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The influence of growing medium composition on pine and birch seedling response during the period of simulated spring drought

Ondřej Špulák, Jana Hacurová*

Forestry and Game Management Research Institute, Research Station at Opočno, Opočno

**Corresponding author: spulak@vulhmop.cz*

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Abstract: Climate change increases the earliness and effect of spring dry spells. The efforts to reduce their negative effects on tree regeneration include improvement in physical properties of soil aimed at an increase in water retention and availability. Clinoptilolite from the group of zeolites belongs among such water-absorbent natural materials. The article aims to assess the effect of clinoptilolite admixture in a growing medium, along with different fertilizer concentration, on the growth of pine and birch seedlings and their development during simulated drought. A common (reference) growing medium was tested along with 4 treatments of the growing medium with clinoptilolite. The birch responded to the fertilizer concentration more intensively than to the clinoptilolite admixture and was more vulnerable to drought damage due to a closer dependence of physiological responses on a decrease in the growing medium moisture. The onset of pine withering was slowest in the reference growing medium. A positive effect of clinoptilolite addition on the growth of pine seedlings was confirmed but its unambiguously positive effect on overcoming of spring dry spells was not demonstrated. Clinoptilolite addition with higher fertilizer rate was found as fully unsuitable.

Keywords: volume moisture; growth; flushing; withering; clinoptilolite; zeolite, dry spells

Along with climate extremities of the last decades, accompanied mainly by irregular distribution of precipitation and rising average temperatures (IPCC 2018) causing an increase in potential evaporation, the importance of water in landscape has been enhanced. Recurrent low amounts of winter precipitation increase the earliness and effect of spring droughts. They become the increasingly frequent limiting factor of forest regeneration in many regions.

Drought (moisture deficit) is the essential limiting factor of physiological processes, plant growth and development because it obviously diminishes the cell production (Gruber et al. 2010; Balducci et al. 2016). It is a condition when soil particles hold the remaining water too tightly to be gained by plants which are dying (Singer, Munns 1996). Severe drought is the disruption of the capillary continuum from the soil to the root because of soil dehydration, which may lead to tree death (Körner 2019). After the soil has dried out, the insufficiently saturated root partially shrinks, which substantially disturbs the root contact with surrounding soil. Physiological drought is often considered a trigger of forest decline. Evidently, under the influence of water deficit trees substantially shorten their growth period and their increment is low, so they do not fulfil their growth potential (Eilmann et al. 2011).

The efforts to decrease the negative impact of drought and spring dry spells on the prosperity

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of artificial regeneration comprise the choice of more resistant tree species, changes in silvicultural techniques of production and planting of artificially regenerated seedlings as well as of site preparation (Linder et al. 2008; Clark et al. 2016). Changes in silvicultural techniques include also the management of soil physical properties to increase its precipitation water retention capacity and therefore water availability to plants of forest regeneration.

The character of the soil substrate (the ratio of components with different porosity and specific gravity) determines physical properties and essentially impacts soil moisture constants (field capacity and permanent wilting point, etc.). It directly influences the moisture regime of plants, hence their capacity to overcome the stages of drought. One of the common methods of soil management is an increase in the proportion of water-absorbent materials with the large inner surface. Water-holding additives to soil, both of natural and synthetic origin, are more intensively tested in agriculture (de Campos Bernardi et al. 2016; Kavvadias et al. 2018; Saha et al. 2020; Islam et al. 2021). However also in forestry research, e.g. positive effects of hydroabsorbent application on seedling production and growth after planting were proved (Ayan 2002; Hueso-González et al. 2016).

Zeolites belong among such natural minerals, i.e. crystalline hydrated aluminosilicates of alkali metals and alkaline earth metals. Thanks to the specific spatial structure of atoms they contain channels and cavities in which matters of all three states (gaseous/liquid/solid) can be intercepted. This large group of minerals is used e.g. to improve some properties of soil in agriculture (Reháková et al. 2004; Girijaveni et al. 2018; Mahesh et al. 2018). Because of their adsorption capacities, zeolites can also be used to remove pollutants and to improve properties such as pH, permeability and structural stability (Sakadevan, Bavor 1998; Girijaveni et al. 2018). One of the above-mentioned effects is higher water retention, which is beneficial especially in sandy soils with the naturally low retention capacity (Shahnazari et al. 2020). Due to large deposits in Slovakia, clinoptilolite is one of the most easily accessible zeolites in Central Europe (e.g. Földesová, Hudec 2007). In its microcrystalline structure it contains a higher proportion of Si.

