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Influence of substrate type and cultivation technology on quantitative characteristic of spruce (*Picea abies* (L.) Karst.) seedlings´ root systems: comparison between quantitative traits

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Abstract

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This work deals with evaluation of influence of four different management methods and substrate types on basic quantitative parameters of root systems in one-year old spruce (*Picea abies* (L.) Karst.) seedlings. There were tested differences between three alternatives consisting of bare-rooted plants and one variant of ball plants grown under applying the technique Lännen Plantek. The bare-rooted seedlings were cultivated in pure peat substrate (control) and in peat substrates supplemented with alginite and Baktomix. The tested traits were the following: root system weight, length, surface, volume, mean diameter and number of endings. For testing differences between the mean values of these characteristics, we used variance analysis. There has been found that in all the evaluated traits, the highest values were obtained with using the method Lännen Plantek. These values were several times higher that the next lower value. The variability of the measured values was about 30%. The lowest mean values were obtained in case of the peat substrate enriched with alginite. There is supposed that alginite caused water-logging of substrate and significantly retarded growth under the wetting regime favourable for all the other variants.

Key words

Norway spruce, root system, bare-rooted seedlings, ball seedlings, alginite, Baktomix

Introduction

Spruce is the woody plant most seriously affected by injurious agents, predominantly abiotic (wind). In recent years (2001–2005), the share of incidental felling in this woody plant was higher than 70% of the total amount (source: National Forest Centre Zvolen). The regeneration of deforested plots with the same woody plant is only possible with a considerable share of artificial regeneration.

Sarvaš et al. (2006) report that the Slovak forestry use bare-rooted seedlings in 97% of the total artificial regeneration. The using of this material takes for granted vigorous bare-rooted plants (Standard STN 48 2211). The growing up of such plants usually requires from two to four years, consequently, the producers are not able to respond the market's demands in an appropriately flexible way. This type of planting material is to a considerable extent dependent on the weather course, especially in case of spring lifting and following planting if the basic methodical rules are not complied with by storage. The cause is reduced vitality of such temporary stored material. If the harm caused by improper storing is coupled with unfavourable weather course after the planting, the result is a stronger transplanting-related shock followed by high losses at forestation. The expenses on repeated forestation in 2001 reached more than 160 mil. Sk, in 2003 the sum was higher than 130 mil. Sk (Anonymus, 2003, 2004).

Injurious effects of factors causing lowering the rooting rate in the planted material can be eliminated by applying hydro-absorbents (syn. soil conditioners, hydrosols) to plants grown in forest nurseries and in forestation process (Sarvašová, 2004; Sarvaš and Tučeková, 2004). The principle of their functioning is in their capacity to take up water, cations and anions and to maintain them in their "exchange" form, important for the uptake by plants. These substances promote the soil sorption complex to the extent enabling sufficient supply of water and nutrients to the woody plants. One of these substances is alginite – a tertiary sedimentary rock of organic origin (Vass et al., 2002).

Another possibility how to raise the total rooting rate in artificial regeneration is using ball plants. This type of planting material offers, in comparison with the bare-rooted one, a number of profits – in terms of biology, technology and commerce (Šmelková, 2001, 2004; Tučeková and Ábelová, 2002; Tučeková, 2003, 2004a), on the other hand, the shortcomings are discussed in Tučeková (2004a), Lokvenc (2001).

Baktomix™ is a macrobiological agent assigned for improving soil fertility. It consists of a mixture of active cultures of soil bacteria *Azotobacter chroococcum, Cellulomonas uda* and *Bacillus megaterium*. The use of this mixture can supply to certain extent or even fully the artificial fertilisers, through increasing the activity of nitrification bacteria resulting in improved decomposition of cellulose and increased amount of bound atmospheric nitrogen (source: http://www.agref.sk/baktomix.html).

