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ORIGINAL PAPER

Effect of different innovative substrate mediums on roots characterization of European beech *Fagus sylvatica* L. and pedunculate oak *Quercus robur* L. seedlings

Odunayo James Rotowa✉, Stanisław Małek, Jacek Banach, Maciej Pach

Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, Al. 29 Listopada 46, 31-425 Kraków, Poland

ABSTRACT

The development of a root system is crucial for the effective establishment of forest tree seedlings. There are various seedling production methods in nurseries adopted by professionals and foresters to guarantee quality root systems aimed at successful forest plantations. This study evaluated the effect of different innovative, peat-free organic substrates (R20, R21 and R22) on the root system and nutrient content in the root zone of European beech and pedunculate oak seedlings. This was done to examine if the newly designed substrate and liquid fertilizer formulated by the University of Agriculture in Krakow (UAK) would successfully grow seedlings that meet the existing characteristics of those raised with peat substrate and solid fertilizer. Although the properties and granulometric composition of the substrates were different during the production process of the seedlings, two different Osmocote fertilizers (solid 3-4M and 5-6M) were applied. Fertilization used in the State Forest nurseries based on the set standard was represented with SR20, SR21 and SR22, while the novel fertilizer developed by UAK was represented with UR20, UR21 and UR22. Meanwhile, SC and UC represent the control substrates (peat) in both cases, respectively. The substrates developed by UAK were adapted to the nutritional requirements of the forest tree seedlings and their suitability was monitored using nursery technology with a covered root system in multi-pot containers. The experiment was laid out in a 2×2×4 (2 species, 2 types of fertilizers and four different substrates) experimental design using five seedlings per treatment. The results of the study indicated that the innovative substrate and fertilizer support root system development and aid sufficient macro element content for seedling production in the nursery. Treatment UR20 recorded the highest mean value of total root length in both species. A significant variation was observed from the analysis of nutrients in the root system. Conclusively, substrate mediums developed under this study have proven to possess qualities not worse than the substrate based on peat because the root system is adequately well developed. This guarantees the quantity and reliability of supplies and could replace high peat in the substrate formula.

KEY WORDS

forest seedlings, peat, peat-free organic substrate, root

✉e-mail: odunrotowa@yahoo.com

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Introduction

Forest tree seedlings can be grown in conventional (ground) nurseries or unique compartments often called container nurseries. Irrespective of the method adopted, seedling development relies on several factors for survival such as the availability and accessibility to light, water, and mineral supplements as well as the type of substrate medium used and its physical properties to grow the seedlings (Alameda and Villar, 2009; Perez-Ramos *et al*., 2010; Kormanek, 2013, Pająk *et al*., 2022a). Different standards are utilized to evaluate the seedlings' suitability for plantation establishment. This could be based on attributes such as height and dry weight. However, previous studies have also suggested other factors including seedling sturdiness quotient (SQ) and shoot-root index (S/R) which better indicate the capability of seedlings for utilization (Haase, 2007; Grossnickle, 2012; Ivetić and Skorić, 2013; Banach *et al*., 2020, 2021).

Peat serves as the primary component in nursery substrates as it is known for its exceptional physical, chemical and biological properties. Its remarkable water retention capacity and consistent, high-quality attributes make it an ideal medium for plant cultivation. However, peat soils accumulate a substantial amount of carbon over time which could have profound climate implications. While forests typically sequester carbon, peatlands can inadvertently release it into the atmosphere. This poses a significant challenge since peatlands store more soil carbon, equivalent to over one-third of the world's total, surpassing even the combined carbon storage of all global forests. When peat is spread on plantations, it quickly transforms into carbon dioxide contributing to elevated greenhouse gas levels and endangering precious ecosystems. The annual excavation of 20,000 cubic meters of peat according to Gruda (2012) further exacerbates environmental degradation. As a result of global environmental concerns associated with the use of peat as a standard nursery substrate, peatland should rather be preserved and not destroyed. The growing emphasis on environmental sustainability necessitates the need to design an innovative peat-free organic substrate with materials that are sustainable, cheap and ecologically friendly as alternatives to peat.

Historically rooting space is measured as a plant resource, yet research on biomass portion versatility related to root volume (RV) is uncommon. However, root volume can be considered an asset for plant growth (McConnaughay and Bazzaz, 1991). A decrease in rooting volume can alter entire plant development based on nutrient accessibility. Mechanical limitations forced to root growth and development by the volume of a container has been a significant issue of concern for forest plants (Landis, 1990; Ferree *et al*., 1992; Beeson, 1993; NeSmith and Duval, 1998; Aphalo and Rikala, 2003; Dominguez-Lerena *et al*., 2006). Root limitation lessens crop development and expansion in shoot/root biomass proportion (NeSmith *et al*., 1992; Hsu *et al*., 1996; Clemens *et al*., 1999). The impact of root limitation in different species has been studied (Endean and Carlson, 1975; Carlson and Endean, 1976; Lamhamedi *et al*., 1998; South *et al*., 2005; Dominguez-Lerena *et al*., 2006). The growth response of seedlings to compact rooting volume may be based on species (NeSmith and Duval, 1998; Climent *et al*., 2008).

