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The effect of acorn scarification on the growth and root system size of *Quercus robur* L. seedlings grown in nursery containers

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Abstract: The mechanical scarification of acorns, although requiring a significant investment of resources, is a process commonly used in container nurseries for seed preparation. Its aim is to increase the number of germinating seeds and to shorten and equalise the length of their germination period. Research results indicate that scarification also affects the production of seedlings with improved biometric parameters. However, there is limited information available on improving the structure of the root system, primarily due to the limited availability of image analysis systems for these plant parts. This study employed modern measurement methods using WinRhizo and WinFolia software (Regent Instruments Inc.; Version Pro, 2022) to comparatively analyse root system parameters, focusing mainly on their structure. The parameters of pedunculate oak (*Quercus robur* L.) seedlings grown in polystyrene containers were compared with and without mechanical scarification, achieved by manually cutting off part of the acorn. After the end of the growing season, the parameters of all analysed seedlings (200 pieces) were determined, and a detailed analysis of the root system was performed on selected average individuals (64 pieces). Scarification resulted in an increase in the number of germinated seeds and grown seedlings, as well as an increase in the height and diameter of the root collar and a reduction in the variation of the obtained seedlings' parameters. Seedlings grown from scarified seeds were also characterised by a greater number of leaves with larger unit mass, which had smaller dimensions. The root system of seedlings grown from scarified seeds exhibited a higher average diameter and total volume of roots, as well as greater total length, surface area, and volume of fine roots, i.e. in the diameter range: $0.5 < D \leq 2.0$ mm. The obtained results confirmed the positive effect of seed scarification on germination and emergence efficiency, as well as on biometric features and the quality of the grown seedlings.

Keywords: pedunculate oak; polystyrene container; root architecture; seed scarification; WinFolia; WinRhizo

Pedunculate and sessile oak are economically important species growing in Poland. They occupy 8% of forest stands, with estimated resources of approximately 182 million m^3 (6.8%) (CSO 2023). The share of these species has increased significantly over the last few decades in Poland (1945: 4.1%; 2005: 7.2%; 2010: 6.9%; 2015: 7.5%; 2020: 7.9%; 2022: 8.0%) (CSO 2005, 2010, 2015,

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2020, 2022). According to an optimistic climate change scenario, pedunculate and sessile oak are predicted to significantly increase their distribution area by 2070, mainly in northern Europe (Dyderski et al. 2018). In Poland, in addition to natural sowing, oaks are introduced in the form of planting in stands or forest crops using seedlings raised in ground, nursery, and container nurseries (Banach et al. 2017). Due to high production costs, growing pedunculate oak seedlings in nursery containers requires acorns characterised by excellent viability to achieve maximum nursery stock yield per unit of production area. Oak seed viability is not very high, typically between 60% and 80% (Suszka et al. 2000), and depends on the environmental conditions in a given year, the stand from which seeds are harvested, and the duration of seed storage, with the minimum acceptable level set at 30% (SRP 2004). The low viability of oak seeds presents a significant challenge in the growing of seedlings due to the high costs associated with acquiring a sufficient number of seeds and their subsequent sorting and selection. Besides requiring the highest possible viability, seeds used for nursery production should also be of appropriate size. Studies indicate that tiny seeds result in poorer emergence and lower seedling survival rates. In contrast, larger seeds usually produce larger seedlings with higher growth rates, although very large acorns do not necessarily produce large seedlings in the future (Kormanik et al. 1998; García-Cebrián et al. 2003; Yi et al. 2015). Seedling size correlates with the number of first-order lateral roots, which is determined by seed size. Seeds with multiple embryos are unacceptable for seedling growing, as they tend to produce the smallest seedlings with inappropriate shoot and root development (Kormanik et al. 1998). Insects must not damage acorns used in the nursery, as reduced cotyledon reserves can adversely affect seedling establishment. Research has shown that damaged cotyledon reserves at the top of the acorn are more important than those at the base, as they are vital for acorn viability. Therefore, damage to the apical cotyledon is considered the most damaging, adversely affecting acorn germination activity (Hou et al. 2010).