Experiments with the use of zeolites were conducted also in the forest nursery sector (e.g. Ayan et al. 2008; Tilki, Memisoglu 2014). It was found that zeolite has many good features that make it very attractive for nursery use as growing media over others (Ayan, Tüfekçioğlu 2006). Some knowledge is available concerning synthetic water-holding additives and simulated drought effects on forest tree seedlings: for example Jamnická et al. (2013) confirmed that an amendment of hydrophilic polymer into growing media significantly improved the photosynthetic performance of drought-stressed beech seedlings. However, the specific importance of clinoptilolite for application and availability of nutrients and for the regulation of the water regime of growing media and forest soils for particular tree species in a drought period has not been tested in greater detail in the forest sector.

The objective of the experiment is (1) to assess the effect of clinoptilolite admixture to a growing medium along with the application of various concentrations of fertilizer on the growth of pine and birch plants and (2) to evaluate the development of these plants during simulated drought (imitating dry condition after outplanting in a forest site) in the context of changes in the volumetric water content of growing medium treatments at the onset of bud flushing (a) and in the late stage of bud flushing (b).

MATERIAL AND METHODS

Experimental growing media and tree species. An experiment was started in spring 2019. Five treatments of growing media were created combining peat, clinoptilolite (fraction 0.5–1 mm) and perlite at a chosen volume ratio and Kristalon fertilizer (Superior Soluble; N, P, K, B, Cu, Mn, Mo, Zn) at two concentrations (Table 1). Growing medium I (common composition used in forest nurseries) was considered as a reference one. These growing media were filled into QP 40T/11.5 trays with the cell volume of 220 mL. Bare-rooted pine seedlings (one year old cultivated in a plastic greenhouse; average height 6.1 cm; average root basal diameter 1.8 mm) using 40 plants per treatment and container-grown birch seedlings (grown for six months in QP 144/6 trays in a plastic greenhouse; average height 5.8 cm; average root collar diameter 1.2 mm) using 100 plants per treatment were transplanted into these growing media. Each tray was individually labelled according to the treatment and placed under supplementary irrigation in the experimental forest nursery of the Forestry and Game Management Research Institute in Opočno.

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Table 1. Composition of tested growing media; growing medium I (common composition) was considered as a reference one

Treatment	Composition	Volume ratio
I	peat + perlite + Kristalon	5 : 1 : Com
II	peat + perlite + Kristalon + clinoptilolite	5 : 1 : Com : 1
III	peat + perlite + Com clinoptilolite	5 : 1: 1
IV	peat + perlite + High clinoptilolite	5 : 1: 1
V	peat + perlite + clinoptilolite	5 : 1: 1

Com – common (recommended) rate of Kristalon (i.e. 10 g per 10 L of moisturizing solution); Com clinoptilolite – clinoptilolite soaked for 24 h before the preparation of growing medium in a Kristalon solution of common concentration (i.e. 10 g per 10 L of solution), mixing moisturized solution with pure water only; High – clinoptilolite soaked for 24 h before the preparation of growing medium in a Kristalon solution of high (fivefold) concentration, mixing with pure water only

Height (in cm) and root collar diameter (in mm) of pine and birch seedlings were measured in autumn 2019. All individuals were measured. The height and root collar diameter (RCD) increments of the tree species growing in the particular treatments of growing medium were statistically compared by Welch's ANOVA followed by Tukey's test. A significance level 0.95 (α = 0.05) was used across the whole study.

Simulated spring dry spells. Experiment 1 simulating the effect of rainless period on tree species was conducted before the onset of bud flushing while during experiment 2 the tree species were subjected to drought in the advanced stage of bud flushing.

Experiment 1 started in January 2020. A tray with 20 seedlings of each tree species was transferred from the outdoor location to a laboratory. To ensure more repetitions of gravimetric measurements (see below) the trays were divided into 2 segments of 4 cells and 2 segments of 6 cells (plants). The growing media were saturated with water by placing them for 20 h into crates with the bottom size of 60 × 40 cm and the water level of 5 cm, each tray was saturated individually. Compared to saturation from above (intensive watering), this method minimized the risk of nutrient leaching from the growing medium and the influence of overmoistening (waterlogging) on the root vitality. After unbound water dripped (4 h), the input weighing of particular tray segments was done (the value was used to calculate the maximum volumetric water content reached) and subsequently according to the previously chosen scheme they were placed into a Fitotron plant growth chamber (Weiss, the United Kingdom) on 18th January 2020. The regime of 12-h alternation of day and night was used. The temperatures of 20 °C (day) and 10 °C (night) were set for 10 days, then they were increased to 23 °C and 13 °C to accelerate the onset of bud flushing and effect of drying out. The module of air humidity control was not available. The seedlings were left without watering. Air temperature and air humidity were measured with external sensors in the upper and lower part of the plant growth chamber (DN4000, Ing. Libor Daneš, the Czech Republic). The experiment in the chamber lasted for 35 days in total.