In Poland, coniferous monocultures have been intensively restructured due to the declining health and quality of trees. European beech *Fagus sylvatica* L. is an Atlantic climate species found throughout central and western Europe (Jaworski, 2019). Oak *Quercus robur* L. is a significant tree species in Polish forests and the majority of European temperate vegetation types. Due to their excellent wood quality, beech and oak are becoming more competitive than several conifers as they are the preferred tree genera in adaptation strategies to climate change for both ecological and economic reasons in Europe (Rotowa *et al*., 2023). Hence, it is of paramount importance to intensify efforts to raise the health and the quantity of sustainable forest stands of these highly sought species.

Therefore, a comparison of the root biomass allocation and ontogenetic parameters of beech and oak seedlings of contrasting substrate treatments were used as the basis for this study. The following hypothesis was tested for beech and oak seedlings grown in a container nursery using organic substrate: the features of beech and oak seedlings grown on a peat-free substrate and liquid fertilizer developed by the University of Agriculture in Krakow are similar to those grown on a standard substrate (peat plus solid fertilizer).

Materials and methods

SUBSTRATE COMPOSITION AND PREPARATION. The peat substrate used for this study as the control variant (C) was produced at Nursery Farm in Nędza (50.167964 N, 18.3138334 E). The substrate composition included peat 93% and perlite 7% with the addition of dolomite (3 kg per m^3 of the substrate) to obtain a pH of 5.5. The peat-free substrates (R20, R21, R22) consisted of a blend of various components including scobs, wood chips, straw, bark, perlite, core wood and mixed silage [%]. These components were combined in varying proportions as shown in Table 1. The peat-free substrates and liquid fertilizer used in the study were prepared under the project POIR.04.01.04-00-0016/20 funded by the National Centre for Research and Development (NCBiR) from National Resources and the European Regional Development Fund entitled 'Innovative technologies for the production of substrate and fertilizer produced from indigenous resources for the production of forestry tree seedlings' which was led by the Department of Ecology and Silviculture, Forest Faculty, the Agricultural University of Krakow. Four substrates (R20, R21, R22 and peat) and two fertilization variants were used. The first was a standard fertilizer in the Suków container nursery (SR20, SR21 and SR22 variants) and the second was a novel liquid fertilizer designed by the University of Agriculture in Krakow (UR20, UR21 and UR22). The peat substrate used in both fertilizer scenarios included two control variants (SC and UC). The substrates were mineralized with a microwave mineralizer MARS CEM in a mixture of HCl (35-38%) and HNO_3 (65%) acids at the Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation in the Department of Ecology and Silviculture and Faculty of Forestry, University of Agriculture in Krakow. In each experimental variant seedlings of both species were grown in 75 Marbet V300 polystyrene containers each containing 53 cells with a volume of 275 cm^3 (Fig. 1). The cells were tapered downward and were equipped with vertical guides for the root systems. The components of the peat that were adapted for the preparation of the substrate used for this study are shown in Table 2, although the particle sizes of the substrate before seed sowing were different (Table 3). The nutrient content present in the substrate was the same before seed sowing, however, it became different at the end of seedling production (Table 4).

SEED SOWING AND GERMINATION. After filling the containers with the various substrates, beech and oak seeds were sown manually in the Suków-Papierna Nursery Farm (Daleszyce Forest District). The seeds were sown on April 19-20, 2022 with the preparation and sowing of seeds

Table 1.
Properties of the organic peat free substrate

Substrate	Scobs	Wood chips	Straw	Wood bark	Perlite	Core wood	Mixed silage
	[%]						
R20	73	10	–	10	4	2	1
R21	20	63	–	10	4	2	1
R22	50	–	10	33	4	2	1

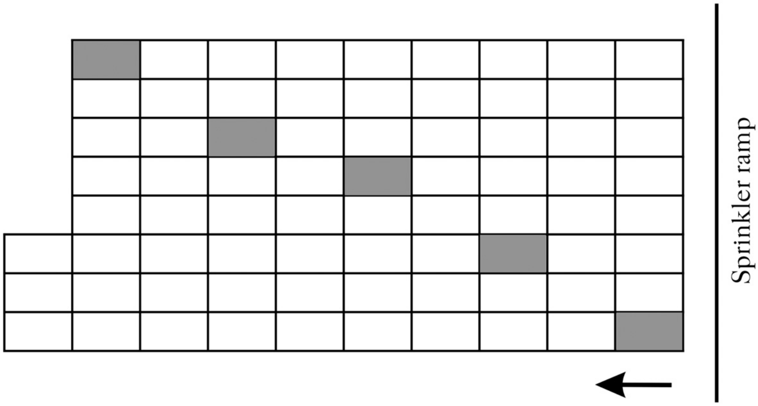

Fig. 1.
Distribution of containers in the production field for one experimental variant; the containers from which seedlings were taken for analysis are marked in grey