Oak is a species characterised by sparse crop years and a lack of long-term storage of acorns. Under natural conditions, germination is uneven, with differences between the first and last acorn emergence lasting up to several weeks. Simultaneous germination and plant development are well known to promote equal access to light and foliar fertilisation while reducing competition among developing seedlings. This lack of uniformity poses a significant problem, especially in intensive nursery production, which typically is usually limited to one growing season (Suszka 2006; Andrzejczyk 2009; Skrzyszewska et al. 2019).

Different methods of weakening, opening or otherwise altering the seed coat, known as scarification, are used to accelerate and improve the uniformity of seed germination. The most commonly used methods include chemical, thermal, and mechanical scarification. Chemical scarification involves immersing the seed in a substance that damages the seed coat, generally an acid such as sulfuric, nitric, or phosphoric acid (Karaguzel et al. 2004; Maldonado-Arciniegas et al. 2018; Monteiro et al. 2021). Thermal scarification includes exposing seeds to hot air, hot water (boiling), or freezing at temperatures as low as −80 °C (Szabla, Pabian 2003; Karaguzel et al. 2004; Kimura, Islam 2012; Campbell et al. 2022). Mechanical scarification generally involves cutting off part of the seed coat opposite the embryo axis or making incisions (Karaguzel et al. 2004; Montaño-Arias et al. 2015; Monteiro et al. 2021). An additional benefit of mechanical scarification is the ability to analyse mummification changes of acorns, which can be determined visually or using automated optical systems (Tadeusiewicz et al. 2017; Tylek et al. 2021). Devices are also encountered that use rotating elements to strike seeds, causing the seed coat to break and facilitating water ingress (Kimura, Islam 2012).

Acorn scarification mainly increases the number of germinating seeds and accelerates this process (Giertych, Suszka 2011; Skrzyszewska et al. 2019). For pedunculate oak, seedlings grown from scarified seeds germinate up to two weeks earlier, with greater uniformity, and experience approximately 20% higher germination rate (Giertych, Suszka 2011). Similar results have been observed for pedunculate oak as well (Skrzyszewska et al. 2019). This is attributed to the removal of a small part of the pericarp and cotyledons, facilitating easier water uptake, seed swelling, and access to oxygen (Branco et al. 2002; Suszka 2006). A negative aspect of excessive seed scarification is the reduced height of seedlings, which persists for up to two

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growing seasons when more than 50% of the cotyledon weight is removed. The effects of cotyledon reduction alone on metabolic transformation and seedling development are not yet fully elucidated (Branco et al. 2002; Suszka 2006; Giertych, Suszka 2011).

In container nurseries, a common pre-sowing procedure is mechanical scarification of acorns, which involves cutting off about one-third of the cotyledon part of the acorn. Following scarification, viable seeds are selected through visual cross-sectional assessment, where acorns showing necrotic spots, drying, insect damage, or disease are removed. Thus, seed scarification and selection increase the proportion of seeds capable of germination (Skrzyszewska et al. 2019).

Scarification is a labour-intensive process as it is difficult to mechanise. Cutting off parts of the glans is often performed with hand secateurs driven by the muscle power of the person cutting. To reduce the effort, secateurs with an electric drive (battery-powered) are used, or other methods such as discs that shear the part of the acorn pressed against it by hand. Attempts to partially mechanise this process are also encountered, involving a mechanism that automatically feeds the acorn into the shearing disc, with the mechanism still being filled by hand (Adamczyk et al. 2018). In Poland, approximately 10 million seeds are scarified annually. Several workers are involved in this process in each nursery, producing oak seedlings. The duration of the work cycle is 2.5 s, so scarifying 1 million seeds requires more than 700 working hours (equivalent to 90 days of 8 h per day) of manual workers (Tylek et al. 2021). Given the above, it becomes interesting to answer two questions: whether the acorn scarification process can be fully mechanised and whether carrying out this costly procedure is justified in improving the number and quality of raised planting material.

