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Growth performance and resistance to ground late frosts of *Fagus sylvatica* L. plantation treated with a brassinosteroid compound

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Abstract

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We measured the initial growth performance of a young plantation of European beech treated with a brassinosteroid compound prior to planting and compared it with a control treatment: we assessed heights, root collar diameters and mortality rate during the period 2012–2015. The trees showed posterior damage by ground frost after a substantial late frost event on the night of 4–5 May 2014. Therefore, we evaluated the post-stress vitality of trees, subsequent height increment from spring to August, and the height range of the damage.

Mean height, root collar diameter, and mortality rate did not show any significantly better performance in the brassinosteroid treatment over the control treatment. Neither did the application treatment have a significant positive effect on the resistance of beech to late frosts. The severe frost damage was most intense at 30 cm above ground, and rapidly declined with increased height.

Keywords: European beech; brassinolide; frost events; vitality of trees; forest plantations

European beech (*Fagus sylvatica* Linnaeus) is a widely distributed forest tree species. With around 8% of the reduced forest area in the Czech Republic (Ministry of Agriculture of the Czech Republic 2015), it is the most common of all broadleaved forest tree species; it is also commercially important. Beech is nowadays used as an ameliorative species in coniferous forest stands and also for reforestation of abandoned agricultural lands (Podrázský, Remeš 2007, 2008; Holubík et al. 2014). Therefore, artificial plantations of beech are very common. Beech serves as a stand stabilizing species, and an addition of 10–20% in a mix is enough to significantly improve the stability of forest stands (Schütz et al. 2006). As a result, the forest area of beech in the Czech Republic has been steadily increasing in the last 10–20 years (Podrázský et al. 2014).

Brassinolides are plant hormones that were first isolated from *Brassica napus* Linnaeus pollen by Grove et al. (1979). There are many types of artificially synthesized analogues to the natural brassinolide which are called brassinosteroids – BRs (Back, Pharis 2003). Brassinolides are considered as prospective stimulants of performance and growth of plants (Bajguz 2011). They naturally occur in various parts of plants (Krishna 2003; Bajguz, Hayat 2009), and they are present in young tissues in higher concentrations (Rao et al. 2002). BRs also influence a range of physiological and morphological reactions in plants (e.g. Sasse 2003). Some experi-

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ments have shown that the application of BRs had positive effects on germination, growth, vitality and stress resistance of agricultural and woody plants, e.g. increased number of fruits in commercial yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa*) O. Degener (Gomes et al. 2006), enhancement of sorghum (*Sorghum vulgare* Persoon) seedling growth under osmotic stress (Vardhini, Rao 2003), cell elongation of rice (*Oryza sativa* Linnaeus) seedlings under low temperatures (Fujii, Saka 2001), seed germination and seedling growth enhancement of maize (*Zea mays* Linnaeus) (He et al. 1991; Singh et al. 2012) and cucumber (*Cucumis sativus* Linnaeus) (Khripach et al. 1999) under chilling stress, increased freezing tolerance of *Bromus inermis* Leysser (Wilen et al. 1995), and increased sugar beet (*Beta vulgaris* Linnaeus) yield (Hradecká et al. 2009). BRs were also reported to improve the seed germination of forest trees (Li et al. 2005) and promote the initiation of embryogenic tissue formation of coniferous trees (Pullman et al. 2003). Further, Khripach et al. (1999) suggested that the general ability of BRs to increase the resistance of plants to unfavourable environmental factors such as extreme temperatures, drought, salinity, or pesticides could be crucial for practical application.

In the conditions of Central Europe, late frosts are an abiotic factor causing substantial losses in forest plantations. The adverse effects of sub-zero temperatures are accentuated in late spring, when the young buds and leaves have not fully matured yet. The frost damage susceptibility differs among the tree species. For instance, the threshold when flushing spruce and pine buds are injured is around –3 and –6°C, respectively (Christersson, von Fircks 1988). European beech is generally known as a frost-sensitive species, although dormant winter buds are well-resistant to the Central European climate extremes, and even the mature foliage is considered to show fairly high freezing tolerance (Kreyling et al. 2012). There is only a short time period, directly after leaf flushing, when beech becomes highly sensitive to frost (Kreyling et al. 2012; Menzel et al. 2015; Lenz et al. 2016). Dittmar et al. (2006) specified the temperature threshold value of –3°C for late frost damage at the beginning of the vegetation period which can negatively affect the radial increment. It is apparent that any visible leaf damage which is able to regenerate and has no effect on the increment can appear also after minor frost. Furthermore, it is not only the absolute minimum temperature value, but also the duration of the frost event (temperature sum), which is important in determining the severity of frost injury (Langvall et al. 2001).