Both the method and technology in plant material growing have impact on development of quantitative and qualitative parameters of both aboveground and underground parts of seedlings and plants. The values of these parameters have direct influence on the viability of the planting material, and, consequently, on rate of successful artificial regeneration. The growing speed in plants is primarily dependent on the uptake of water and dissolved nutrients, which in its turn depends on the root system's size. The correlation between the two factors is positive.

Material and methods

Experimental seeding trials were carried out on two plots: the first situated in the Arboretum Borová Hora, the second in the Forest Nursery Centre (FNS) Jochy, Semenoles Liptovský Hrádok. Both plots were established on May 2, 2006, following the technology *Lännen Plantek*. There was used the spruce seed provided by the School Forest Enterprise (SFE) Technical University in Zvolen: 093/01/03 EVK 01563ZV577 15/2003. The germination per cent was 99%, purity was 95%.

In the Arboretum Borová Hora, three experimental mini-plots, each 1m^2 in size, were sawn each with a 35 g batch of Norway spruce seed. Each plot was divided into three equal blocks. The seeding substrate was pure peat substrate from Suchá Hora (Orava region, N Slovakia). The first plot was supplemented with hydro-absorbent alginite in amount of 3 kg m^{-2} , the second with Baktomix 4 l m^{-2}, the third was left as control. The seeds were covered with a thin peat layer. The germination was observed on May 21, 2006. Over the whole vegetation period, the plots were treated in accordance with common agricultural engineering. Beginning with the first October week 2006, 35 seedlings from each block were lifted carefully in such a way as to avoid impairment of their root systems.

The seed of the same origin was also sawn in boxes with peat substrate at the FNC Jochy, Semenoles Liptovský Hrádok. The Norway spruce seed in number of 162 was planted into the trays with 81 cells with parameters listed in Table 1. The seedlings were cultivated in shelters, where there were wetted with fertilisersenriched water. In August and September, they were transported to open area with the aim to promote appropriate maturing of shoots and winter-resistance of the woody plant. On October 19, 2006, there was taken a sample consisting of 35 Norway spruce seedlings from the experimental seeding into Lännen Plantek trays.

The values of quantitative morphological characteristics of root systems of the examined one-year-old seedlings were determined using the direct destructive method. The root systems of the sampled plants were first of all thoroughly rinsed with water to remove the substrate. At the same time, it was necessary to take care and prevent tearing-off and loss of parts. If such prepared material was not possible to further process and analyse on the same day, it was stored in a fridge immersed in water for at most three days – to avoid its degradation (drying-out, growth of moulds, respiration, etc.). The washed seedlings were measured in diameter at their root collars with an accuracy of 0.05 mm, and separated into the aboveground and underground parts. The root length, volume and surface area as well as the number of root tips was determined using the software WinRhizo 2004a™ (Fig 1). The computer programme itself represents standard methods for determining these parameters. The output consists of the data about

Table 1. Parameters of Plantek-F seedling trays used in test sowing

Name	Number of cells per tray	Tray dimensions [mm]	Internal cell dimensions [mm]	Cell volume [cm^3]	Plant density [No m^{-2}]
PL 81F	9 × 9	385 × 385 × 73	41 × 41 × 73	85	549

the total length, average thickness, total surface area, volume and number of endings of the system or the section analysed. The output is provided in form of a text file easily importable into each table processor (eg Excel).

Fig 1. Example of a root system prepared for analysis with the WinRhizo software package (control, 1. block, 6. individual exemplar – weight 0.0623 g, root system's length 228.05 cm, surface area 27.67 cm^2, mean diameter 0.39 mm, volume 0.267 cm^3 and number of root tips 740)

The scanned root system was then dried up at a temperature of 60 °C for 72 hours with the aim to prevent loss, except free water, of other volatile substances, eg aromatic oils and turpentine which would discard the measurement reliability. Finally, the weight was determined with an accuracy of 0.001 g.