The answer to the first question is that mechanising scarification is challenging because a mechanised system would need to precisely cut off part of the acorn based on measurements taken of the nut. Another challenge is the selection of suitable seeds from those unsuitable for sowing, carried out manually by the operator performing the scarification. A technical solution for these challenges has been developed under the name 'acorn scarifier' (Tadeusiewicz et al. 2018). This system operates by initially photographing the acorn to measure its basic dimensions (length, width, area) using image analysis methods to calculate the aspect ratio. It then cuts off a portion of the acorn to the appropriate depth on the appropriate side of the nut (the nut is automatically oriented in the machine). After cutting, another photograph is taken inside the nut. Based on this, seeds are automatically categorised for usefulness using an algorithm that compares the recorded image with images of abnormal sections contained in the system's database (Jablonski et al. 2016).

The second question is answered by the scientific results cited earlier, which confirm that acorn scarification, when carried out correctly, improves the production process of oak seedlings, regardless of the scarification method. Information on the effect of scarification on the grown seedlings is obtained through detailed measurements, considering both the root system and the assimilation apparatus of the seedlings. The information collected makes it possible to assess differences between individuals of the same species and to determine their response to environmental factors (Böhm 1985; Kormanek 2013; Kormanek et al. 2013a, b, 2015b). Such measurements are conducted with simple measuring instruments such as a linear gauge and calliper, laboratory balances for determining wet and dry mass, and displacement methods for assessing the volume of individual seedling parts. Planimetric and image analysis methods are also used to determine seedling parameters and their fragments. Recent years have seen rapid development in image analysis methods, especially for the assimilation apparatus and root system, driven by advancements in optoelectronics, measurement microprocessor systems, and computer-based computational methods (Gocławski et al. 2009; Kormanek et al. 2015a). Modern image-analysis measurement methods enable the acquisition of information that was difficult and sometimes even impossible to obtain using previously used methods. Key parameters assessed include length, area, leaf, and needle aspect ratio for the assimilation apparatus, and length, area, and volume of total roots, as well as length, area, and volume of roots of varying diameters, average root diameter in the root system, number of bifurcations, terminations, and root intersections for root systems. In addition to this information, contemporary programs such as WinFOLIA™ (Version Pro, 2020), WinSEEDLE™ (Version Pro, 2022),

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or WinRhizo™ (Version Pro, 2024) allow colour analysis of captured images, facilitating inference about the plant's condition, nutritional status, or analysis of disease symptoms, which aids in identifying the agent and degree of plant infection (Kormanek et al. 2015a, b). The work presented here used modern image analysis methods of scanned leaves and root systems to analyse seedlings grown from scarified and nonscarified seeds. The study aimed to determine the effect of acorn scarification on the growth parameters of pedunculate oak (*Quercus robur* L.) seedlings grown in polystyrene containers, with a particular emphasis on assimilation apparatus and root system architecture. The study verified two research hypotheses regarding seedlings grown from simultaneously sown seeds: (*i*) there exists a significant difference in the growth parameters of seedlings from scarified and nonscarified acorns; (*ii*) acorn scarification positively influences seedling root system architecture.