Extraordinary temperature extremes are primarily reached during clear (cloudless) nights and days (e.g. Oke 1970; Gallo et al. 2011; Leslie et al. 2014). The spatial distribution of low temperatures corresponds well with local terrain forms, and frost events are more frequent in so called frost hollows, i.e. shallow valleys and immersed basins (Lindkvist, Lindqvist 1997). The zone near the ground is particularly treacherous (Špulák, Balcar 2013). As a result, height and diameter increments deteriorate and mortality rate can increase (Kuneš et al. 2014). Some tree species are more susceptible to late frosts than others – beech is counted as predisposed (Strasser 2011) among other common broadleaves. The susceptibility increases following the cumulation of several negative factors such as drought and heat during the day (Geiger 1950; Steyrer 2011), and the sensitivity to late frosts varies across the species distribution range (Kreyling et al. 2012; Novotný et al. 2015).

The aim of this paper is:

(*i*) to assess mortality and growth (height and root collar diameter) performance of a European beech plantation treated before planting with a brassinosteroid compound: 2-alpha-3-alpha-17-beta-trihydroxy-5-alpha-androstan-6-one (BR);

(*ii*) to verify whether BR treatment improved the resistance of beech foliage to a late ground frost event in the second year after planting, and to study how it influenced vitality and growth performance of the trees in the subsequent vegetation period.

MATERIAL AND METHODS

The experimental design was similar to an analogous experiment with *Pinus sylvestris* Linnaeus, published by Nováková et al. (2014). The BR compound we used was a synthetically prepared analogue of naturally originated 24-epibrassinolide (Kohout et al. 2002, 2004). In October 2011, one part of the nursery seedbed with beech seedlings [two-year-old root-pruned planting stock from Provenance Region 10 – Central Bohemia (Decree No. 83/1996 Coll.), Forest Vegetation Zone 3 – *Quercus-Fagus* (Forest Management Institute 2003)] was treated with the BR compound. The application was done by mechanized spraying of BR solution on the top of the seedbed. The dose corresponded to 4 mg BR·ha^{-1}. Before application, the BR was dissolved in water with dimethyl sulfoxide. The other part of the field with beech seedlings was left untreated as a control.

In April 2012, the treated and control seedlings were lifted from the nursery and transplanted to

Table 1. Climatic data on the experimental plot measured by an automatic station

Year	Average annual temperature measured at different levels above ground (°C)			Sum of annual precipitation (mm)	Precipitation in vegetation period (mm)
	200 cm	100 cm	30 cm		
2013	8.8	8.9	8.4	747	535
2014	10.4	10.6	10.0	563	379
2015	10.6	10.7	10.2	451	199

the planting site. The experimental plot was situated at the Truba Research Station close to Kostelec nad Černými lesy, Czech Republic (50°0.36'N, 14°50.25'E, altitude 365 m a.s.l.). The surface was flat, the soil of a sandy-loam character, and the area was exposed to direct sunlight for most of the day. Generally, the stand conditions represented afforestation of abandoned agricultural land. The area was protected by a fence.

In 2013, an automatic meteorological station was installed in the immediate neighbourhood of the experimental plantation – LEC 3010 data logger (Libor Daneš, Czech Republic). The overview of basic climatic conditions in 2013–2015 is shown in Table 1. Precipitation during the whole year was measured by a standard non-heated tipping bucket rain gauge with the circular surface of 500 cm^2, i.e. diameter ≈ 25.3 cm. Precipitation in the vegetation period was calculated as the sum of daily precipitation in the period of April to September.

The experimental plantation was established at spacing of 1 × 1 m and the rows of the control and treated seedlings alternated. In total, 655 seedlings were planted, including 310 BR-treated and 345 control trees. Initial measurements of height and root collar diameter were performed in spring 2012 (initial values). Periodic measurements of height and root collar as well as records of mortality rates were taken annually in autumn 2012–2015 (after the vegetation period). Mortality rates were calculated as the percentage of dead seedlings related to the initial numbers of plants.

The plantation was affected by a late frost event during the clear and calm night of 4–5 May 2014, which coincided with the bud-break and unfolding of leaves. The detailed progress of temperatures is shown in Fig. 1.

Measurement to examine the frost damage was done one week after the event, after the leaves had fully opened and the damage was still obvious despite regeneration processes. For the purpose of the damage evaluation, the near-ground zone was divided into height levels of 5 cm intervals. We registered the level of the lower and upper height limits of the damage that was marked by burnt leaves and buds. The upper limit bounded the near-ground zone negatively affected by frost. The lower limit bounded the lowest area damaged, below which the tree was mostly protected by grass. The mean extent of damage was calculated as the difference between the upper and lower limits of the damage. The height of grass and herbaceous weed vegetation during the time of the frost event varied approximately between 20 and 40 cm. The density of weed vegetation was variable.

The seedling height was measured to the nearest 1 cm and vitality was assessed according to the scale published by Kuneš et al. (2011) (Table 2).