The statistical processing of the data was carried out with using the one-way variance analysis (ANOVA), for experiment designed in random blocks with different numbers of blocks in different variants. In terms of calculation of sum of squares, the method is the same as the one-way ANOVA with arranging without blocks. The block is embedded into calculation as a dummy second factor. The influence of this factor is not tested any more, its embedding, however, entails a considerable reduction in the residual variance. The value F is calculated as the ratio of the mean square (MS) of deviations associated with influence of the examined factor and MS associated with the blocks. The ANOVA analysis is followed by the Duncan's test in case when comparison between the variants is necessary or by the Dunnett's test if the modified variants are compared with the control. This method was used for verification of statistical significance of the total values (unpublished data).

This work presents first partial results of the experiment aimed at influence of various substrates and management methods used in spruce seedlings growing from seed.

Results

The size of root systems was compared based on comparisons between the mean values of the most important biometrical variables. The list of these values as well as fundamental data about the overall variability are summarised in Tables 2–7.

Examining the tables we can see that the differences between the mean values obtained in case of the Lännen – Plantek system and that obtained in case of bare-rooted plants grown in different modified substrates were manifold. The differences between the bare-rotted plants grown in different substrates were much smaller.

The variability of the measured values of the individual variables is rather low. As we can see in Tables 2–7, the values of variation coefficients reach about 30%, and the differences between the measured characteristics within one variant are much bigger than the differences between the variants in frame of one characteristic. The most variable characteristic was always the number of root tips; the lowest variability was observed in the mean root diameter. The exception was the variant Lännen, which might potentially be caused by the lower sample size. For each examined characteristic, the statistical significance of differences between the variants was tested with one-way variance analysis with randomised blocks. The results are listed in Table 8.

The results of variance analysis, namely the very high F-values, point out a high statistic significance of differences in means between the individual variants. The high value of the sum of squares the for variant (factor), and, consequently, the mentioned high F-value reflect the highly significant difference between the mean values in the variant Lännen and the mean values obtained in case of other – bare-rooted variants.

The comparison between the mean values of the studied biometric variables in individual variants and evaluation of statistical significance of the differences was carried out using the Duncan's test the output of which ranks the parameters into the individual homogeneous groups (Tables 2–7).

According to the results of the Duncan´s test carried out for the total length of roots, there have not been detected significant differences between the substrate supplemented with alginite and the control. Other homogeneous group comprised the control and Baktomix-enriched substrate. At the same time, also the difference in length between the substrate supplemented with alginite and the substrate supplemented with Baktomix was found statistically significant. The ball plants obtained using the Lännen Plantek methods were remarkably different from all the other variants (Table 2).

The classification of the variants into the homogeneous groups for the trait "root system surface area" is unambiguous. The first distinct group represents the variant with alginite in which there were found the smallest values of root system surface. The difference between the variant with Baktomix and the control was found to be random only (Table 3). The variant Lännen Plantek with ball plants is distinctly different from all the others. There are no overlapping homogeneous groups.

The evaluation of differences in average volume resulted in identification of the same relation as in case of root surface – the final ordering of the variants in case of this variable is increasing too, and the variants are clustered in three homogeneous groups with random within-group differences. The root volume of plants grown in alginite-enriched substrate was the smallest of all the variants. Baktomix caused almost no changes to the root volume compared with the control. Because the results of the Duncan´s test classified the last two variants (Baktomix and control) into one homogeneous group, we can declare the considered difference as random only.

Positive effect on root system formation in terms of volume has only the growing technology Lännen Plantek, for which there was found a mean value more than three times higher than the closest lower mean value. This variant also reached the highest value of volume density: 0.335 g cm^{-3} (Table 4).

The mean diameter of roots of the whole root system was the quantitative characteristic with the lowest variability (see Table 5). At the same time, this trait shows the smallest relative differences between the variants, except the pot-planting variant Lännen. The variance analysis revealed that, with regard to mean root thickness, the treated variants can be classified into two homogenous groups. The first consists of bare-rooted plants irrespective of substrate; the other group comprises ball plants only. Nevertheless, affiliation of the variant with Baktomix to the same group with control and alginite is close to the limit of statistical significance ($p = 0.056$). On the other hand, the hypothesis about difference in means between the control and the variant with alginite can be rejected with much more reliability.