A higher diurnal temperature amplitude was detected in the upper part of the plant growth chamber than in its lower part (by 3.8–4.5 °C) in both experiments. Relative air humidity was generally high, more than 85% during the night at the beginning and ca. 80% at the end of experiments. During the day, the air humidity was lowered by 31% on average in the upper part of the chamber and by ca. 15% in the lower part as compared to the night values. In both experiments a difference between daytime and nighttime air humidity gradually increased. The drying out of growing media was completed in a laboratory at an average temperature of 21 °C and air humidity of 37%.

The analyses include weighing of tray segments and registration of the degree of seedling bud flushing (according to an empirical scale, Table 2) and withering (according to the scale 0 – no symptoms of withering; 1 – withering, but with revitalization potential; 2 – dry, without revitalization potential). The above-mentioned attributes were evaluated at the interval of 1–2 days at the same hour, except weekends. After each measurement the position of the treatments in the plant growth chamber was changed according to a cyclical scheme to compensate for the influence of assumed air temperature and air humidity differences in the chamber interior.

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Table 2. Empirical scales of pine and birch bud flushing – description and flushing index

Species	State	Index
Pine	not flushing	0
	bud swelling	1
	bud burst beginning (green needles clearly showing through the bud scales)	2
	advanced bud burst (confined bundle of needles growing from buds)	3
	elongation beginning (open bundle of needles, beginning of their extension growth)	4
	elongation growth of new flushes	5
Birch	not flushing	0
	bud swelling	1
	leaf flushing	2
	leaf opening	3

Experiment 2 aimed at the effect of dry spell on the advanced stages of bud flushing that began on 10th February 2020. The identical number of plants, growing medium treatments and tree species mentioned above, including the division of trays into segments, was located in a plant growth room at a temperature of around 25 °C and relative air humidity of ca. 65%, with 12-h alternation of day and night and regular watering. Bud flushing stages (Table 2) of both tree species was evaluated. After a fortnight, the growing media were saturated with water and the input weighing of tray segments was done according to the identical method (see above). On 26th February 2020 the trays were placed into a plant growth chamber (23 °C and 13 °C; day and night temperature regime, respectively), without irrigation, and regular weighing of tray segments was started including the subsequent cyclical change of the position in the chamber. Evaluation of bud flushing and degree of plant withering was continued. The experiment in the plant growth chamber lasted also for 35 days.

The experiments ended by spontaneous drying out of growing media in laboratory conditions. The weight of the growing medium dried in this way was used to calculate volumetric water content ($g \cdot cm^{-3}$) on the particular dates of measurement.

RESULTS

Tree species growth. At the end of the growing season (2019), significantly lower increments of height and of root collar diameter of pine seedlings were confirmed in the treatments of growing medium I (reference; see Table 1) and V in comparison with the other growing media (Table 3). The increment of birch seedlings was related more obviously with the presence and concentration of the initial fertilizer rate. The significantly lowest growth was observed in birch plants in growing medium V (without fertilizer addition), while the most intensive increment with respect to root collar diameter was confirmed in the reference growing medium (treatment I; Table 3).

Experiments of drought simulation. Bud flushing and withering of pine and birch seedlings. Birch flushing was earlier and faster compared to pines in both experiments. In experiment 1 both tested

Table 3. Height increment and root collar diameter (RCD) increment of pine and birch seedlings after one year of experimental cultivation in relation to growing media treatments (for treatment explanation see Table 1)

Tree species	Treatment	Increment of height (cm)		Root collar diameter (cm)	
		mean	SD	mean	SD
Pine	*P*	< 0.001		< 0.001	
	I	6.9[a]	0.4	0.78[a]	0.11
	II	8.3[c]	1.0	1.06[b]	0.18
	III	7.6[b]	0.9	1.06[b]	0.20
	IV	7.9[bc]	1.1	1.11[b]	0.18
	V	6.9[a]	0.8	0.88[a]	0.17
Birch	*P*	< 0.001		< 0.001	
	I	14.3[c]	4.6	2.63[c]	0.58
	II	14.9[c]	4.4	2.27[b]	0.39
	III	10.0[b]	2.9	2.18[b]	0.40
	IV	14.9[c]	4.4	2.33[b]	0.39
	V	7.5[a]	2.1	1.94[a]	0.41

SD – standard deviation; a, b, c – statistically heterogeneous groups of data for a tree species

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species flushed most intensively on growing medium II. Pine flushing was the slowest in treatment IV, also flushing of birches in treatments IV and III lagged behind (Figure 1). During experiment 1 the flushing cycle was completed in birches. Unlike them the flushing of pine was strongly limited by gradual withering (Figure 2).