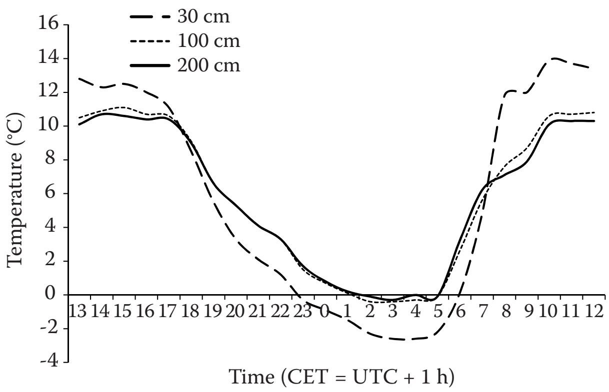

Fig. 1. Temperatures at three above-ground levels during the frost episode of 4–5 May 2014 on the experimental plot

Table 2. The scale for seedling vitality assessment in the early years after planting, according to Kuneš et al. (2011)

A	Excellent health status – the transplant shock overcome, marked height increment, full foliage, no damage
B	Good health status – revived height growth and good prospects of future development
C	Partially deteriorated health status – no significant height increment as a result of persisting transplant shock
D	Markedly deteriorated health status – defoliation, manifestations of serious transplant shock, uncertain survival prospects
E	Dead tree

Statistical analyses. The analysed characteristics included the data related only to trees which had survived until autumn 2015. Data relating to the trees already dead by autumn 2015 were excluded retrospectively. Some seriously damaged live trees (especially those injured by a brush cutter during weed cutting) were also excluded (10 trees).

The Fisher's exact test was applied for the comparison of mortality rates between treatments within a particular period of measurement. The χ^2 test was applied to find any differences in the distribution of trees within vitality classes between the two treatments. To test the increment between spring and autumn 2014 (after the frost event), the Wilcoxon signed-rank test was applied. The probability of damage in respective height zones was calculated as a number of damaged/number of undamaged trees for each height class individually.

RESULTS

Mortality rate

On all monitored dates, BR treated plants showed an insignificantly (P = 0.244) lower mortality rate than the control. The high initial mortality in summer 2012 can be ascribed to a transplant shock, as the plantation showed a gradual decrease in total mortality as the experiment progressed (Fig. 2), i.e. the increase of the cumulative mortality slowed down.

Height, root collar diameter

On all monitored dates, the mean height of the BR treatment insignificantly surpassed the control treatment in absolute numbers. Both treatments showed a stable increment (Fig. 3a). The differences in the periodical height increment from 2012 to 2015 were not significant (P = 0.127).

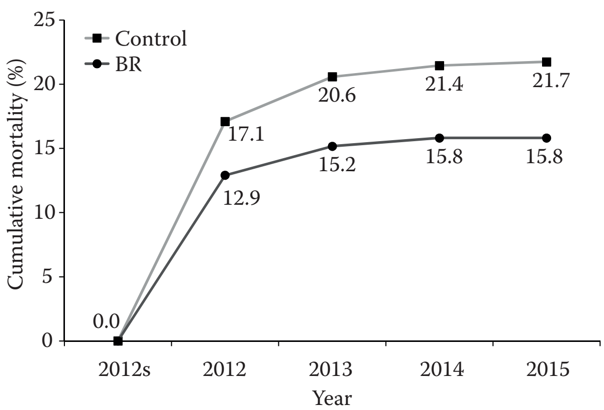

Fig. 2. Cumulative mortality rates of European beech seedlings recorded in spring 2012 (2012s) and autumn 2012–2015 in the brassinosteroid treatment (BR) and control

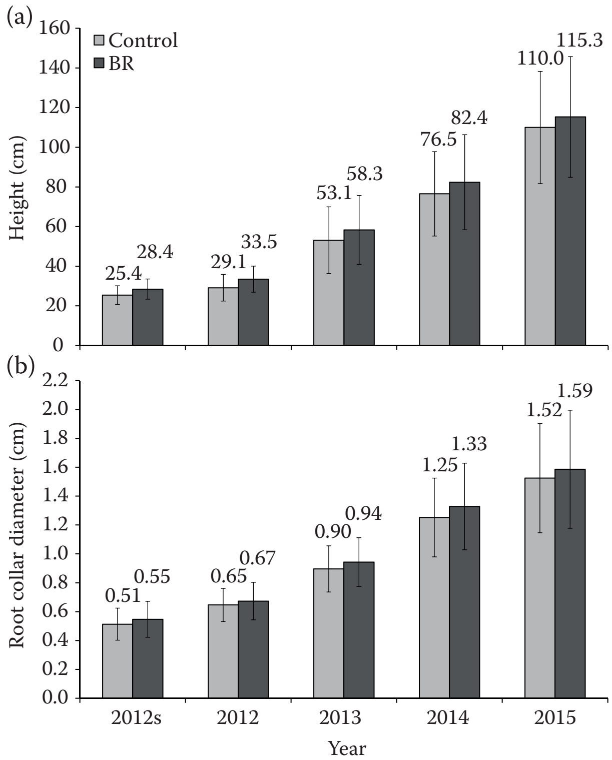

Fig. 3. Mean heights (a), means of root collar diameters (b) of European beech seedlings recorded in spring 2012 (2012s) and autumn 2012–2015 in the brassinosteroid treatment (BR) and control, error bars depict standard deviations