Further we evaluated constant weight of the root systems. This characteristic, unlike those above discussed, can be measured quite easily and can provide an authentic pattern for biomass creation in different conditions. In case of weight of the whole root systems, the individual variants are grouped in the same way as in case of the overall length. The substrate with alginite and the control create one homogeneous group. The second group consists of the control and substrate with Baktomix. The third group again consists only of the ball plants grown using the method Länen Plantek (Table 6).

The number of root tips is a variable reflecting on one hand the root system's growth potential, on the other also its suction capacity. For this reason, in spite of the fact that this variable is quantitative, it rather describes the root system's quality. At the same time, it is the characteristic with the highest variability. In Table 7 we can see that the ball seedlings have almost four times more root tips than the other variants. The significance of differences between the endings number was evaluated by means of variance analysis. From the results listed in Table 8 we can conclude about the high significant differences between the variants. According to the results of the Duncan´s test (Table 7), the bare-rooted seedlings form one homogeneous group. From this group are different, with a high probability, ball seedlings obtained by means of the Länen Plantek method.

Tables 2–7 also contain relative shares of the important biometric variables. From their values it follows that the most distinct characteristic is the root mass, representing in the variant Lännen more than 580% of the root mass in the control. As for the other characteristics, in the Lännen variant they are two or even four times higher compared to the other variants.

Table 2. Comparison of basic statistical characteristics between individual variants, root length (cm)

Variant	Statistical characteristic						
	Mean	s_x	s_x [%]	Min	Max	%	Duncan*
Control	219.20	67.71	30.89	71.50	461.50	100.00	AB
Lännen	805.50	265.98	33.02	367.60	1,454.70	367.50	C
Alginite	192.70	50.32	26.12	97.90	390.90	87.90	A
Bactomix	243.00	71.65	29.49	95.80	442.00	110.90	B

*Differences between variants labelled with the same symbol are not statistically significant ($\alpha = 0.01$).

The bare-rooted variant with Baktomix differs from the control positively in all the evaluated characteristics, except volume and average length the values of which are lower in comparison with the control. These differences, however, are not statistically significant.

Table 3. Comparison of basic statistical characteristics between individual variants, root surface (cm^2)

Variant	Statistical characteristic						
	Mean	s_x	s_x [%]	Min	Max	%	Duncan*
Control	25.48	7.42	29.13	9.16	51.50	100.00	B
Lännen	94.50	28.28	29.93	47.08	156.58	370.90	C
Alginit	21.30	5.30	24.88	11.78	42.02	83.60	A
Bactomix	26.49	7.92	29.89	11.52	52.18	104.00	B

*Differences between variants labelled with the same symbol are not statistically significant (α = 0.01).

Table 4. Comparison of basic statistical characteristics between individual variants, root volume (cm^3)

Variant	Statistical characteristic							
	Mean	s_x	s_x [%]	Min	Max	%	Duncan*	g cm^{-3}
Control	0.24	0.07	32.58	0.09	0.46	100.00	B	0.22
Lännen	0.89	0.25	28.62	0.42	1.34	375.50	C	0.34
Alginite	0.19	0.05	27.48	0.10	0.42	79.70	A	0.23
Bactomix	0.23	0.07	31.55	0.11	0.51	97.50	B	0.25

*Differences between variants labelled with the same symbol are not statistically significant (α = 0.01).

Table 5. Comparison of basic statistical characteristics between individual variants, root diameter (mm)

Variant	Statistical characteristic						
	M	s_x	s_x [%]	Min	Max	%	Duncan*
Control	0.37	0.02	6.32	0.32	0.44	100.00	A
Lännen	0.79	0.22	28.25	0.33	1.22	212.60	B
Alginite	0.35	0.03	8.81	0.31	0.53	94.90	A
Bactomix	0.35	0.02	5.88	0.30	0.42	93.30	A

*Differences between variants labelled with the same symbol are not statistically significant (α = 0.1).