In experiment 2, when the planting stock was flushed in the plant growth room under full watering before drying out was started, the situation was different. Pines of treatment IV flushed faster at first, but delayed flushing of buds was observed on reference growing medium I (Figure 1). From the beginning of March, however, differences between treatments were small. The bud flush of birches grown in different growing medium treatments was very balanced from the beginning, only in the final stage of flushing in the second half of February the delayed development was observed in growing medium III (Figure 1). Birches, with the exception of several individuals from treatment III, completely flushed in the plant growth room before they were placed in the plant growth chamber.

In experiment 1 the most satisfactory development in relation to withering of both tree species was observed in the reference growing medium (Figure 2). Differences between treatments were greater in birch compared to pine when the most intensive withering was observed in treatment IV and also in treatment II.

In experiment 2 the beginning of pine withering was later than in birches. Pine withering was slower in the reference growing medium, while differences in withering of the other treatments were low. Birch withering, similarly like in experiment 1, was faster in growing medium IV, the best development was found in treatment V.

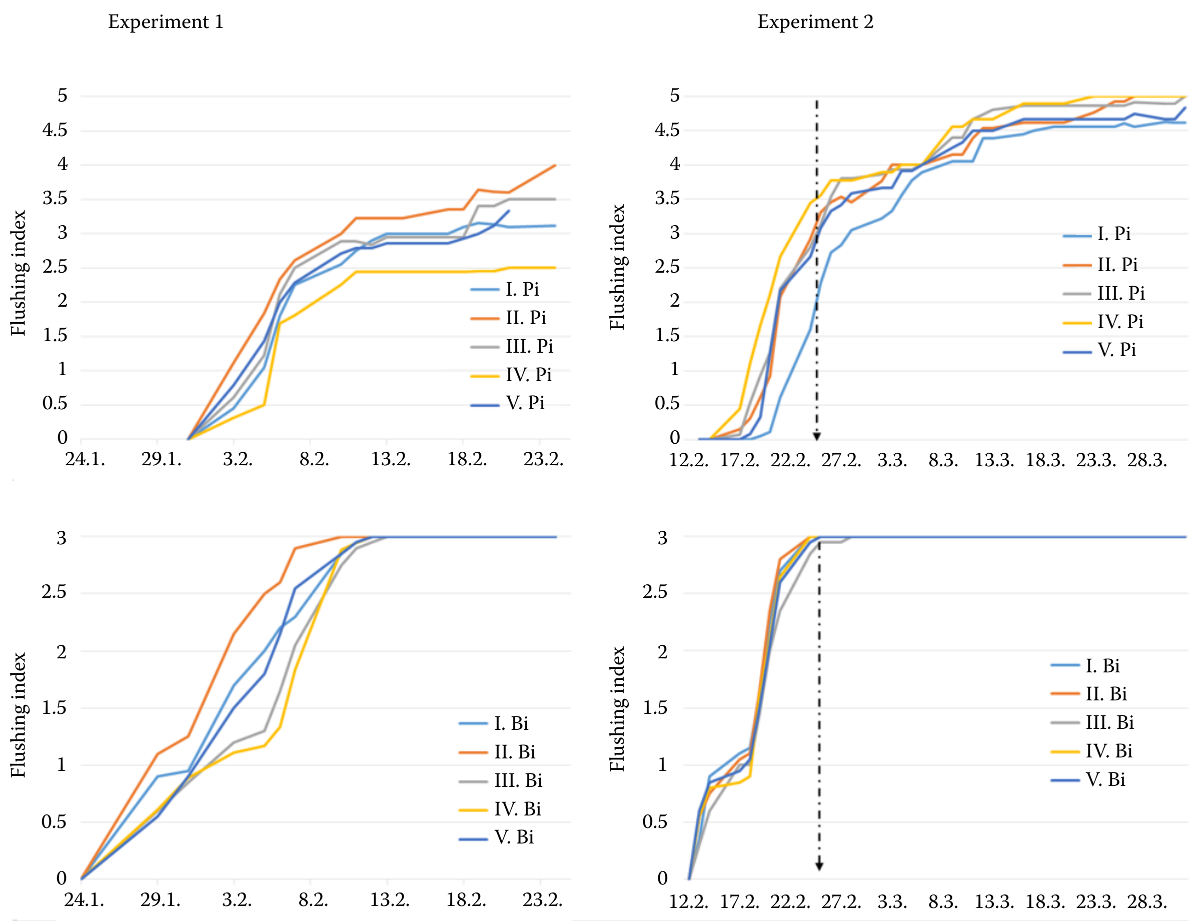