In the increment of mean root collar diameter (Fig. 3b), there was a pattern of an advance in the BR set, but the differences were insignificant (P = 0.434), as in the case of mean height.

Impact of the frost event

The duration of the frost episode was 7 hours at 30 cm above ground (Table 1). The minimum temperature reached –2.6°C and the sum of sub-zero hour temperatures was –12.4°C. At 100 cm above ground, the duration was 4 hours, the minimum temperature reached –0.4°C and the sum of sub-zero hour temperatures was –1.2°C. At 200 cm above ground, the duration was 4 hours, the minimum temperature –0.3°C, and the sum of sub-zero hour temperatures –0.5°C. The following day, the maximum temperature reached 17.7, 15.0 and 14.2°C at 30, 100, and 200 cm above ground, respectively. The pyranometer SG 005 (C.T.M. Praha, spol. s.r.o., Czech Republic) showed this was an almost clear to partly cloudy day. The values of solar irradiance were relatively high, corresponding to

Table 3. Characteristics of the experimental beech plantation after a frost event in the night of 4–5 May 2014

	Control	BR
Total number of trees	268	253
Number of intact trees	44	44
Number of damaged trees	224	209
Mean height of trees ± SD (cm)	61.3 ± 17.4	66.6 ± 18.9
Mean annual increment after frost event ± SD (cm)	16.3 ± 11.9	16.7 ± 12.7
Mean range of damage ± SD (cm)	31.2 ± 14.9	29.7 ± 14.9
Mean height of the lower limit of damage ± SD (cm)	11.6 ± 13.3	11.9 ± 13.0
Mean height of the upper limit of damage ± SD (cm)	42.8 ± 12.0	41.7 ± 12.1
Maximal range of damage (cm)	75	70

SD – standard deviation, BR – brassinosteroid treatment

direct sunlight (maximum 851 W·m^{-2} at 10:00), but fluctuated during the day due to clouds.

The characteristics of the plantation one week after the frost event are shown in Table 3. The mean height was higher in BR treatment, however, the absolute upper limit of damage was lower for BR treatment. The mean range of damage was 5% lower in the BR treatment compared to the control treatment. Trees that showed negative increment (breakage, dry terminal) were excluded from the calculation of mean annual increment after the frost event.

The number of damaged trees according to above ground level was rising from 5 up to 30 cm above ground with the highest number of damaged trees (Fig. 4). At higher levels, the number rapidly decreased. No trees were damaged at the level above 70 and 75 cm in the BR and control treatment, respectively. As the damage of individual trees could possibly infringe on more than one height level, the figure does not represent the probability distribution in the statistical sense. The subsequent height increment from spring did not differ between treatments according to the Wilcoxon test (P = 0.8).

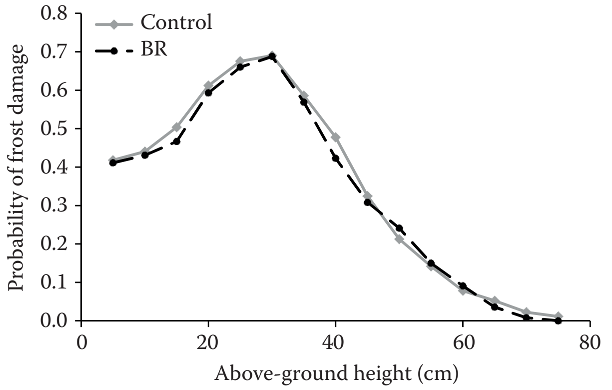

Fig. 4. Distribution of damage to European beech seedlings among height levels and treatments, showing the frost damage probability. Each tree was counted according to its range of damage at various height levels. The highest probability of frost damage was at 30 cm above ground level. The graph is not a distribution function *sensu stricto*, as the sum of probabilities of all heights is not equal to 1. As an example, trees damaged at 20–40 cm were used for calculating damage ratios for 20, 30 and 40 cm. Statistical significance was not tested given the very similar values for control and brassinosteroid treatments (BR)

When we consider the vitality of trees (A – best vitality, E – dead, details in Table 2), both treatments showed an equal pattern with the most abundant average quality 'B', followed by 'C' and 'A' (Fig. 5). The χ^2 test could not prove any difference in the distribution of individuals into categories by their vitality status (P = 0.58).

