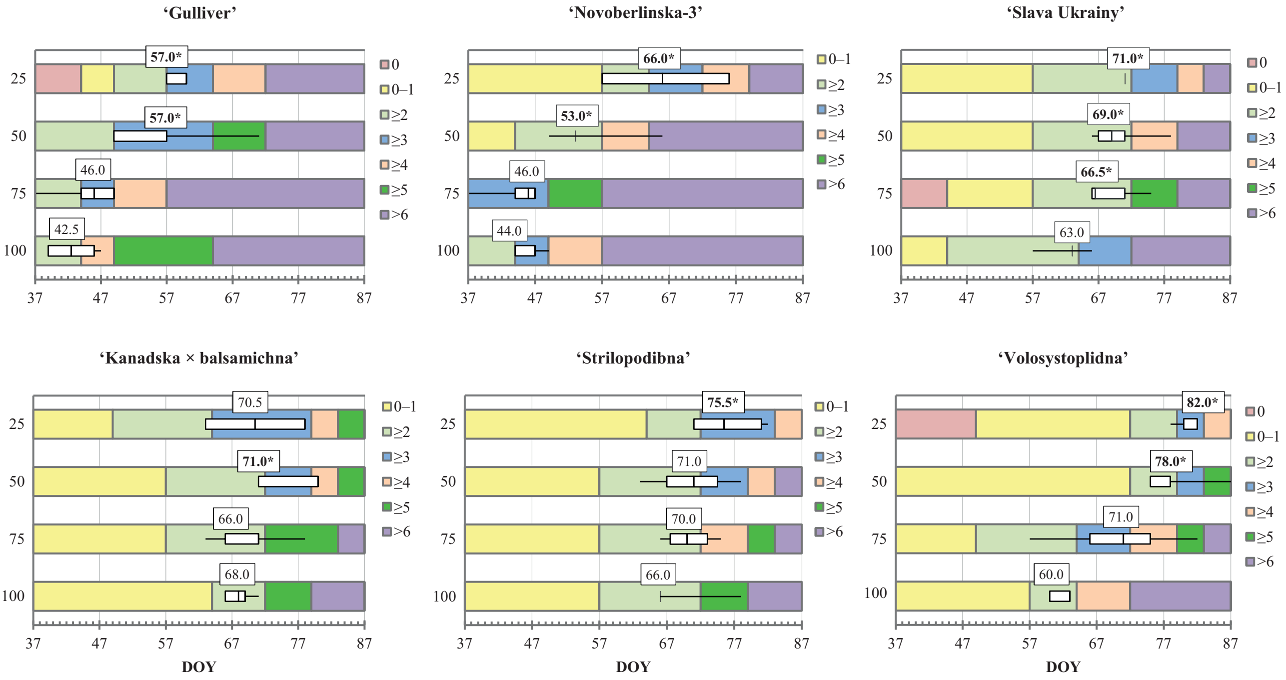

Fig. 4. Effects of drought stress on the spring phenology stages (0–6) of six poplar clones. 100 – control plants were watered normally, 75, 50, and 25 – corresponding watering % from the control volume. DOY – day of the year. On the box and whisker plots, median values (DOY) for reaching stage 3 of bud development are presented; the box represents the values within the 25–75% quartiles, and the whiskers show the minimum and maximum values. * – denotes significant differences between control and water-deficient variants within each clone for the dates of reaching stage 3 of bud development after Mann-Whitney tests ($p < 0.05$). N = 3–6 for each variant