Figure 1. Average bud flushing of pines (Pi, upper graphs) and birches (Bi, lower graphs) according to treatments in experiment 1 (left) and 2 (right); the arrow in experiment 2 indicates the date of transfer from the plant growth room to the plant growth chamber (for treatment explanation see Table 1; for the empirical scale see Table 2)

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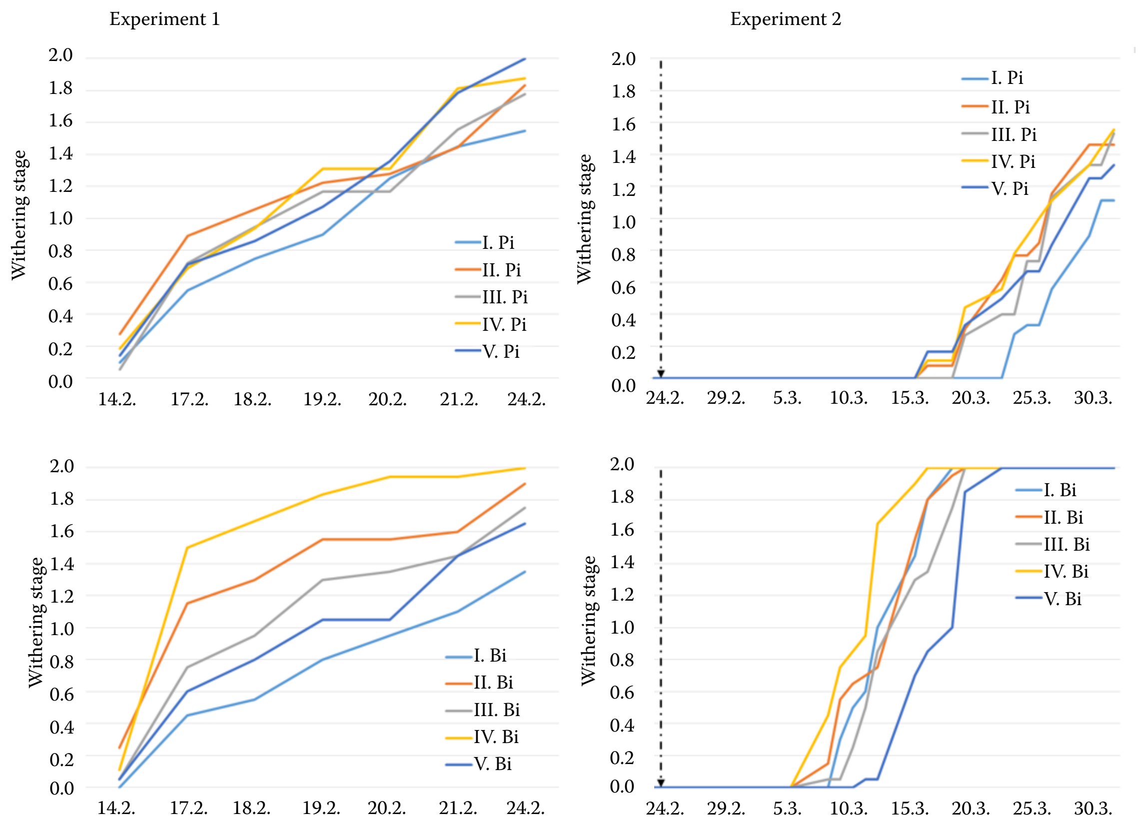

Figure 2. Average withering of pines (Pi, upper graphs) and birches (Bi, lower graphs) according to treatments in experiment 1 (left) and 2 (right); in experiment 2 only the period after transfer to the plant growth chamber is illustrated (for treatment explanation see Table 1)

0 – not withering; 1 – withering, but revitalization is possible; 2 – dry, revitalization is not possible

Development of volumetric water content of growing media. In both tree species the lowest values were usually measured in growing media III and IV, with the exception of growing medium III in birch in experiment 2. Growing medium II in both experiments had the highest values of maximum volumetric water content reached in birch while in pine the maximum water content was reached in growing medium I and II in experiment 1, and in growing medium V in experiment 2 (Table 4). In pine significance of the differences in maximum volumetric water content of growing media reached after saturation disappeared during a short period of several days, in birch it lasted longer.