MATERIAL AND METHODS

Container filling and subsequent seedling cultivation (one growing season) took place at the Sowin Nursery Farm in the Gidle Forest District (50°58'39.8"N, 19°29'11.7"E) in the year 2017, following standard production procedures. The experiment utilised 8 Marbet V300/53 polystyrene containers (Marbet Green, Poland; Table 1), filled with a peat-perlite substrate (95% peat, 5% perlite) on an automated Urbinatii Ypsilon line (Urbinati S.l.r., Italy). Subsequently, 50 acorns of pedunculate oak (*Quercus robur* L.) collected from a seed stand registered in the National Register of Forestry Basic Material under number MP/1/47555/07 were manually sown into each container. Four containers (200 seeds) were sown with scarified seeds (SC variant), and another four with nonscarified seeds (NoSC variant). The scarification process involved manually cutting 15% of the seed length, performed by the most experienced worker in scarification, who assessed seed viability based on the appearance of tissues in the resulting cross-section (Figure 1). Only healthy acorns, showing no signs of mummification, damage from various fungal and insect pests, or other disease symptoms indicative of reduced viability, were used for container sowing. After sowing, the containers remained in the outdoor production field for 2 weeks, then were transferred to a vegetation hall for 6 weeks, before being returned to the open area for the remainder of the production season, during which they received periodic fertilisation and watering (Figure 2; Table 1). The temperature in the vegetation hall was maintained at about 25 °C using warm air heating, irrigation in the morning and evening using sprinkler irrigation boom with a variable number of work cycles to reach the level of container water capacity from 60% to 75%.

At the end of the growing season (21 September), the height *Wp* (cm) of each raised seedling was measured with a linear gauge to an accuracy of ±1 mm, and the diameter at the root collar *Dp* (mm) with an electronic calliper to a precision of ±0.01 mm. The assimilation apparatus was analysed after scanning the leaves on an Epson Photo V800 shadowless scanner (Seiko Epson Corporation, Japan) using WinFolia software. The number of leaves *Ln* (pcs), the sum of the leaf area *Lsa* (cm^2), the average leaf area *La* (cm^2), and the dimensions of the leaf blade, including length *Ll* (cm) and width *Lw* (cm), were determined. After drying the leaves in a Memmert UF 110 laboratory dryer (Memmert GmbH, Germany) (65 °C, 48 h) and weighing them on a Radwag PS 210.X2 analytical balance (Radwag, Poland) with an accuracy of ±0.001 g, the average leaf dry weight *Ldm* (g) was determined for each seedling. For root system analysis, eight seedlings were select-

Table 1. Container parameters and procedure for producing pedunculate oak seedlings

Variant	*Ln* (pcs)	*Lsa* (cm^2)	*La* (cm^2)	*Ll* (cm)	*Lw* (cm)	*Ldm* (g)
SC	9.31 ± 5.08	43.50 ± 2.66	13.98 ± 0.20	6.80 ± 0.05	3.18 ± 0.03	1.166 ± 0.037
NoSC	8.74 ± 4.75	52.60 ± 2.41	15.19 ± 0.19	7.09 ± 0.04	3.37 ± 0.02	0.985 ± 0.026
t-test (*P*)	$F = 2.043$	$t = 2.484$	$F = 19.040$	$F = 19.771$	$F = 27.706$	$t = 4.414$
F-test (*P*)	$P = 0.153$	$P = 0.013$	$P = 0.000$	$P = 0.000$	$P = 0.000$	$P = 0.000$

SC – scarified seeds; NoSC – nonscarified seeds; *Ln* – number of leaves; *Lsa* – total leaf area; *La* – mean leaf area; *Ll* – mean leaf length; *Lw* – mean leaf width; *Ldm* – mean leaf dry weight

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Figure 1. Manual scarification of acorns carried out at Sowin Nursery Farm of the Gidle Forest District

ed from each container (64 seedlings total) whose height and diameter at the root neck were closest to the average parameters of the seedlings growing in that container [mean height of the aboveground part (*Wpsa*) and diameter at the root collar (*Dpsa*) determination]. Once selected, the seedlings were removed from their cells, and their root systems were cleaned of substrate by washing them under running water and drying them with paper towels. The seedlings were then labelled, individually packed in plastic bags, and stored at +4 °C to prevent root desiccation. Scanning the root systems took approximately 21 h (64 root systems × 20 min). The root system of each seedling was scanned using an Epson Photo V800 shadowless scanner, and the images were analysed using WinRhizo software. For each root system, total root length *Rl* (cm), root area *Rsa* (cm^2), root volume *Rv* (cm^3), average root diameter *Rd* (mm), length : volume ratio *Rl* : *Rd* (cm^{-2}), number of terminations *Re* (pcs), intersections *Rc* (pcs), and bifurcations *Rf* (pcs) were determined. The length *L* (cm), area *A* (cm^2), and volume *V* (cm^3) of the roots were then determined in 10 intervals of their diameter *D* (mm):