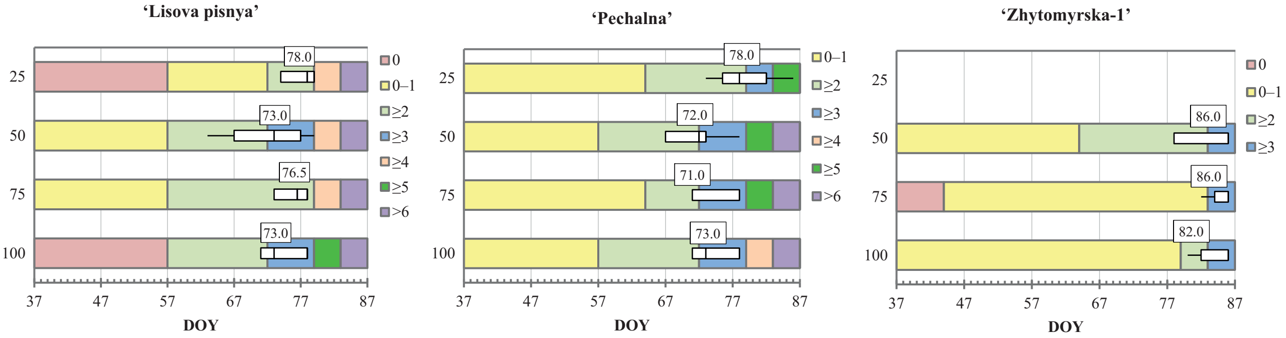

Fig. 5. Effects of drought stress on the spring phenology stages (0–6) of three willow clones. Symbols have the same meaning as in Fig. 4

impacted by drought stress as it was the only clone affected after both, severe and even moderate stress on the last day of scoring. In this clone, the day of reaching stage 3 was delayed by 18 and 20 days after moderate and severe stress, respectively, compared to the control.

All willow clones demonstrated no statistical differences in spring bud developmental stages after drought stress, neither at the intermediate (Fig. 5) nor at the final stage of analysis (Table 2).

Discussion

Our experiments were designed as a model to study the effects of summer drought on fast-growing trees. We demonstrated that severe drought stress significantly reduced survivability in both poplars and willows, confirming their vulnerability under extreme water deficit conditions, while moderate and light drought stress were not as critical.

Bud break dates varied considerably between poplars and willows. We have determined that the BB stage in poplars depended on water availability in the previous summer season, and severe drought stress caused the most delays in BB stages of all clones (Fig. 4). Moderate stress delayed bud development in some ways in all clones except 'Strilopodibna'. Meanwhile, light drought stress did not change the timing of BB in all clones except 'Slava Ukrainy'. The results obtained agree with those of Signorelli et al. (2022), where the authors demonstrated that water availability directly and strongly affected the bud burst rate in *Vitis vinifera* when provided in the range of 35 to 85% of field capacity. From 85% of field capacity, the BB rate became independent of water content. Conversely, at extremely low water levels (<35% of field capacity), BB did not occur (Signorelli et al., 2022).

The most unexpected result of the current study was the slower bud development in control willows compared to control poplars. Most likely, because of this delay, we did not observe the effects of drought stress on the bud development in willows. In contrast, in our previous study at the experimental plot, we observed that buds in willows developed faster than those in poplars (Khoma & Kutsokon, 2019). By combining these studies in outdoor and indoor conditions, we will attempt to explain why we observed no dependencies between the timing of bud development and water deficiency in willows.

It is assumed that "temperature and stress factors may variably interact with photoperiod in controlling the timings of phenological events in woody species, including willow and poplar" (Orlandi et al., 2020). Therefore, we suppose that the light regime, an important factor for BB initiation (Ghelardini et al., 2014; Orlandi et al., 2020), was limited in our experiment in the laboratory space with an unregulated environment. This probably had a more substantial impact on the willows, masking the effects of the drought treatment. Furthermore, we should also mention that the winter chilling requirement could be another driver for successful BB initiation (Orlandi et al., 2020), as winter warming significantly delayed bud burst in *P. cathayana* (Yu et al., 2018). Inadequate chilling could slow both the rate and speed of bud burst in birch and aspen trees (Pletsers et al., 2015). In our experiment, potted plants were stored under an average temperature of 5–7 °C during the period of dormancy, which, perhaps, may not be sufficient for the chilling threshold of willows.

Compared to poplars, willows are generally more adapted to colder climates (Bonosi et al., 2013). Murray et al. (1989), analysing the BB of 15 tree species in Great Britain, discovered that *P. trichocarpa* and *Crataegus monogyna* had the lowest level of dormancy, i.e. shorter thermal time to bud burst and lower chilling requirement than *S. viminalis*. Although reduced light exposure and insufficient chilling may have influenced bud developments in willows, these factors were not explicitly measured in this study, and further research is needed to determine their precise effects.

Interactions between various factors can make predicting tree responses and performance difficult. For example, a study by Inoue et al. (2020) showed that in trembling aspen *P. tremuloides*, a longer photoperiod extended the growing season by delaying autumn phenology, while water stress significantly reduced growth. In this case, photoperiod signals would extend the growing season, but water limitation would constrain the potential growth response. However, the combined effects of multiple factors are often poorly understood.