In experiment 1, the progress of volumetric water content of reference growing medium I was similar in pines and birches. In experiment 2 the complete bud flushing of birches led to a significantly faster moisture loss of reference growing medium (I) already at the beginning of the environment-controlled phase compared to pine (Figure 3).

Table 4. Maximum volumetric water content reached after saturation of the growing media in experiment 1 and experiment 2 ($g \cdot cm^{-3}$) (for treatment explanation see Table 1)

Tree species	Treatment	Experiment 1		Experiment 2	
		mean	SD	mean	SD
Pine	*P*	0.01		0.03	
	I	0.689^{b}	0.015	0.693^{ab}	0.012
	II	0.690^{b}	0.015	0.685^{ab}	0.018
	III	0.636^{a}	0.008	0.683^{a}	0.005
	IV	0.656^{ab}	0.021	0.681^{a}	0.010
	V	0.661^{ab}	0.027	0.715^{b}	0.013
Birch	*P*	< 0.001		< 0.001	
	I	0.642^{b}	0.012	0.671^{bc}	0.024
	II	0.690^{c}	0.005	0.702^{c}	0.012
	III	0.583^{a}	0.013	0.671^{bc}	0.008
	IV	0.585^{a}	0.010	0.626^{a}	0.007
	V	0.618^{ab}	0.034	0.655^{ab}	0.014

SD – standard deviation; a, b, c – statistically heterogeneous groups of data for a tree species

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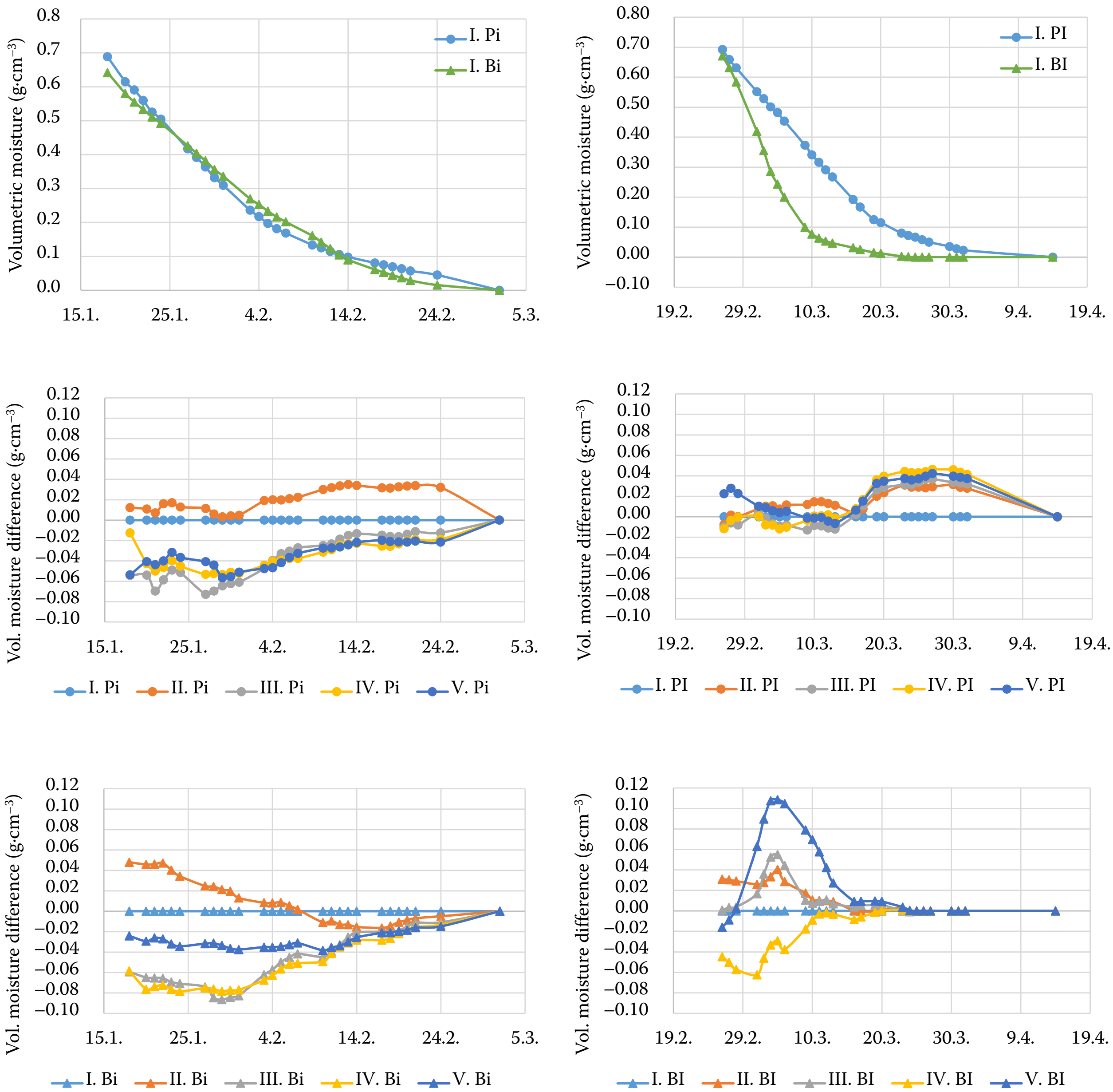