Table 2.
Mean and standard deviation values of the organic substrate properties

Substrate	Water capacity [%]	Average [litre/min]	Variation factor [%]	Bulk density [g/cm^3]	Solid density [g/cm^3]	Air capacity [%]	Porosity [%]
R20	53.02 ±2.42	0.595 ±0.150	25.2	0.127 ±0.009	1.56 ±0.000	38.90 ±2.90	91.85 ±0.60
R21	45.39 ±3.60	0.781 ±0.114	14.6	0.103 ±0.013	1.61 ±0.000	48.14 ±4.20	93.62 ±0.83
R22	50.71 ±2.11	0.594 ±0.150	25.3	0.113 ±0.009	1.62 ±0.000	42.35 ±2.61	93.04 ±0.55
Control	71.44 ±2.83	0.417 ±0.145	34.9	0.091 ±0.006	1.59 ±0.000	22.89 ±3.15	94.25 ±0.39

Table 3.
Mean and standard deviation values of granulometric composition of the substrate before sowing

Substrate	>10 mm	10-5 mm	5-2 mm	2-1 mm	1-0.5 mm	0.5-0.25 mm	0.25-0.1 mm	>0.1 mm
R20	0.05 ±0.10	3.77 ±1.57	14.45 ±5.90	30.53 ±9.72	24.45 ±2.24	17.72 ±7.49	7.71 ±4.16	1.69 ±0.98
R21	0.00 ±0.00	6.40 ±1.37	25.44 ±1.91	30.90 ±1.11	19.11 ±0.90	12.16 ±0.31	5.12 ±0.41	0.96 ±0.11
R22	0.08 ±0.13	3.03 ±0.45	14.15 ±2.36	33.36 ±2.36	25.11 ±1.07	17.02 ±3.21	7.11 ±1.72	1.48 ±0.28
Control	0.00 ±0.00	11.27 ±0.37	25.08 ±1.18	27.77 ±1.05	16.20 ±1.05	8.42 ±0.56	3.81 ±0.43	1.88 ±0.21

carried out by workers at the container nursery. To enhance the germination process, oak seeds were scarified just before sowing which involved the removal of approximately one-third of the seed in the cotyledon part. In contrast, beech seeds underwent a stratification process without the use of a stratification medium with temperature maintained at +3°C and humidity at 31%. The seeds used for all substrate variants, regardless of species, were sourced from the same provenance and came with separate certificates of origin (MR/65848/21/PL for oak and MR/63313/20/PL for beech). After sowing, the containers were placed in a vegetation hall for four weeks and then transported to an external production field. During the growth of the seedlings manual weeding was employed. The seedlings were grown for five months following the procedure used in the container nursery (Szabla and Pabian, 2009). During the seedling growth period the total rainfall was only 78 mm, therefore, to replenish the water deficit irrigation was applied using an automatic RATHMAKERS Gartenbautechnik sprinkler ramp.

Osmocote fertilizer was applied once during substrate preparation before sowing at a total dose of 3 kg m^{-3} of each substrate medium, prepared as a mixture of Osmocote 3-4M (2 kg) and

Table 4.
Nutrient content of substrates before seed sowing and after seedling production

Substrate	C	N	P	K	Ca	Mg	Na
	[%]						
Before sowing							
R 20	48.01	0.297	0.031	0.159	0.452	0.055	0.040
R 21	46.34	0.507	0.068	0.271	0.601	0.072	0.035
R 22	48.9	0.447	0.043	0.404	0.857	0.059	0.042
Control	45.85	0.709	0.015	0.058	1.307	0.585	0.068
After seedling production							
Beech							
UR20	44.148	0.434	0.030	0.066	0.677	0.055	0.016
UR21	42.518	0.5323	0.049	0.072	0.854	0.055	0.015
UR22	42.93	0.578	0.043	0.074	1.179	0.066	0.018
UC	39.784	0.651	0.019	0.071	1.543	0.525	0.072
SR20	42.167	0.596	0.093	0.129	0.721	0.068	0.018
SR21	39.978	0.996	0.134	0.161	0.985	0.087	0.020
SR22	42.167	0.756	0.110	0.156	1.463	0.086	0.023
SC	40.987	0.844	0.096	0.162	1.695	0.476	0.075
Oak							
UR20	44.703	0.383	0.028	0.563	0.594	0.065	0.015
UR21	44.969	0.418	0.032	0.597	0.627	0.042	0.015
UR22	45.422	0.493	0.032	0.650	0.966	0.060	0.015
UC	41.863	0.654	0.016	0.654	1.392	0.472	0.060
SR20	45.455	0.519	0.059	0.991	0.589	0.056	0.014
SR21	43.313	0.942	0.121	1.626	0.879	0.088	0.020
SR22	45.096	0.872	0.114	1.703	1.139	0.081	0.018
SC	41.425	0.805	0.076	1.798	1.424	0.441	0.069

S – State Forests fertilization, U – University fertilization, R – novel substrates, C – control substrate (peat-perlite)