Because of the supposed "neutralization" of the impact of drought treatment on bud development in willows by unaccounted factors, such as light and chilling requirements, we could only estimate the drought tolerance for poplar clones. Thus, the clone 'Volosystoplidna' was the most sensitive to the drought. All other poplar clones also suffered from water deficiency, mainly from severe stress, although to a lesser degree. It is difficult to determine the reasons for the differences in drought tolerance between the clones as we did not assess these mechanisms in the current work. However, the increased drought tolerance are commonly linked with the drought-adaptive abilities such as increased root growth, reduced leaf area, control of transpiration through the regulation of stomatal conductance, deposition of cuticular waxes to limit non-stomatal transpiration etc. under the control of signalling pathways and drought-responsive genes (Larchevêque et al., 2011; Himes et al., 2021; Polle et al., 2019; Rosso et al., 2023).

In another Ukrainian study, clones 'Strilopodibna' and 'Gulliver' were classified as having low drought tolerance according to summarizing of several physiological indicators measured under laboratory conditions (leaf tissue water content, water deficit, leaf water-holding capacity, and electrical conductivity) (Vysotska, 2023). However, the research also reported variability in their tolerance levels, from higher to lower, depending on the specific parameter assessed (Vysotska, 2023). In our study, clone 'Strilopodibna' also exhibited low drought tolerance, whereas clone 'Gulliver' demonstrated higher drought tolerance in terms of bud development. These findings suggest that drought response may be trait-dependent and vary between physiological and phenological indicators.

For many plant species, phenology is known to be a variable trait with a high degree of heritability. Studying the leaf phenology of 17 poplar clones, Pellis et al. (2004) found that the same clones had a significantly earlier or later bud burst than other clones almost every year. Estimation of 27 larch clones also demonstrated clear differences in their bud development (Sierota et al., 2017). Ishchuk et al. (2020) defined three phenological groups of poplar in terms of BB. They discovered that "bud burst and leaf emergence was recorded in the second half of April in correspondence with the accumulation of a temperature sum of 45–118 °C, depending on the species".

We suppose that the differences in drought tolerance of various clones may be negatively linked with their productivity. Results from our previous study (Kutsokon et al., 2022) demonstrates, that in term of stem diameter (during the first year) clones 'Gulliver', 'Novoberlinska-3', and 'Slava Ukrainy' were among the less actively growing in nursery. In the current study, these three clones were more drought-tolerant, demonstrating the fastest "recovery" of bud development after drought stress as they reached stage 6 at the end of the scoring. On the contrary, clones 'Kanadska × balsamichna', 'Volosystoplidna', and 'Strilopodibna were among the most actively growing in the nursery. However, in the current study, these three clones were more drought-sensitive, demonstrating delayed bud development after drought stress. We suspect that the reason for such delay in the most actively growing clones are that they are more demanding for water and optimal growth conditions. It may be caused by the decline in photosynthetic activity under drought stress and even damage to the photosynthetic apparatus under severe stress, as demonstrated in poplar (Brilli et al., 2007; Liang et al., 2019). However, photosynthetic activity likely has the most significant biological importance at the later stages of bud development (Sierota et al., 2017).

Similar results were determined by Larchevêque et al. (2011) when studying potted *P. balsamifera* and its two hybrids. They identified that the hybrid with the highest biomass production had a riskier strategy because it kept its stomata open under drought conditions. Likewise, more productive genotypes of *P. deltoides* × *P. nigra* demonstrated lower drought tolerance and a decrease in biomass under drought, while less productive clones exhibited a wider range of stress tolerance (Monclus et al., 2006). Similarly, Vander Mijnsbrugge et al. (2016) showed that smaller plants of *Q. petraea* have a higher chance of survival. Therefore, even the most drought-tolerant clones may not achieve high productivity under either well-watered or drought conditions, but they are likely to survive severe drought climates better than clones that are highly productive under optimal conditions. Due to this, high variability among the plants may be advantageous (Vander Mijnsbrugge et al., 2016), as smaller plants will survive harsh summer conditions, and bigger plants will benefit in the years with higher precipitation.

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