Effects of brassinosteroids on prosperity of Scots pine seedlings

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ABSTRACT: We tested the influence of exogenous application of brassinosteroids (BRs) on survival, growth and biomass production of Scots pine (*Pinus sylvestris*) seedlings. BRs are natural substances, supposedly having many positive effects on plants such as improving growth, stress tolerance, survival and biomass production. One part of the seedlings was treated with a low concentration of synthetically prepared BRs and the other part was designated as the control without any treatment. Mortality, height and root collar diameter were measured in two subsequent years, the root-to-shoot ratio of biomass volume was determined in autumn 2013. The application of BRs significantly slowed height and radial growth of pine. The height increment of the BRs-treated seedlings was lower by 11% between 2012 and 2013 compared to the control. The increment in root collar diameter (2012–2013) in the BRs-treated seedlings was lower by 13% than in the control. The BRs-treated pines also experienced a significantly higher mortality compared to the control. No significant influence of BRs on biomass production was found.

Keywords: plant hormones; seedlings; *Pinus sylvestris*; growth; biomass production

Brassinosteroids (BRs) are plant hormones that belong to the class of polyhydroxy steroids and are structurally related to the animal and insect steroid hormones (Bajguz 2011). They were first isolated from the pollen of *Brassica napus* (Grove et al. 1979; Cortes et al. 2003; Hradecká et al. 2009).

Low concentrations of brassinosteroids were found across the whole plant kingdom throughout all parts of plant bodies including pollen, flower buds, fruits, seeds, vascular cambium, leaves, shoots and roots (Bajguz 2011). Young plant tissues contain a higher amount of brassinosteroids than the mature tissues (Rao et al. 2002). Pollen and immature seeds are the richest sources of BRs with a range of 1–100 $\mu g \cdot kg^{-1}$ of fresh weight, while shoots and leaves usually have lower amounts of 0.01–0.1 $\mu g \cdot kg^{-1}$ of fresh weight (Bajguz, Tretyn 2003; Bajguz 2011).

Research studies dealing with brassinosteroids revealed that these hormones are able to elicit a broad spectrum of both positive and undesirable physiological and morphological responses in plants, including stem elongation, leaf bending and epinasty (Krishna 2003; Bajguz, Hayat 2009; Bajguz 2011), floral initiation, development of flowers and fruits (Hayat, Ahmad 2003), induction of ethylene biosynthesis and proton pump activation, synthesis of nucleic acids and proteins, regulation of carbohydrate assimilation and allocation and activation of photosynthesis (Bajguz, Hayat 2009; Bajguz 2011).

Brassinosteroids play a crucial role in plant development and can also promote the tolerance to a range of abiotic stresses, including salt and drought stress, temperature extremes and pathogen attacks (Clouse, Sasse 1998; Krishna 2003; Divi et al. 2010). Although much has been learned about their role in plant development, the mechanisms by which BRs control plant stress responses and regulate stress-responsive gene expression are not fully explained (Divi et al. 2010). Sakurai et al. (1999) pointed out that BRs acted in interaction with other phytohormones. Several brassinosteroid analogues with a structure similar to the natural BRs were shown to be useful in agriculture (Zullo et al. 2003).

Low temperatures and frosts are important limiting factors for growth and survival of plant species. In the temperate zone, spring is often a critical period of the

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year, because young developing leaves are only little frost resistant, but air temperatures may still drop below the freezing point (Larcher, Hackel 1985). Ali et al. (2006); Behnamnia et al. (2009) and Fariduddin et al. (2009) pointed out the importance of exogenous application of BRs to stressed plants.

In many cases, positive effects of BRs on the germination of agricultural plants were shown (Artecy 1996; Procházka et al. 1998; Štranc et al. 2008; Procházka et al. 2011).

The effects of external brassinosteroid application are now studied and used particularly in agriculture (Vardhini, Rao 1997, 2003; Annuradha, Rao 2001; Hradecká et al. 2009; Divi et al. 2010). The practical application of BRs is being done in Japan, where these hormones are used in large agricultural areas to improve the yield of crops such as wheat and rice (Brosa 1999). Brosa (1999) informed that BRs are natural, nontoxic products which are applied in extremely low doses and therefore suitable from the ecological and environmental points of view.

Although Hong et al. (2005) suggested that the application of brassinosteroids in forestry could be possible, there is a lack of experience with the effects of brassinosteroids on forest trees. In Europe there has basically been no intensive research focusing on the issue. The aim of this article is to assess whether the exogenous applications of BRs are able to promote initial survival, growth and biomass production of *Pinus sylvestris* plantation.