Osmocote 5-6M (1 kg). The composition of the Osmocote fertilizer 3-4M was the following: N – 16% including 7.1% N-NO_3^- and 8.9% N-NH_4^+; P_2O_5 – 9%, K_2O – 12%; MgO – 2.0%, and microelements (B, Fe, Cu, Mn, Zn, Mo); 5-6M: N – 15%; including 6.6% N-NO_3^- and 8.4% N-NH_4^+; P_2O_5 – 9.0%; K_2O – 12%; MgO – 2.0%; and microelements (B, Fe, Cu, Mn, Zn, Mo). The novel liquid fertilizer applied was based on two distinct compositions. The first variant consisted of N at 4.78%, P_2O_5 at 1%, K_2O at 2.64%, CaO at 2.65%, MgO at 1.4%, SO_3 at 0.71%, and Na_2O at 0.14%. This fertilizer was administered initially with a total volume of 3.14 dm^3 (0.048 $dm^3 \cdot 1\ m^{-2}$). The second fertilizer variant contained N at 0.798%, P_2O_5 at 0.166%, K_2O at 0.440%, CaO at 0.441%, MgO at 0.234%, SO_3 at 0.118%, and Na_2O at 0.023%. The second fertilizer was applied with a total volume of 15.09 dm^3 (0.229 $dm^3 \cdot 1\ m^{-2}$). Over the course of seedling production the first fertilizer variant was applied eight times at 10-day intervals, while the second variant was applied fifteen times at 5-day intervals. It is important to note that the fertilization regimes remained consistent for both beech and oak seedlings.

Parameter assessment and analysis of nutrients. At the end of the nursery's production cycle, a thorough examination of seedlings was conducted. Due to limitations stemming from the availability of seedling parts for laboratory testing, a specific selection process was employed. Five seedlings, characterized by standard vigor and biometric parameters, were carefully chosen from each of the eight treatment groups for the purpose of data collection. This resulted in a total assessment of 40 seedlings for the two species used in the experiment (beech and oak). To obtain seedlings for measurements, five containers in each treatment were selected with 1 seedling taken from each container. These selected containers were distributed diagonally across the experimental field. After measuring the height and root collar diameter of the seedlings, one representative seedling was chosen from each of these subsamples for further analysis which included the evaluation of root system parameters and nutrient content.

Biometric data was collected on root collar diameter (RCD), total root length (TRL), root surface area (RSA), average root diameter (ARD), and root volume (RV). All these root parameters were analyzed using WinRHIZO software in the Laboratory of Forest Biotechnology, Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, Poland.

Subsequently, the roots of the sampled seedlings were dried at 105°C for 48 h, ground into a powder form and analysed for their N, S, and C content using a LECO CNS TruMac analyzer and P, K, Ca, and Mg contents using a Thermo iCAP 6500DUO ICP-OES following mineralization in nitric and hydrochloric acids at a ratio of 3:1. The concentrations of most chemical elements (expressed in percentages) were determined using spectrometer ICP OES with the exception of C and N which were determined using the TruMac LECO apparatus. The analyses were performed at the Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation in the Department of Ecology and Silviculture and Faculty of Forestry, University of Agriculture in Krakow, Poland.

Statistical Analysis. The experiment consisted of two species (beech and oak), two fertilizers (solid and liquid) and four substrates for each variant (R20, R21, R22 and control). This was laid out in a 2×2×4 experimental design using five seedlings per treatment. To show the comparative performance between the treatments, the collected data (after verifying that it met the assumptions of ANOVA) were subjected to mean and variance analysis (ANOVA). At the same time, the Duncan Multiples Range Test (DMRT) was applied to locate where the significant difference occurred in the treatments at $p<0.05$. This was done to confirm the substrate variant that differed significantly from the others according to the selected biometric features and elemental content in the root systems of both species. A correlation test was further carried out to quantify the strength of the linear relationship between the analyzed variables.

Results

Root characteristics of beech and oak seedlings grown in different types of organic substrates. The root collar diameter of beech seedlings showed evident variation among the eight treatments. Treatment SR22 recorded the highest mean value of 5.95 ±0.75 mm followed by 5.51 ±0.84 mm recorded in SC, while the lowest mean value of 4.00 ±0.58 mm was recorded in the UR22 samples. The results of variance analysis showed that significant variations exist among the treatments. The results for total root length revealed a different trend from the results of root collar diameter. Treatment UR20 recorded the highest mean value (1436.84 cm) followed by the SR20 variant, and the lowest value for this parameter was recorded in the UR21 variant which had a significant difference. The analysis of average root diameter and volume showed that the highest values were recorded in variant UR21 of 0.47 mm and 1.15 cm^3, respectively (Table 5).

Treatment SC recorded the highest mean value of 2.78 mm for the root collar diameter of oak seedlings. The results of the parameters assessed revealed that UR variants has competitive growth and in some cases performs better especially on total root length and surface area. Excellent growth trend was also recorded in the SC variant, especially in average root diameter and root volume.

Table 5.
Mean and standard deviation of studied root system parameters of *F. sylvatica* and *Q. robur*