DISCUSSION

There are relatively few studies of BR application related directly to the growth of forest trees. The author team's research of the application of BRs to

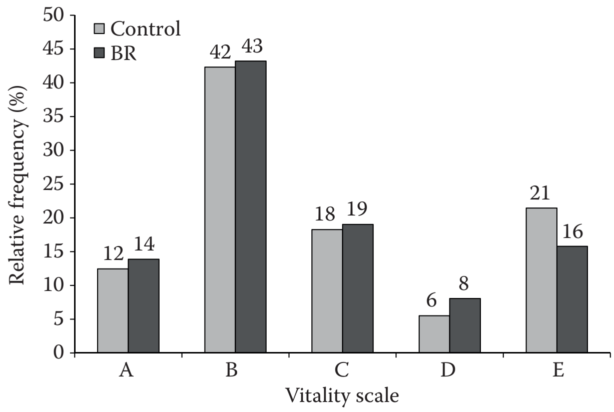

Fig. 5. Distribution of seedlings in brassinosteroid (BR) and control treatments categorized into vitality classes after the late frost event

A – tree with the best vitality, B – tree with good health status, C – tree with partially deteriorated health status, D – tree with markedly deteriorated health status, E – dead tree, for details see Table 2 forest trees has so far less convincing results than other authors investigating agricultural plants: Nováková et al. (2014) reported a substantially increased mortality rate of Scots pine seedlings planted on abandoned agricultural land after the application of a synthetically prepared analogue of 24-epibrassinolid. BR in that case also slowed the height and radial growth. The effect was significant in two subsequent measurements, which suggests a long-term effect of BR. However, in the second study (Nováková et al. 2015), based on data gathered in a forest nursery, BR treatment decreased the mortality rate of the same species, but general effects on height and radial growth were rather inhibiting again. In the same study, the influence of BR treatment on Norway spruce seedlings was slightly beneficial, depending on the chosen concentration, but mostly insignificant. The medium concentration (4 $mg \cdot ha^{-1}$) showed the best results. In relation to our study, it could be suggested that the application could have decreased the planting shock in terms of the mortality rate. But for such a conclusion more research on various species needs to be done.

We also studied adverse effects of late frosts on European beech, as it is the principal broadleaved stand-forming tree species on large areas of low, middle and high elevations in Central Europe. The recorded frost event (4–5 May 2014) was the strongest in terms of stress influence with the most serious impact on vegetation during the whole monitored period (2013–2015). Other frost events were either less pronounced or occurred in a period of lower vegetation sensitivity. The lowest temperature (measured at 30 cm above ground) reached –2.6°C (duration 2 h, Fig. 1). This event caused the foliage injury of most trees within the plantation. Subsequently, the damaged trees regenerated and the annual height and diameter increment did not differ in comparison with previous seasons.

During the field reconnaissance we noticed a slightly reduced amount of frost injuries in the BR treatment. When only the range of damage (lower and upper limits) was assessed, the influence of BR treatment on frost sensitivity was negligible. The process leading to increased freezing tolerance involves plasma membrane lipids showing lower phase transition temperature and higher unsaturation degree when treated with a brassinolide, leading to higher fluidity under low temperature, as found in mango fruit. Therefore "proteins and lipids are involved in brassinolide-mediated responses to cold stress" (Li et al. 2012). Many other physiological changes were also described, for example increases in leaf water content, relative water content and water potential (Li et al. 2008). Resulting from this, a series of processes including the decrease of intracellular CO_2 was described increasing the effectivity of photosynthesis under heat stress (Thussagunpanit et al. 2015).

The frost damage to the lowest parts of trees (i.e. below 20 cm above) was rather reduced, because the shelter provided by grass and herbaceous weeds protected them: where the beech leaves were sheltered in dense grass, the damage was negligible. A similar shelter effect of the vegetation such as grass (less efficient) was described by Petritan et al. (2011) in *Rubus* Linnaeus shrubs and woody shrubs (more efficient). In our study, the upper limit of frost damage was at about 60 cm above ground, which corresponded with the recorded temperature progress. At the monitored heights of 100 and 200 cm above ground, the minimum temperatures reached only –0.4 and –0.3°C, respectively. Thus we can generally deduce the temperature threshold of about –1.5 to –2°C, which is able to cause visible but not severe damage to young (flushing) European beech foliage. The upper above-ground limit of frost damage naturally differs with the intensity of the frost event. In this respect, the zone from 0 to 100 cm above ground is determined by various studies, e.g. Geiger (1950) and Špulák and Balcar (2013), as the zone of the air layer where the daily temperature amplitude (cold at night but hot in the day-time) is most extended.