– interval $0 < D \leq 0.5$;
– interval $0.5 < D \leq 1.0$;
– interval $1.0 < D \leq 1.5$;
– interval $1.5 < D \leq 2.0$;
– interval $2.0 < D \leq 2.5$;
– interval $2.5 < D \leq 3.0$;
– interval $3.0 < D \leq 3.5$;
– interval $3.5 < D \leq 4.0$;
– interval $4.0 < D \leq 4.5$;
– interval $4.5 < D$.

Figure 2. View of the experiment

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After drying in an oven at 105 °C and weighing on a Radwag PS 210.X2 analytical balance with ±0.001 g, the dry weight of the roots *Rdm* (g) was determined. For the parameters of seedlings, the Student's *t*-test was used after checking the assumptions of normality of distribution of parameters (Shapiro-Wilk test) and homogeneity of variance (Levene's test). For the parameters *Ln*, *La*, *Ll*, *Lw* not equal in number, a one-way analysis of variance was performed after checking the assumptions (Luszniewicz, Słaby 2003; Rabiej 2012). Statistical analysis of root length *Rl*, root volume *Rv*, and root area *Rsa* in each diameter class *D* (mm) was performed in three root thickness ranges, i.e. very fine roots $0 < D \leq 0.5$, fine roots $0.5 < D \leq 2.0$, and coarse roots $D > 2.0$ mm (Makita et al. 2011; Farahnak et al. 2020). All statistical analyses were performed for significance level $P < 0.05$, using Statistica (Version 12, 2006) program. The relative percentage change in root parameters from root diameter is presented, and the regression relationship of the change in *PRl* (percentage increase of root length), *PRsa* (percentage increase of root area), and *PRv* (percentage increase of root volume) from root diameter is determined. The relative percentage change was calculated as the difference of the parameter in the SC variant and in the NoSC variant, divided by the value of the parameter in the NoSC variant multiplied by 100.

RESULTS

The average number *ns* of all seedlings (Table 2) grown from scarified seed (SC) was 40.3 ± 0.9 units, and from nonscarified seed (NoSC) it was 34.5 ± 2.5 units, respectively, while the success rate (*u*) was 80.5 ± 3.8% for SC and 69.0 ± 5.0% for NoSC. Significantly more seedlings, by 16.7%, were grown in the SC variant, as confirmed by analysis of Student's *t*-test ($t = 3.159$; $P < 0.019$). Mean shoot height *Wp* and diameter at the root neck *Dp* for all seedlings were higher in the scarified (SC) variant, which was also confirmed by *t*-test analysis (Table 2).

In the scarified acorn (SC) variant, the raised seedlings had, on average, more leaves (*Ln*) with higher dry weight (*Ldm*). In contrast, their total leaf area (*LSa*) and average individual leaf area (*L*a), as well as average leaf length (*Ll*) and width (*Lw*), were lower compared to the nonscarified acorn (NoSC) variant (Table 3).