Treatment	RCD [mm]	TRL [cm]	RSA [cm^2]	ARD [mm]	RV [cm^3]
			F. sylvatica		
SR20	4.25 ±0.69c	1412.40 ±199.04ab	140.61 ±22.21a	0.31 ±0.01de	1.12 ±0.16bc
UR20	4.35 ±0.77d	1436.84 ±145.29a	134.99 ±12.58a	0.30 ±0.01e	1.01 ±0.16b
SR21	5.26 ±0.87d	825.19 ±70.63 ab	102.22 ±10.78ab	0.39 ±0.02ab	1.02 ±0.16bc
UR21	5.16 ±0.57d	647.47 ±37.07b	95.24 ±8.41ab	0.47 ±0.04a	1.15 ±0.16ab
SR22	5.95 ±0.75b	1067.23 ±204.39a	137.49 ±15.90a	0.43 ±0.04ab	1.47 ±0.16a
UR22	4.00 ±0.58d	656.20 ±98.60b	72.24 ±6.29b	0.36 ±0.02bc	0.64 ±0.16c
SC	4.37 ±0.42d	897.99 ±134.33bc	96.09 ±14.86ab	0.34 ±0.02cd	0.83 ±0.16ab
UC	5.51 ±0.84a	686.06 ±175.92a	82.93 ±18.69b	0.41 ±0.02ab	0.81 ±0.16bc
Total	4.86 ±0.92	953.67 ±66.99	107.73 ±6.13	0.38 ±0.01	1.01 ±0.16
P-value.	0.00**	0.00**	0.00**	0.00**	0.02*
			Q. robur		
SR20	1.66 ±0.32c	730.57 ±177.88a	113.94 ±18.81a	0.53 ±0.06a	1.51 ±0.33a
UR20	0.96 ±0.07d	894.97 ±112.22a	106.18 ±12.40a	0.38 ±0.01a	1.00 ±0.12a
SR21	1.19 ±0.13d	631.41 ±90.58a	111.33 ±12.35a	0.57 ±0.04a	1.60 ±0.22a
UR21	1.16 ±0.19d	619.11 ±125.43a	122.63 ±19.92a	0.67 ±0.14a	2.16 ±0.57a
SR22	2.15 ±0.49b	603.21 ±78.10a	109.63 ±11.48a	0.60 ±0.04a	1.61 ±0.19a
UR22	0.87 ±0.06d	820.04 ±179.87a	110.89 ±15.09a	0.46 ±0.04a	1.24 ±0.16a
SC	2.78 ±0.54a	843.29 ±134.75a	167.23 ±27.81a	0.64 ±0.08a	2.67 ±0.48a
UC	1.15 ±0.19d	494.63 ±126.36a	83.23 ±17.95a	0.99 ±0.49a	1.45 ± 0.33a
Total	1.49 ±0.69	704.65 ±128.15	115.63 ±16.69	0.61 ±0.16	1.65 ±0.44
P-value.	0.00**	0.38ns	0.12 ns	0.46ns	0.39ns

S – State Forests fertilization, U – University fertilization, R – novel substrates, C – control substrate (peat-perlite), RCD – Root collar diameter, TRL – Total root length, RSA – Surface area, ARV – Avg. Diameter, RV – Root Vol. The same letter in the same column are not significantly different while figures with different letter are significantly different at p=0.05

According to the results of ANOVA, significant variation was recorded in the entire root collar diameter based on different substrate formulations. Beyond the root collar diameter and total root length, root surface area, average root diameter, and root volume also varied based on different treatments (Table 5).

A significant positive correlation was observed between root collar diameter and average root diameter and root surface area for beech roots. A negative relationship was observed for oak between root length and average root diameter which was substantial, while most of the other parameters showed significantly positive correlations. Additionally, all assessed parameters consistently exhibited a negative correlation with the substrate treatments with the implication that as the seedlings grow older, the rate of nutrient absorption reduces. This observed negative correlation indicates an inverse relationship between the substrate treatments and the root biometrics parameters (Table 6).

Content of macro elements. The concentration of elements in the root systems differed between treatments and tree species. The highest macro element concentration in the beech root system was in the C variant with a total mean value of 46.23%. The highest mean value of C content was 47.56% recorded in treatment UR20, and the lowest mean value (44.71) was recorded in treatment UR21. The result was the same for oak roots. The highest mean value of 46.04% was recorded in treatment UR20, and the lowest mean value (44.22%) was recorded in treatments SR22 and UR22, respectively. The root system's lowest macro element concentration was in S with a total average mean value of 0.047% in beech and 0.042% in oak roots. The highest mean

Table 6.
The results of correlation analysis of biometric root parameters

Parameter assessed	Root collar diameter	Root surface area	Average root diameter	Root volume	Root length	Treatment
			Beech			
Root collar diameter	1					
Surface area	0.337*	1				
Average root diameter	0.371*	–0.160	1			
Root volume	0.530**	0.821**	0.419**	1		
Root length	0.092	0.901**	–0.552**	0.498**	1	
Treatment	–0.310	–0.175	0.058	–0.118	–0.154	1
			Oak			
Root collar diameter	1					
Surface area	0.362*	1				
Average root diameter	0.058	–0.223	1			
Root volume	0.407**	0.718**	0.391*	1		
Root length	0.041	0.745**	–0.491**	0.145	1	
Treatment	–0.706**	–0.256	–0.043	–0.285	–0.0004	1

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level

S values (0.067%) were recorded for beech roots in treatment UC while that of oak (0.055%) was recorded in SR22. The N content in roots was highest in variants SC and SR22 of beech and oak roots, respectively. N showed significant variation amongst treatments in beech but not in oak roots (Table 7). A significant negative correlation exists between N and Mg in beech roots, between N and P, N and S, and P and K in oak roots. (Table 8)

Discussion

The analysis of biometric features and content of macro elements of the root systems of the beech and oak seedlings under study suggests that the innovative substrate mediums and fertilizers utilized effectively promote root system development. In essence, the characteristics of beech and oak seedlings cultivated in a peat-free substrate, coupled with the liquid fertilizer developed by the University of Agriculture in Krakow, closely resemble those of seedlings grown on the conventional substrate comprising peat and solid fertilizer.