The high variability of sensitivity to frost damage in this species, which is not related to climatic conditions of provenances or species phylogeography, needs to be considered (Hofmann et al. 2015). The hydraulic frost resistance of European beech is characterized by increased embolism in winter, followed by a decrease before bud flushing, in contrast to, for instance *P. sylvestris*, which is characterized by complete resistance (Charrier et al. 2013). Gömöry and Paule (2011) pointed out the presence of the compromise between two contradictory strategies in terms of the date of bud flushing, which are the effective use of vegetation period and protection against late frost.

In our case, the late frost event hit the plantation during bud flushing, when the trees are most vulnerable to frost damage (Dittmar et al. 2006); the course of bud flushing was described for example by Robson et al. (2013) and Dittmar and Elling (2006). The date of bud flushing in European beech (late successional species) is strictly determined by the photoperiod besides the temperature (Lechowicz 1984; Basler, Körner 2012; Lenz et al. 2016), namely it is driven by chilling and forcing temperatures with an interaction effect of the photoperiod on the forcing rate (photoperiod co-control of leaf-out). It is more pronounced when the chilling requirement is partially satisfied, rather than when buds are fully chilled (Vitasse, Basler 2013). This dual regulating system minimises the risk of encountering a frost event during bud flushing (Caffarra, Donnelly 2011). When defoliation caused by late frost occurs, European beech can recover by the second flushing, but the quality (content of nutrients, sucralose) is decreased, and that can have a negative impact on growth (Gömöry, Paule 2011).

CONCLUSIONS

This study, monitoring the growth performance of European beech plantation, did not find any significant effects of the exogenous application of BR in the forest nursery on the topsoil in autumn on the initial growth of young beech trees during the whole monitored period after planting on a sandy, sun-exposed site. No obvious differences were revealed in terms of seedling height and root collar increments. The late frost event two years after planting (in spring 2014) proved to be most severe at 30 cm above ground. The response of trees to the event in terms of vitality was similar in both treatments.

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References

Back T.G., Pharis R.P. (2003): Structure-activity studies of brassinosteroids and the search for novel analogues and mimetics with improved bioactivity. Journal of Plant Growth Regulation, 22: 350–361.

Bajguz A. (2011): Brassinosteroids – occurrence and chemical structures in plants. In: Hayat S., Ahmad A. (eds): Brassinosteroids: A Class of Plant Hormone. Dordrecht, Heidelberg, London, New York, Springer Science + Business Media B.V.: 1–28.

Bajguz A., Hayat S. (2009): Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiology and Biochemistry, 47: 1–8.

Basler D., Körner C. (2012): Photoperiod sensitivity of bud burst in 14 temperate forest tree species. Agricultural and Forest Meteorology, 165: 73–81.

Caffarra A., Donnelly A. (2011): The ecological significance of phenology in four different tree species: Effects of light and temperature on bud burst. International Journal of Biometeorology, 55: 711–721.

Charrier G., Cochard H., Améglio T. (2013): Evaluation of the impact of frost resistances on potential altitudinal limit of trees. Tree Physiology, 33: 891–902.

Christersson L., von Fircks H.A. (1988): Injuries to conifer seedlings caused by simulated summer frost and winter desiccation. Silva Fennica, 22: 195–201.

Dittmar C., Elling W. (2006): Phenological phases of common beech (*Fagus sylvatica* L.) and their dependence on region and altitude in southern Germany. European Journal of Forest Research, 125: 181–188.

Dittmar C., Fricke W., Elling W. (2006): Impact of late frost events on radial growth of common beech (*Fagus sylvatica* L.) in southern Germany. European Journal of Forest Research, 125: 249–259.

Forest Management Institute (2003): Přehled lesních typů a souborů lesních typů v ČR (typologická tabulka). Available at http://www.uhul.cz/images/typologie/tab_LT_web_F.pdf (accessed Sept 20, 2016).

Fujii S., Saka H. (2001): The promotive effect of brassinolide on lamina joint-cell elongation, germination and seedling growth under low-temperature stress in rice (*Oryza sativa* L.). Plant Production Science, 4: 210–214.

Gallo K., Hale R., Tarpley D., Yu Y. (2011): Evaluation of the relationship between air and land surface temperature under clear- and cloudy-sky conditions. Journal of Applied Meteorology and Climatology, 50: 767–775.

Geiger R. (1950): The Climate Near the Ground. Cambridge, Harvard University Printing Office: 482.

Gomes M.M.A., Campostrini E., Leal N.R., Viana A.P., Ferraz T.M., Siqueira L.D., Rosa R.C. C., Neto A.T., Nuñues-Vázquez M., Zullo, M.A.T. (2006): Brassinosteroid analogue effects on the yield of yellow passion fruit plants (*Passiflora edulis* f. *flavicarpa*). Scientia Horticulturae, 110: 235–240.