MATERIAL AND METHODS

In autumn 2011, one part of *Pinus sylvestris* seedlings (two-year-old root-pruned planting stock of Central Bohemian origin) grown in a forest nursery was treated with brassinosteroid-2-alfa-3-alfa-17-beta-trihydroxy-5-alfa-androstan-6-one that was a synthetically prepared analogue of 24-epibrassinolid (Kohout et al. 2002; Kohout et al. 2004). It was applied 4 mg of the brassinosteroid per 1 ha. Before application the brassinosteroid was dissolved in 210 l of water with added dimethyl sulfoxide (DMSO). The other part of the seedlings was left untreated as the control. In spring 2012, the seedlings of both treatments were transplanted from a nursery to an experimental plot on an afforested farmland at the Truba Research Station close to Kostelec nad Černými lesy (50°0.37'N, 14°50.21'E), the Czech Republic. The experimental plot was protected by fencing. The altitude of the site was 365 m a.s.l., mean annual air temperature (2013) at 30, 100 and 200 cm above the ground was 8.6, 9.0 and 8.8°C, respectively, and the annual precipitation (2013) equalled to 800 mm.

The plantation was established at a spacing of 1 × 1 m. We planted 784 control seedlings and 694 seedlings treated with brassinosteroids. The rows of the control and treated seedlings alternated. Initial measurements of height and root collar diameter were performed in spring 2012 (initial values). Periodical measurements of height and root collar as well as records of mortality rates were done in autumn 2012 and autumn 2013. The values of relative height and relative calliper (radial) growth describe the relative increments (in %) of height and root collar diameter over the period 2012–2013 in relation to the initial values that were recorded in spring 2012. The mensurational characteristics consisted of the data only relating to the trees alive in the autumn of 2013. Data belonging to the trees dead until autumn 2013 were retrospectively excluded. The mortality rates were calculated as the percentage of dead seedlings related to the initial numbers of plants.

In autumn 2013, the above-ground and below-ground biomass volume was inspected. Twelve seedlings from each treatment were randomly chosen. These seedlings were excavated including their root systems and the soil from roots was carefully washed away. Seedlings were then dissected into roots and shoots, the volume of the above-ground and below-ground biomass was measured and the root-to-shoot ratio of biomass volume was calculated.

Height, root collar diameters, relative height growth, relative radial growth, biomass volume and root-to-shoot ratio were analysed using the Mann-Whitney-U test. Mortality of seedlings was analysed using a binomial test. The chosen significance level was 0.05 for all statistical analyses. The statistical analyses were conducted in STATISTICA12 (StatSoft Inc., Tulsa, USA) software.

RESULTS

The BRs treatment showed significantly lower values of mean height throughout the evaluated period and the difference successively increased (Fig. 1).

As depicted in Fig. 2, significant differences between treatments in root collar diameter were recorded in spring and autumn 2012. The control was, nonetheless, reducing the head start of the BRs treatment in root collar diameter. In autumn 2013, the treatments changed their order. Although the differences between treatments were not significant in autumn 2013 (P = 0.093), the control treatment overtook the lead.

Increments in height and root collar diameter are shown in Table 1. Particularly in these parameters it is well demonstrable that the BRs treatment grew significantly slower. The mean values of the height

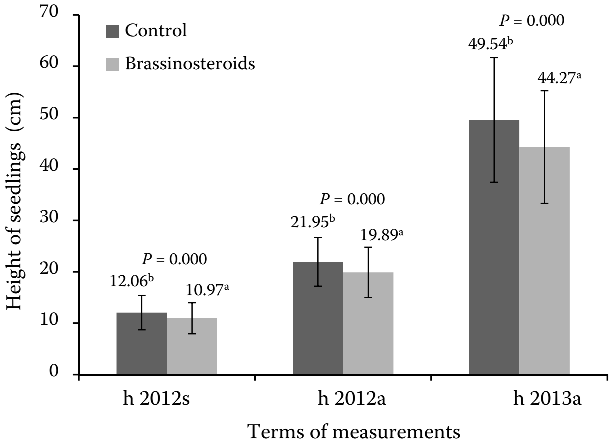

Fig. 1. Mean Scots pine seedlings height of compared treatments (control and BRs) in spring 2012 (h 2012s), autumn 2012 (h 2012a) and autumn 2013 (h 2013a), respectively. Error bars depict standard deviation. Significant differences ($P < 0.05$) between treatments (letter indexes behind values) were proved on all dates of measurements