In the present study, however, a significant positive correlation was discovered to exist among the biometrics features. Analysis of variance showed considerable variation in the entire root collar diameter. This could be attributed to the variation in properties and granulometric composition of the substrate since the lower density of substrates allows better root penetration and nutrient transportation (Arvidsson, 1999; Pająk *et al.*, 2022a). Beyond the root collar diameter and total root length, root surface area, root average diameter, and root volume also varied between treatments resulting in the significant formation of a viable root system. This is consistent with the results of previous studies by Kormanek *et al.* (2015) in the root growth of *Quercus petraea* (Matt.) Liebl. seedlings. Tworkoski *et al.* (1983) reported a reduction in the growth of *Quercus alba* L. with variations in the compartment medium and density. It can further be deduced from the results obtained that these seedlings could be planted in the forest as the root system is adequately well-developed. These results, therefore, corroborate other studies on forest tree species grown in containers (Sands and Bowen, 1978; Corns, 1988; Pająk *et al.*, 2022a).

There is no doubt that when tree seedlings are well nourished it increases their performance in the forest. To this end, nourishment has been considered a significant property in seedling

Table 7.
Percentage of macro elements in the roots of beech and oak seedlings for each treatment (±SD)

Treat-ment	C	N	P	K	S	Ca	Mg
	[%]						
			F. sylvatica				
SR20	45.68 ±2.00a	0.622 ±0.011ab	0.145 ±0.057a	0.389 ±0.115a	0.037 ±0.011a	0.494 ±0.115a	0.119 ±0.057a
UR20	47.56 ±2.00a	0.663 ±0.115ab	0.071 ±0.011a	0.323 ±0.115a	0.049 ±0.011a	0.648 ±0.057a	0.158 ±0.088a
SR21	45.75 ±2.00a	0.563 ±0.011abc	0.148 ±0.011a	0.434 ±0.115a	0.031 ±0.011a	0.499 ±0.115a	0.120 ±0.115a
UR21	44.71 ±2.00a	0.340 ±0.057c	0.054 ±0.011a	0.408 ±0.115a	0.032 ±0.011a	0.408 ±0.057a	0.087 ±0.005a
SR22	46.61 ±2.00a	0.695 ±0.115a	0.134 ±0.057a	0.402 ±0.115a	0.057 ±0.011a	0.442 ±0.057a	0.111 ±0.057a
UR22	45.98 ±3.06a	0.388 ±0.057bc	0.060 ±0.011a	0.374 ±0.115a	0.042 ±0.011a	0.367 ±0.011a	0.092 ±0.001a
UC	47.18 ±2.00a	0.682 ±0.115a	0.039 ±0.011a	0.305 ±0.115a	0.067 ±0.011a	0.243 ±0.057a	0.127 ±0.057a
SC	46.38 ±2.00a	0.689 ±0.115a	0.137 ±0.057a	0.390 ±0.115a	0.064 ±0.011a	0.251 ±0.057a	0.154 ±0.115a
Total	46.23 ±2.00	0.580 ±0.037	0.098 ±0.014	0.378 ±0.035	0.047 ±0.004	0.419 ±0.034	0.121 ±0.014
p-value	0.801ns	0.051*	0.201ns	0.993ns	0.237ns	0.22ns	0.948ns
			Q. robur				
SR20	44.860 ±2.00a	0.501 ±0.173a	0.087 ±0.005a	0.464 ±0.115a	0.036 ±0.011a	0.382 ±0.115a	0.108 ±0.057a
UR20	46.043 ±1.50a	0.414 ±0.000a	0.049 ±0.011a	0.418 ±0.115a	0.032 ±0.011a	0.364 ±0.115a	0.105 ±0.057a
SR21	44.790 ±2.00a	0.690 ±0.115a	0.158 ±0.057a	0.682 ±0.115a	0.044 ±0.011a	0.357 ±0.115a	0.119 ±0.057a
UR21	44.530 ±2.00a	0.509 ±0.115a	0.109 ±0.057a	0.492 ±0.115a	0.036 ±0.001a	0.364 ±0.115a	0.121 ±0.011a
SR22	44.220 ±2.00a	0.831 ±0.115a	0.163 ±0.057a	0.576 ±0.115a	0.055 ±0.005a	0.362 ±0.057a	0.107 ±0.057a
UR22	44.220 ±2.00a	0.412 ±0.577a	0.055 ±0.011a	0.585 ±0.115a	0.048 ±0.011a	0.389 ±0.115a	0.100 ±0.057a
SC	44.600 ±3.00a	0.688 ±0.115a	0.113 ±0.057a	0.446 ±0.115a	0.054 ±0.011a	0.232 ±0.011a	0.130 ±0.057a
UC	44.380 ±1.00a	0.436 ±0.115a	0.035 ±0.011a	0.408 ±0.000a	0.036 ±0.011a	0.300 ±0.057a	0.139 ±0.057a
Total	44.705 ±2.13	0.560 ±0.449	0.096 ±0.015	0.509 ±0.036	0.042 ±0.003	0.344 ±0.030	0.116 ±0.016
p-value	1.000ns	0.126ns	0.286ns	0.605ns	0.702ns	0.944ns	0.999ns