Figure 3. Volumetric soil moisture content of the growing media in the plant growth chamber; reference growing media (I) above, differences in volumetric moisture content of growing media II – V from the reference one for pine in the middle and for birch in the bottom (< 0 indicate higher volumetric water content compared to the reference growing medium), symbols represent the dates of measurements (for treatment explanation see Table 1)

The pattern of differences in volumetric water content bvriments. In experiment 1 the volumetric water content of the reference growing medium of both tree species was higher than the water content of the other growing media except growing medium II (in birch only until the middle of the studied period). In experiment 2 water content of the respective reference growing medium was mostly lower in comparison with the other media, except growing medium IV in birch. The water content pattern of growing medium V in birch in experiment 2 was very different from the other treatments (Figure 3).

DISCUSSION

The observed effect of clinoptilolite admixture to the growing medium on either pine or birch

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growth in the growing season 2019 was not direct – a significant growth improvement seems to be always connected with the presence of fertilizer. Based on preceding studies evaluating the effects of zeolites in agriculture (e.g. Reháková et al. 2004; Sakadevan, Bavor 1998; Girijaveni et al. 2018) it was expected that the clinoptilolite admixture would improve both its hydric properties (water holding capacity, field capacity, porosity) and nutrient availability (Girijaveni et al. 2018) and these would directly influence the growth and morphological parameters of plants. Tilki and Memisoglu (2014) also reported no significant differences between the growing media in the diameter growth of Scots pine. And Ayan and Tüfekçioğlu (2006) demonstrated the lowest height growth in the growing media with zeolite (20%), without extra addition of fertilizer, which is in line with our study, when birch grew most slowly in treatment V with no fertilizer. Ayan and Tüfekçioğlu (2006) also observed that root collar diameter differed significantly with types of growing media and was also significantly lower in the growing medium with zeolite added. Clinoptilolite had to be coupled with fertilization in our study to increase the growth of both species during the first year of cultivation.

Relatively small differences in growth, despite of their statistical significance, suggest that under suitable moisture conditions the differences between the tested treatments of growing medium are not crucial for the growth of pine seedlings. In our experiment birch showed better growth in fertilizer-enriched growing media with optimum moisture regime. But in the drought period the growing medium enriched with fertilizer (IV) appeared to be the most limiting in both tree species. In this growing medium bud flushing was delayed in birch and the withering of both tree species was accelerated at the same time. If the growing medium is not supplied enough water, the plants are not able to efficiently utilize all nutrients contained in it because nutrient availability and their translocation under limited water availability are reduced (Girijaveni et al. 2018). The interaction between water and nutrients affects tree growth and survival. Water and nutrient relations are tightly bound (Pallardy 2008). In general, higher nutrient availability leads to the poor development of tree species roots, which is a disadvantage in comparison with much better developed root systems on poorer soils. Gessler et al. (2016) concluded that the features of tree species that developed at a nutrient rich site underlie a higher risk of embolism due to the imbalance in reduced water availability and need.