Gömöry D., Paule L. (2011): Trade-off between height growth and spring flushing in common beech (*Fagus sylvatica* L.). Annals of Forest Science, 68: 975–984.

Grove M.D., Spencer G.F., Rohwedder W.K., Mandava N., Worley J.F., Warthen J.D., Steffens G.L., Flippen-Anderson J.L., Cook J.C. (1979): Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature, 281: 216–217.

He R.Y., Wang G.J., Wang X.S. (1991): Effects of brassinolide on growth and chilling resistance of maize seedlings. In: Cutler H.G., Yokota T., Adam G. (eds): Brassinosteroids: Chemistry, Bioactivity and Applications. Washington, D.C., American Chemical Society: 26–35.

Hofmann M., Durka W., Liesebach M., Bruelheide, H. (2015): Intraspecific variability in frost hardiness of *Fagus sylvatica* L. European Journal of Forest Research, 134: 433–441.

Holubík O., Podrázský V., Vopravil J., Khel T., Remeš J. (2014): Effect of agricultural lands afforestation and tree species composition on the soil reaction, total organic carbon and nitrogen content in the uppermost mineral soil profile. Soil and Water Research, 9: 192–200.

Hradecká D., Urban J., Kohout L., Pulkrábek J., Hnilička R. (2009): Využití brassinosteroidů k regulaci stresu během růstu a tvorby výnosu řepy cukrové. Listy cukrovarnické a řepařské, 125: 271–273.

Khripach V.A., Zhabinskii V.N., Groot, A.E. (1999): Brassinosteroids: A New Class of Plant Hormones. San Diego, Academic Press: 456.

Kohout L., Kasal A., Chodounská H. (2004): Použití 2α,3α,17β-trihydroxy-5α-androstan-6-onu pro ovlivňování stresu rostlin. [Utilisation of 2α,3α,17β-trihydroxy-5α-androstan-6-one for influencing of plant stress.] PV 2001-2436 (2.7.2001) – CZ pat. No. 294 343 (4.10.2004).

Kohout L., Kasal A., Chodounská H., Slavíková B., Hniličková J. (2002): The use of 2α,3α,17β-trihydroxy-5α-androstan-6-one for plant development regulation. PCT pat. appl. No. PCT/CZ02/00041 (2.7.2002) (WO2003003834) and EU patent 1 401 278 (16.1.2003).

Kreyling J., Thiel D., Nagy L., Jentsch A., Huber G., Konnert M., Beierkuhnlein C. (2012): Late frost sensitivity of juvenile *Fagus sylvatica* L. differs between southern Germany and Bulgaria and depends on preceding air temperature. European Journal of Forest Research, 131: 717–725.

Krishna P. (2003): Brassinosteroid-mediated stress responses. Journal of Plant Growth Regulators, 22: 289–297.

Kuneš I., Baláš M., Millerová K., Balcar V. (2011): Vnášení listnaté příměsi a jedle do jehličnatých porostů Jizerských hor. Lesnický průvodce 9/2011. Jíloviště-Strnady, VÚLHM: 36.

Kuneš I., Baláš M., Zahradník D., Nováková O., Gallo J., Nárovcová J., Drury M.L. (2014): Role of planting stock size and fertilizing in initial growth performance of rowan (*Sorbus aucuparia* L.) reforestation in a mountain frost hollow. Forest Systems, 23: 273–287.

Langvall O., Nilsson U., Örlander G. (2001): Frost damage to planted Norway spruce seedlings – influence of site preparation and seedling type. Forest Ecology and Management, 141: 223–235.

Lechowicz M.J. (1984): Why do temperate deciduous trees leaf out at different times? Adaptation and ecology of forest communities. The American Naturalist, 124: 821–842.

Lenz A., Hoch G., Körner C., Vitasse Y. (2016): Convergence of leaf-out towards minimum risk of freezing damage in temperate trees. Functional Ecology, 30: 1480–1490.

Leslie A.D., Mencuccini M., Perks M. (2014): Frost damage to eucalypts in a short-rotation forestry trial in Cumbria (England). iForest – Biogeosciences and Forestry, 7: 156–161.

Li B., Zhang C., Cao B., Qin G., Wang W., Tian S. (2012): Brassinolide enhances cold stress tolerance of fruit by regulating plasma membrane proteins and lipids. Amino Acids, 43: 2469–2480.

Li K.R., Zhang W.B., Li H.K. (2005): Effect of natural brassinolide on germination of *Ailanthus altissima* seeds. Forestry Studies in China, 7: 12–14.

Li K.R., Wang H.H., Han G., Wang Q.J., Fan J. (2008): Effects of brassinolide on the survival, growth and drought resistance of *Robinia pseudoacacia* seedlings under water-stress. New Forests, 35: 255–266.

Lindkvist L., Lindqvist S. (1997): Spatial and temporal variability of nocturnal summer frost in elevated complex terrain. Agricultural and Forest Meteorology, 87: 139–153.