S – State Forests fertilization, U – University fertilization, R – novel substrates, C – control substrate (peat-perlite); figures with the same letter in the same column are not significantly different while figures with different letter are significantly different at p=0.05, separately for each species

Table 8.
The results of correlation analysis of macro element content

Elements	C	N	P	K	S	Ca	Mg
			Beech Root				
C	1						
N	0.598	1					
P	–0.241	0.418	1				
K	–0.767*	–0.350	0.652	1			
S	0.683	0.682	–0.144	–0.593	1		
Ca	0.042	–0.001	0.194	0.094	–0.547	1	
Mg	0.419	0.825*	0.431	–0.234	0.607	–0.212	1
			Oak Root				
C	1						
N	0.037	1					
P	0.156	0.880**	1				
K	0.002	0.482	0.768*	1			
S	–0.345	0.746*	0.630	0.467	1		
Ca	–0.064	–0.263	0.081	0.410	–0.334	1	
Mg	0.177	0.069	–0.154	–0.372	–0.066	–0.780*	1

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level quality (Burdett, 1983; Landis, 1985; Puttonen, 1989; Ritchie *et al*., 2010; Hawkins, 2011). Although the results of nutrient content show no significant differences among the substrates and fertilizer treatments (except N in beech), the percentage value of nutrient content amongst the species and treatments falls within the commonly used percentage value that meets the needs of plant growth which is consistent with studies by Baule and Fricker (1973) who reported the demand for Ca and K as high in forest trees. Dzwonko (1990) reported that beech seedlings develop better in a substrate rich in Ca, Mg, and K. Balcar *et al*. (2011) and Pająk *et al*. (2022b) reported that the use of dolomite (which contains both Mg and Ca) to fertilize beech trees has a positive effect on the growth of the seedlings, especially root systems.

Other studies have reported that the use of Mg has enhanced yields by 8.5% in numerous experiments across different nations of the world regardless of the species of tree, soil and substrate conditions, and other factors in China (Wang *et al*., 2020) and Poland (Pająk *et al*., 2022b). Furthermore, Wang *et al*. (2020) reported that using Mg fertilizers is more efficient at improving growth and yields than using N, P, and K. The average mean value of Mg in this study (0.121% and 0.116% for beech and oak root, respectively) falls slightly below 0.165% as reported by Pająk *et al*. (2022b) on the root system of European beech. Banach *et al*. (2013) reported that the structure of the substrate was vital for proper growth in beech seedlings. The type of fertilizer used for seedling production is also very important for this species, as demonstrated by Banach *et al*. (2021). It can, therefore, be said that the substrate medium and fertilizer developed and used to raise these forest seedlings is rich in essential macro elements, especially the control variants of the treatments possibly due to the addition of dolomite additives that were added to increase the pH of the substrates.

The results of various element contents obtained from the root systems of the studied beech and oak seedlings indicates that the substrates mediums used and fertilizers applied (especially the novel design) enhanced the root system in a manner that has the ability to increase Mg uptake by seedlings further as they grow beyond nursery stage. This could be related to the low density of the organic substrates used. This is supported by the results reported of Pająk *et al*. (2022b) that an improved root system resulted in increased Mg uptake in *F. sylvatica* seedlings from lower substrate densities which in turn resulted in a better proportion of dry-weight above- to below-ground parts of the seedlings. Potassium likewise plays a significant role in stacking the phloem and carbohydrate transportation in plants. Its deficiency may likewise result in an expansion in the dry-weight S: R proportion as reported for *Phaseolus vulgaris* L. and *Betula pendula* Roth cuttings (Cakmak, 2013). Treatment with dolomite decreases soil acidification and increases the Mg content in the plant. Additionally, dolomite promotes enzymatic action in the peat substrate as confirmed by different studies carried out in forest nurseries on F. *sylvatica* (Lasota *et al*., 2021; Pająk *et al*., 2022a) and *Q. robur* (Lasota *et al*., 2021).

Conclusions

The results of the root biometric features indicate that the different substrate treatments caused significant variation in root length, root surface diameter, and average root volume in beech and oak seedlings. The effects of these treatments were visible in differences in root formation and macro element concentrations in the root system. Interestingly, the novel peat-free organic substrate and fertilizer mediums developed by the University of Agriculture in Krakow have shown strong competitiveness with organic peat. The newly designed substrate and liquid fertilizer formulation by the University of Agriculture in Krakow for beech and oak seedlings grown in container nurseries using organic substrate met the existing characteristics of those raised with peat substrate and solid fertilizer. The root system was adequately well developed to tap into the soil for nutrients and water necessary for plant growth which further guarantees plant growth and survival.

Authors contributions

O.J.R. – paper concept, methodology, manuscript preparation, data collection, statistical analysis, literature review, writing; S.M. – funding acquisition, project administration, reviewing, and editing; J.B. – data editing, writing, review; M.P. – literature review, writing, editing, and review.