Table 6. Comparison of basic statistical characteristics between individual variants, root mass (g)

Variant	Statistical characteristic						
	Mean	s_x	s_x [%]	Min	Max	%	Duncan*
Control	0.05	0.02	31.00	0.02	0.11	100.00	AB
Lännen	0.30	0.08	25.68	0.15	0.43	584.30	C
Alginite	0.04	0.01	27.65	0.02	0.08	84.30	A
Bactomix	0.06	0.02	30.91	0.01	0.11	111.80	B

*Differences between variants labelled with the same symbol are not statistically significant (α = 0.01).

Table 7. Comparison of basic statistical characteristics between individual variants, number of root tips (n)

Variant	Statistical characteristic							
	Mean	s_x	s_x [%]	Min	Max	%	Duncan*	n cm^{-1}
Control	720.00	234.50	32.58	224.00	1660.00	100.00	A	3.30
Lännen	2,702.00	1,052.87	38.96	1,242.00	5,700.00	375.30	B	3.40
Alginite	687.00	269.40	39.20	280.00	2591.00	95.40	A	3.60
Bactomix	737.00	244.90	33.22	211.00	1731.00	102.40	A	3.00

*Differences between variants labelled with the same symbol are not statistically significant (α = 0.01).

Table 8. Basic values of one-way variance analysis with block experiment for each variant

Source of variability	df	F	p
Length (cm)	3	48.3	0.0001
Surface (cm^2)	3	59.2	0.0001
Volume (cm^3)	3	76.7	0.0001
Mean diameter (mm)	3	1,007.0	0.0001
Weight (g)	3	162.7	0.0001
Root tips number (n)	3	67.5	0.0001
For all variants	block 2, residuum 344		

Discussion

Comparing the growth of root system between the bare-rooted and ball plants we can see that the potted material was significantly better than the bare-rooted in all the evaluated quantitative characteristics. Šmelková and Tichá (2003) report for an experiment with larch the mean root dry weight obtained in variant Lännen representing 145% of dry weight obtained with bare-rooted seedlings. Similar results also reached Tichá (2004) studying spruce seedlings grown in mineral soil and in pots Jiffy 7 Forestry™. In favour of the potted technology, the number of lateral roots being 1.4 times higher than in bare-root technologies gives evidence. The author further reports even a more marked increase – up to four times observed in dry mass of root system of the potted plants. In our experiment (see part *Results*), the difference between the variant Lännen and the control reached almost a value of six.

The lowest mean values of the measured variables (apart from mean diameter) were obtained in the bare-root variant with alginite. This variant is statistically significantly different from the other variants in root system's surface area and volume; in terms of root length and mass, it forms one homogeneous group with the control. The same result also obtained Sarvašová (2006) in her experiment with seedlings of *Abies alba* when she found that the influence of alginite on growth of these seedlings was insignificant in terms of root length and root mass. In experiments with the artificial hydroabsorbent TerraCottem™ coupled with precisely controlled watering, the same author (1999) confirmed for spruce seedlings grown in containers synergic effect of watering and amount of TerraCottem in the substrate in case of all the measured variables, except the aboveground axis. The influence of watering regime was found more pronounced than the influence of amount of the hydroabsorbent.

Takáčová et al. (2006) who carried out experiments with artificial hydroabsorbent Stocksorb Micro™ admixed to substrates used in the Lännen technology, did not observe influence of this substance on growth of aboveground parts of the seedlings. In this case, the differences resulted from differences in photoperiod.