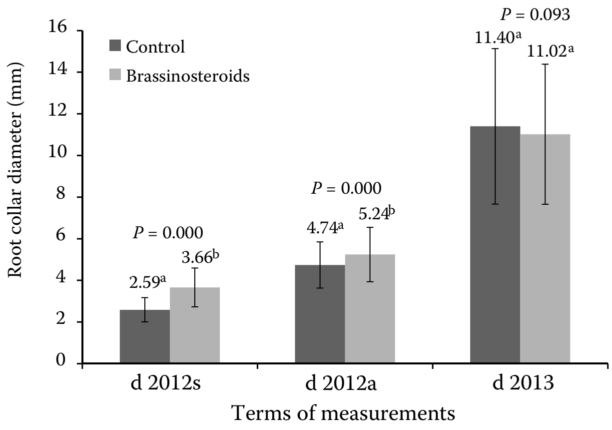

Fig. 2. Mean root collar diameters of compared treatments (control and BRs) in spring 2012 (d 2012s), autumn 2012 (d 2012a) and autumn 2013 (d 2013a), respectively. Error bars depict standard deviations. Significant differences ($P < 0.05$) between treatments (letter indexes behind values) were recorded in spring 2012 (d 2012s) and autumn 2012 (d 2012a)

increment between spring 2012 and autumn 2013 in the control and in the BRs treatment were 37.5 cm and 33.3 cm, respectively. These values were significantly different ($P = 0.000$).

The mean values of the root collar increment between spring 2012 and autumn 2013 in the control and in the BR treatment were 8.81 cm and 7.36 cm, respectively. Values in the BRs treatment were significantly ($P = 0.000$) lower than those in the control.

The mortality rate in the BRs treatment proved to be significantly higher than the mortality rate in the control treatment during both monitored years (Fig. 3).

As for biomass volume (Fig. 4), the statistical analyses did not reveal any significant differences, despite the above-ground biomass being greater in the control than in the BRs treatment. The root-to-shoot ratio in the control and BRs treatment was 19.2 and 20.2, respectively. The difference between treatments was neither large nor significant ($P = 0.285$).

DISCUSSION

The aim of the study was to assess whether the exogenous application of BRs is able to promote initial survival, growth and biomass production of *Pinus sylvestris* plantation, an important commercial tree species. As mentioned above, there was a lack of experience with BRs effects on woody trees. The works of Mandava (1988); Rao et al. (2002);

Table 1. Height and root collar diameter increments of control (C) and brassinosteroids (BRs) treatment in 2012, 2013 and over the period 2012–2013

Period	Variant	Increment h (cm)			Increment d (mm)		
		mean	median	SD	mean	median	SD
2012	C	9.46	10	3.89	1.90	2	1.10
	BR	8.92	9	3.91	1.58	1	1.02
	significance	*			*		
2013	C	27.59	27	9.85	6.66	7	2.95
	BR	24.38	24	8.38	5.78	6	2.57
	significance	*			*		
2012–2013	C	37.48	37	11.7	8.81	9	3.59
	BR	33.3	34	10.53	7.36	7	3.05
	significance	*			*		

*$P < 0.05$, SD – standard deviation

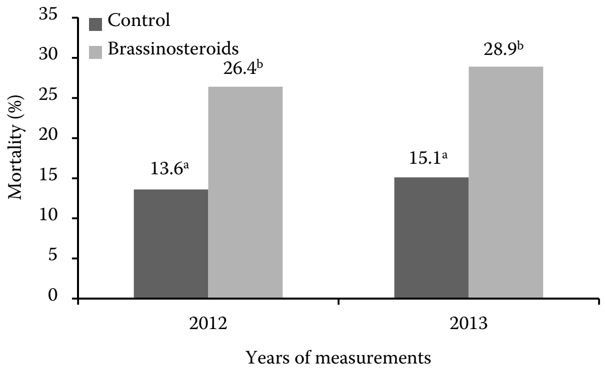

Fig. 3. Development of overall mortality rates between 2012 and 2013 in compared treatments. Significant differences ($P < 0.05$) between treatments (letter indexes behind values) were found on both dates of measurement

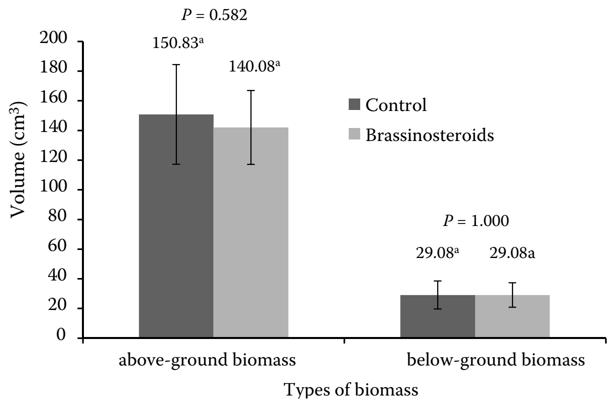

Fig. 4. Volumes of the above-ground and below-ground biomass of Scots pine seedlings in the compared treatments recorded in autumn 2013. No significant differences ($P < 0.05$) were found between treatments (letter indexes behind values)

Pereira-Netto et al. (2006) and Bajguz (2007) report a stimulating effect of BRs on the stem elongation of many (usually agricultural) plants. Prušáková et al. (1995) informed about an increased stem diameter in barley after BRs applications and thus also an increased resistance to lodging.