Menzel A., Helm R., Zang C. (2015): Patterns of late spring frost leaf damage and recovery in a European beech (*Fagus sylvatica* L.) stand in south-eastern Germany based on repeated digital photographs. Frontiers in Plant Science, 6: 110.

Ministry of Agriculture of the Czech Republic (2015): Zelená zpráva o stavu lesa a lesního hospodářství České republiky v roce 2014. Prague, Ministry of Agriculture of the Czech Republic: 108.

Nováková O., Kuneš I., Gallo J., Baláš M. (2014): Effects of brassinosteroids on prosperity of Scots pine seedlings. Journal of Forest Science, 60: 388–393.

Nováková O., Gallo J., Baláš M., Špulák O., Kuneš I. (2015): Vliv brassinosteroidů na výškový přírůst, fluorescenci chlorofylu a mortalitu sazenic smrku ztepilého a borovice lesní v podmínkách lesní školky. Zprávy lesnického výzkumu, 60: 122–129.

Novotný P., Frýdl J., Čáp J. (2015): Zhodnocení kvalitativních parametrů buku lesního (*Fagus sylvatica* L.) na sedmi provenienčních výzkumných plochách ve věku 25 let. Zprávy lesnického výzkumu, 60: 14–23.

Oke T.R. (1970): The temperature profile near the ground on calm clear nights. Quarterly Journal of the Royal Meteorological Society, 96: 14–23.

Petritan I.C., von Lüpke B., Petritan A.M. (2011): Influence of shelterwood and ground vegetation on late spring frost damages of planted beech (*Fagus sylvatica*) and Douglas-fir (*Preudotsuga menziesii*) saplings. Baltic Forestry, 17: 227–234.

Podrázský V., Remeš J. (2007): Humus form status in close-to-nature forest parts comparing to afforested agricultural lands. Lesnícky časopis – Forestry Journal, 53: 99–106.

Podrázský V., Remeš J. (2008): Rychlost obnovy charakteru lesních půd na zalesněných lokalitách Orlických hor. Zprávy lesnického výzkumu, 53: 89–93.

Podrázský V., Zahradník D., Remeš J. (2014): Potential consequences of tree species and age structure changes of forests in the Czech Republic – review of forest inventory data. Wood Research, 59: 483–490.

Pullman G.S., Zhang Y., Phan B.H. (2003): Brassinolide improves embryogenic tissue initiation in conifers and rice. Plant Cell Reports, 22: 96–104.

Rao R.S., Vidya Vardhini B., Sujatha E., Anuradha S. (2002): Brassinosteroids – a new class of phytohormones. Current Science, 82: 1239–1245.

Robson T.M., Rasztovits E., Aphalo P.J., 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. Agricultural and Forest Meteorology, 180: 76–85.

Sasse J.M. (2003): Physiological actions of brassinosteroids: An update. Journal of Plant Growth Regulation, 22: 276–288.

Schütz J.P., Götz M., Schmid W., Mandallaz D. (2006): Vulnerability of spruce (*Picea abies*) and beech (*Fagus sylvatica*) forest stands to storms and consequences for silviculture. European Journal of Forest Research, 125: 291–302.

Singh I., Kumar U., Singh S.K., Gupta C., Singh M., Kushwaha S.R. (2012): Physiological and biochemical effect of 24-epibrassinoslide on cold tolerance in maize seedlings. Physiology and Molecular Biology of Plants, 18: 229–236.

Špulák O., Balcar V. (2013): Temperatures at the margins of a young spruce stand in relation to aboveground height. iForest – Biogeosciences and Forestry, 6: 302–309.

Steyrer G. (2011): Abiotische Schäden im Winter und Frühjahr. Forstschutz Aktuell, 53: 30–31.

Strasser L. (2011): Schwarzes Laub und braune Nadeln – Spätfrost in Bayern. Forstschutz Aktuell, 53: 28–29.

Thussagunpanit J., Jutamanee K., Kaveeta L., Chai-arree W., Pankean P., Homvisasevongsa S., Suksamrarn A. (2015): Comparative effects of brassinosteroid and brassinosteroid mimic on improving photosynthesis, lipid peroxidation, and rice seed set under heat stress. Journal of Plant Growth Regulation, 34: 320–331.

Vardhini V., Rao R.S. (2003): Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. Plant Growth Regulation, 41: 25–33.

Vitasse Y., Basler D. (2013): What role for photoperiod in the bud burst phenology of European beech. European Journal of Forest Research, 132: 1–8.

Wilen R.W., Sacco M., Gusta L.V., Krishna P. (1995): Effects of 24-epibrassinolide on freezing and thermotolerance of bromegrass (*Bromus inermis*) cell cultures. Physiologia Plantarum, 95: 195–202.

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