From these results we can conclude that the influence of hydroabsorbents on values of morphological characteristics of the seedlings does not depend on amount of the applied substance only, but also on interactions of these chemicals with other factors – primarily the watering regime, soil type and woody plant species. For this reason, the positive influence of hydroabsorbents is more evident in case of their use at drier and poorer localities. Promising is also their use in protection of root system in transport of and manipulation with planting material. Our results show that in conditions of sufficient water supply, the influence of alginite is rather negative, because most mean values of the evaluated parameters in root systems (not regarding statistical significance of the differences) were the lowest in this variant. The case can be in the water-logged substrate entailing lack in soil oxygen to which the spruce is especially sensitive (Köstler et al., 1968).

Tučeková (2004b) reports the first data obtained in testing the microbiological soil conditioner Bacto-Fil B™. The data show that the weight increment in transplants grown in nursery-beds supplemented with this substance was higher by 100%, the amount of hair roots was higher by 30%. The author, however, gives no information either about roots which were considered as hair roots or about the parameter used in evaluation of these roots´ "richness". The values of root system's length in case of bare-rooted variant grown in the substrate with Baktomix admixture were higher by 10.9%, the values of mass by 11.8% than in the control; on the other hand, the volume reached 97.5% of the control, only. These differences, however, are not statistically significant, and based on the results of the Duncan's test, this variant forms one homogeneous group with the control.

Our experimental material evaluated in this paper does not reach the values of biometric characteristic required for a standard planting material according to the Standard STN 48 2211 (Silviculture, seedlings and plants of forest woody plants). Even in case of the ball plants grown in the variant, the requirements of the Standard have not been met – due to too small diameter of the root collar having had the mean value of 1.219 mm, and the maximum of 2.25 mm.

The obtained results are high reliable and precise, and they can be used with profit in planning the following experiments.

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Vplyv substrátu a technológie pestovania na kvantitatívne charakteristiky koreňových systémov semenáčikov smreka (*Picea abies* (L.) Karst.): porovnanie kvantitatívnych znakov

Súhrn

Táto práca analyzuje vplyv rozlične modifikovaných substrátov a technológie Lännen Plantek na rast koreňových systémov 1-ročných smrekových semenáčikov. Substrát pre voľnokorenné semenáčiky bol vytvorený zmesou rašeliny a kompostu. Doňho bol potom primiešaný bakteriálny prípravok Baktomix® (4 l/m^2) a ílovitý minerál alginit (3 kg m^{-2}). Čistý substrát slúžil ako kontrola. Voľnokorenné sadenice boli porovnávané s krytokorennými vypestovanými technológiou Lännen Plantek. Zisťovali sa nasledujúce parametre: hmotnosť koreňového systému v sušine, dĺžka koreňov, veľkosť ich povrchu, počet zakončení koreňa (koreňových špičiek) a jeho objem. Tieto parametre boli zisťované pomocou programu WinRhizo a následne boli analyzované.

Variabilita jednotlivých hodnôt bola pomerne nízka, pohybovala sa okolo 30 %. Variant Lännen Plantek dosahoval vo všetkých sledovaných parametroch niekoľkokrát vyššie hodnoty ako voľnokorenné semenáčiky. Rozdiely v týchto znakoch medzi samotnými voľnokorennými variantmi boli, aj napriek malým diferenciám v ich hodnotách, väčšinou štatisticky významné, a to vďaka spomínanej nízkej variabilite. Pri biometrických znakoch priemerná hrúbka a počet zakončení tvoria všetky tieto tri varianty homogénnu skupinu, v ostatných hodnotených znakoch patria do rovnakej skupiny Baktomix spolu s kontrolným variantom, alginit s kontrolou pri hodnotách parametrov hmotnosť a dĺžka koreňového systému. Diferencie medzi Baktomixom a alginitom sú signifikantné v hmotnosti, dĺžke, ploche povrchu aj veľkosti objemu. Použitie alginitu pôsobí pri rovnakom závlahovom režime skôr nepriaznivo na rastové schopnosti koreňov, štatisticky významne pri $\alpha = 0{,}01$ sa od ostatných variantov líši nižšími hodnotami povrchu a objemu koreňa.

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