Positive effects of stem elongation were shown in the cuttings of *Phaseolus vulgaris*, when treated with BRs in lanolin paste. On the other hand, the undesirable side effects, such as curvature, swelling or splitting, were recorded. In more concrete terms, the BRs treatment caused elongation, curvature and swelling at a lower concentration than 0.01 mg and splitting at a higher concentration than 0.1 mg (Mandava et al. 1983).

Bao et al. (2004) reported that application of BRs 1–100 nM (with auxin) increased the growth of lateral roots in *Arabidopsis*. The authors referred the best results after application of 10 nM solution. However, on the other hand, the dose of 1–100 nM inhibited the elongation of primary roots. Also Kim et al. (1994) mentioned that application of BRs had an inhibitory effect on the radish hypocotyl elongation.

In many studies, the BRs applied in various concentrations ranging from 0.05 μM (Janeczko et al. 2007) to 15 mg·ha^{-1} (Korableva et al. 2002) showed a positive effect on the tolerance of plants to stresses. For example, the positive effect of BRs on thermal stressed plants were documented in the case of rise seedlings (Fujii et al. 2001) and cucumber (*Cucumis sativus*), on water-stressed wheat (*Triticum aestivum*) (Sairam 1994) and radish (*Raphanus sativus*) (Kommavarapu et al. 2013). As for woody species, Li et al. (2008) tested the soaking of *Robinia pseudoacacia* seedlings in BRs solutions (0–0.4 mg·l^{-1}), the results showed that soaking roots in BRs prior to planting significantly increased the survival and growth of seedlings (reduced water and transplant stresses). The best results were in the 0.2 mg·l^{-1} BRs treatment.

In our study, the experimental exogenous application of BRs to *Pinus sylvestris* seedlings led to much less promising results. The increments of height and root collar diameter were significantly lower in the BRs treatment than in the control (Table 1). Mortality rate of pine seedlings in the BRs treatment was also significantly higher than that in the control (Fig. 3) and the volume of biomass was not influenced. This particular result seems to be in contrast with the findings of the other authors reporting the ability of BRs to raise the survival rate of plants and to protect them against stresses (Kagale et al. 2006; Li et al. 2008; Divi et al. 2010).

The BRs application in our study did not promote growth performance and survival of *Pinus sylvestris* after transplanting. Moreover, the BRs effects were often even inhibiting. At this initial stage of research on woody trees, it is surely not to say that these phytohormones are not utilisable for forestry purposes. Our experiment, however, documents that the BRs application is not a universal panacea. It seems essential to distinguish between types of stresses as reported by Bajguz et al. (2009). It is also highly probable that the BRs effects depend on species as mentioned by Gomes (2011). The concentrations/dose and timing of the BRs are also a vital topic (Gomes 2011).

The dose of BRs in our experiment (4 mg·ha^{-1} applied as 0.02 mg·l^{-1} solution) is based on exclusively agricultural experiences and we cannot exclude the hypothesis that for plants of forest trees the concentration might be too high. On the other hand, Li et al. (2008) used a substantially higher concentration for the dipping of *Robinia* seedlings and the results were positive. As Gomes et al. (2011) documented, the date of application and dose of applied BRs play an important role. Of course, the response of various species to a particular form of BR treatment might be different.

As shown above, the BRs were applied at various concentrations, in different ways, to several plant species and for various purposes in research studies. It can be seen that these phytohormones surely influence the growth performance of plants. We also found out that the influence does not necessarily have to be positive (Table 1).

To inspect the potential of BRs for forestry, it seems crucial to focus the research on (principal) tree species and define situations when trees are mostly stressed and BRs could be of any help. The question of optimal BR concentration, date and way of application should be opened and also successively answered in an experimental way.

CONCLUSIONS

Height growth and radial growth of pines in the BRs treatment were significantly lower compared to the control. The mortality rate of pines in the BRs treatment was significantly higher than that in the control. As for biomass volume, no significant differences between the BRs treatment and control were found, however, the control pines showed slightly higher values of this parameter.

Our results documented that the response of a tree species to BRs application does not have to be always positive. Further research is desirable to evaluate the potential of BRs for forestry purposes. The response of principal tree species to BRs treatment should be inspected and the task to find an optimal BRs concentration, date and way of application should be addressed.

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