Conflict of interest

The authors declare no conflict of interest.

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STRESZCZENIE

Wpływ różnych podłoży na charakterystykę korzeni sadzonek buka zwyczajnego *Fagus sylvatica* L. i dębu szypułkowego *Quercus robur* L.

W celu zagwarantowania rozwoju systemów korzeniowych szkółkarze stosują wiele metod produkcji materiału szkółkarskiego. W pracy oceniono wpływ innowacyjnych beztorfowych podłoży organicznych (R20, R21 i R22) na rozwój systemu korzeniowego i zawartość składników pokarmowych w korzeniach sadzonek buka zwyczajnego i dębu szypułkowego. Zastosowane podłoża różniły się właściwościami fizycznymi, składem granulometrycznym oraz zawartością składników pokarmowych (tab. 1-4). W trakcie hodowli sadzonek zastosowano 2 warianty nawożenia: standardowe nawożenie mieszaniną 2 nawozów Osmocote (3-4M i 5-6M) oraz nawożenie nawozem dolistnym opracowanym przez Uniwersytet Rolniczy w Krakowie (URK). Warianty podłoża ze standardowym nawożeniem (S) zostały oznaczone jako SR20, SR21 i SR22, natomiast z nawożeniem URK (U) jako UR20, UR21 i UR22. W obu przypadkach jako warianty kontrolne (SC, UC) zastosowano substrat torfowo-perlitowy. Sadzonki obydwu gatunków były hodowane w kontenerach styropianowych w szkółce kontenerowej Suków-Papiernia (Nadleśnictwo Daleszyce) w 8 wariantach substratowo-nawożeniowych. Schemat ustawienia kontenerów na polu produkcyjnym dla wariantu oraz miejsce pobierania sadzonek do analiz przedstawiono na rycinie 1. Sadzonki użyte do analiz wyhodowano w projekcie POIR.04.01.04-00-0016/20 finansowanym przez Narodowe Centrum Badań i Rozwoju ze środków krajowych oraz Europejskiego Funduszu Rozwoju Regionalnego „Innowacyjne technologie produkcji substratów i nawozów produkowanych z rodzimych surowców do produkcji sadzonek drzew leśnych". Po zakończeniu produkcji dla każdego z 8 wariantów wybrano po 25 sadzonek (5 z kontenera) o standardowym wigorze i parametrach biometrycznych. Każdej z nich zmierzono średnicę w szyjce korzeniowej (RCD) suwmiarką elektroniczną, natomiast przy użyciu oprogramowania WinRHIZO określono całkowitą długość korzeni (TRL), powierzchnię korzeni (RSA), przeciętną średnicę korzeni (ARD) oraz ich objętość (RV). Korzenie każdej sadzonki wysuszono i zmielono, a następnie oznaczono (w %) zawartość N, S i C przy użyciu analizatora LECO CNS TruMac oraz P, K, Ca i Mg przy użyciu analizatora Thermo iCAP 6500DUO ICP-OES. Analizy chemiczne przeprowadzono w Laboratorium Geochemii Środowiska Leśnego i Terenów Rekultywowanych, natomiast biometryczne

w Laboratorium Biotechnologii Leśnej Katedry Ekologii Lasu i Hodowli Lasu Uniwersytetu Rolniczego w Krakowie.

Stwierdzono, że rodzaj podłoża w połączeniu ze sposobem nawożenia wpłynął na rozwój systemu korzeniowego oraz na zawartość makroelementów w korzeniach analizowanych gatunków. U sadzonek dębu i buka wyhodowanych w wariancie UR20 odnotowano najwyższą średnią wartość całkowitej długości korzeni (tab. 5). Analizowane parametry biometryczne korzeni buka istotnie dodatnio korelowały ze sobą, natomiast z RCD istotnie dodatnio korelował tylko parametr ARD. W przypadku dębu RCD dodatnio korelował z dwoma parametrami: RSA i RV. W przeciwieństwie do buka odnotowano u dębu ujemną korelację między TRL i ARD, natomiast większość pozostałych parametrów wykazywała istotną dodatnią korelację (tab. 6).

Analiza zawartości makroskładników w systemie korzeniowym sadzonek wyhodowanych w poszczególnych wariantach nawożenia i zastosowanego substratu wykazała różny ich poziom, ale w zbliżonych zakresach. Jedynie u buka stwierdzono istotny wpływ wariantu produkcyjnego na zawartość N. Korzenie buka miały wyższą zawartość C niż dąb, z maksimum w wariancie UR20, natomiast zawartość N i K była na ogół wyższa w korzeniach dębu (tab. 7). Nie stwierdzono istotnej pozytywnej interakcji pomiędzy analizowanymi elementami, z wyjątkiem kilku przypadków, zwłaszcza dla N i P w sadzonkach dębu (tab. 8). Na podłożach innowacyjnych (beztorfowych) z zastosowaniem nawożenia dolistnego zaproponowanego przez URK uzyskano zbliżone wartości analizowanych parametrów systemów korzeniowych w wariancie z substratem torfowym. Wskazuje to na możliwość częściowego lub całkowitego zastąpienia torfu wysokiego nowym składnikiem w składzie podłoża szkółkarskiego.