In spite of the forced air circulation there were some differences in the actual temperature and moisture in the plant growth chamber within its interior. The reason will be the location of the environment-control unit controlling temperature in the upper part of the chamber; the effect of an external temperature of the laboratory that was usually higher than the controlled night temperatures also played its role and could influence the air stratification. Therefore the successive change of positions of experimental treatments during the experiment was crucial for an adequate course of the experiment. By the end of both experiments air humidity in the chamber reached relatively high values (ca. 80% during the night and 50% during the day), which made the conditions of the plant growth chamber more favourable compared to the real properties of a clearcut during spring dry spells where the air humidity can drop to 20% by day (Chen et al. 1993, Aussenac 2000). This difference can be caused by the absence of a continuous exchange of air between the growth chamber and ambient air (wind). So the results reported in this study would probably be reached in a shorter period and consequences of drought would be more pronounced in the field conditions.

In both experiments the bud flushing was earlier in birch. The observed earlier flushing of birch compared to pine is not regular. For example, Schmitt et al. (2004) evaluated the phenology of mixed mature stands of pine and birch in Finland and described the later onset of pine bud flushing by 1–6 days depending on the site. During experiment 1 (drought before the flush onset) no new shoots of the pines completed full flushing. On the contrary, if the drought stage occurred at the time of expanded buds (experiment 2), in spite of transition to a rainless regime, full elongation of buds was observed on 80% of surviving pines. It is possible that the plant was able to make a certain reserve of water: e.g., Bréda and Granier (1996) described some amount of stored stem water in the plant at the beginning of the vegetation period. The slower onset of bud flushing in pine that increased the water requirement for transpiration more slowly, could also decelerate pine responses to the growing medium composition.

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Comparison of the maximum volumetric water content of growing media reached after saturation did not indicate an unambiguous improvement in hydric properties (e.g., water holding capacity) and functioning of the growing medium after clinoptilolite addition. The imbalanced results may be influenced by the operating volume method of tray filling. Changes in the growing medium during winterization, and also the specific development of tree species roots in trays could be other sources of differences. The described facts could lead to an increase in the variability of the growing medium porosity (Singer, Munns 1996). Capillary conductivity of the growing medium with clinoptilolite will probably be lower than in the growing medium with higher peat content.

A decrease in the volumetric water content of growing media reflected the bud flushing of both tree species while it was related with withering especially in birch. If birch was exposed to a rainless period before the end of dormancy (experiment 1), its pattern of withering was similar like in pine but it was more sensitive to the growing medium composition. Drought after bud flush (experiment 2) accelerated birch withering compared to pine by about 10 days and the former tree species started dying after ca. a fortnight without watering. An increased water demand for transpiration during the leaf area expansion (Bréda, Granier 1996) led to a faster drop of water content in growing media. This finding is consistent with the fact that in experiment 2 the slower growth of birch (hence smaller leaf area) in growing medium V poor in nutrients (Table 3) resulted in a lower decrease in volumetric water content with a positive impact on the delay of birch withering. Unlike conifers, broadleaved species do not interrupt either gas exchange or growth in drought (e.g. Leuschner et al. 2001; Leuschner 2009). In pine the differences in the rate of withering between treatments were minimal. Conifers are tree species with isohydric strategy (Lyr et al. 1992; Zweifel et al. 2009; Pretzsch et al. 2014), their photosynthesis is constrained by unfavourable moisture regimes because they respond to drought by stomatal closure (Waring, Franklin 1979). Conifers (especially pine) were proved to be able to compensate climatic constrictions becoming more adapted to survival in drought-prone environments (del Castillo et al. 2018). The isohydric response is at first advantageous (in short-time drought). But in long-term drought the conifers become more vulnerable to drought stress than broadleaves because they are exposed to the risk of organism overheating or starvation (Lyr et al. 1992; Zweifel et al. 2009).

CONCLUSION

The experiment revealed that the response of birch growth and withering to the fertilizer concentration was more obvious than to the clinoptilolite admixture. Birch bud flushing was earlier and faster with small differences between the growing media. But in general, birch was more vulnerable to drought damage due to a closer relationship between its flushing, growth and decrease in the moisture content of growing medium. The effect of the combination of clinoptilolite with higher fertilizer rate on birch withering during drought stress was negative. Bud flushing in pine was less balanced between the growing media, and clinoptilolite admixture in the growing medium rather accelerated the bud flush. The slowest pine withering was observed in the reference growing medium without clinoptilolite. Reduction of volumetric water content in growing media responded to the stage of flushing when the experiment of dry period was applied.

In the experiment the positive effect of clinoptilolite addition on the growth of pine seedlings was confirmed but the positive effect of clinoptilolite admixture in the growing medium on overcoming the episode of spring drought in pine and birch seedlings/plants was not demonstrated unambiguously. However, its negative effect was observed only in the combination of clinoptilolite admixture with higher fertilizer rate.

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