The mean height of the aboveground part (*Wpsa*) of the seedlings selected for further analyses was

Table 2. Number of seeds sown and mean ± SD: Success rate, shoot height, and diameter at root neck of raised seedlings (significance level $P < 0.05$)

Variant	Seeds	Grown seedlings			
	n (pcs)	*ns* (pcs)	*u* (%)	*Wp* (cm)	*Dp* (mm)
SC 1–4	200	40.3 ± 1.9	80.5 ± 3.8	21.1 ± 5.5	5.4 ± 1.11
NoSC 1–4	200	34.5 ± 2.5	69.0 ± 5.0	18.3 ± 6.1	4.8 ± 1.16
t-test (*P*)	–	$t = 3.159$	$P = 0.019$	$t = 5.994$ $P = 0.000$	$t = 3.811$ $P = 0.000$

SD – standard deviation; SC – scarified seeds; NoSC – nonscarified seeds; *n* – number of seeds sown; *ns* – number of healthy seedlings grown; *u* – success rate (%); *Wp* – height of the aboveground part (shoot) of the seedlings; *Dp* – diameter at the root neck of the seedlings

Table 3. Mean ± SD parameters of the assimilation apparatus of all grown seedlings (significance level $P < 0.05$)

Variant	*Ln* (pcs)	*Lsa* (cm^2)	*La* (cm^2)	*Ll* (cm)	*Lw* (cm)	*Ldm* (g)
SC	9.31 ± 5.08	43.50 ± 2.66	13.98 ± 0.20	6.80 ± 0.05	3.18 ± 0.03	1.166 ± 0.037
NoSC	8.74 ± 4.75	52.60 ± 2.41	15.19 ± 0.19	7.09 ± 0.04	3.37 ± 0.02	0.985 ± 0.026
t-test (*P*)	$F = 2.043$	$t = 2.484$	$F = 19.040$	$F = 19.771$	$F = 27.706$	$t = 4.414$
F-test (*P*)	$P = 0.153$	$P = 0.013$	$P = 0.000$	$P = 0.000$	$P = 0.000$	$P = 0.000$

SD – standard deviation; SC – scarified seeds; NoSC – nonscarified seeds; *Ln* – number of leaves; *Lsa* – total leaf area; *La* – mean leaf area; *Ll* – mean leaf length; *Lw* – mean leaf width; *Ldm* – mean leaf dry weight

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higher by an average of 5.1%. In comparison, the diameter at the root collar (*Dpsa*) was higher by an average of 0.2% in the SC variant compared to the NoSC variant, as confirmed by *t*-test (Table 4).

Analysis of root system parameters indicates that total root volume (*Rv*) and average root diameter (*Rd*) were higher in the scarified acorn variant (SC) compared to the nonscarified variant (NoSC) (Table 5).

When comparing root parameters across classes of mean diameter (*Rd*), only fine roots, i.e. those with a thickness of $0.5 < D \leq 2.0$, had statistically more significant total length (*Rl*), surface area (*Rsa*), and volume (*Rv*) in seedlings grown from scarified seeds (SC) (Table 6).

In Figures 3–5 showing *Rl* length, *Rs*a area, and *Rv* root volume as a function of their diameter *D*, it is noticeable that the *Rl* length, *Rsa* area, and *Rv* root volumes are smaller in the nonscarified variant (NoSC) than in the scarified variant (SC).

The change in these parameters due to scarification was confirmed by the relative percentage increase in *PRl* length, *PRsa* area, and *PRv* volume of roots of seedlings grown from the scarified variant (SC) compared to roots of seedlings grown from nonscarified seed (NoSC) (Figure 6). The growth changes as a function of root diameter followed a parabolic course, with the most significant changes occurring in the 2.0 mm to 2.5 mm diameter range, reaching 65%. Acorn scarification increased the proportion of very fine and fine roots in the root system, which are longer and have greater surface area and volume in the SC variant compared to the NoSC variant.

Table 4. Mean ± SD of success rate, shoot height, and diameter at root neck of seedlings selected for detailed root system analyses (significance level $P < 0.05$)

Variant/container	Seedlings selected for next analysis		
	nsa (pcs)	*Wpsa* (cm)	*Dpsa* (mm)
SC 1–4	32	21.5 ± 1.7	5.39 ± 0.32
NoSC 1–4	32	18.6 ± 2.6	5.00 ± 0.53
t-test (*P*)	–	$t = 5.994$ $P = 0.000$	$t = 3.810$ $P = 0.000$

SD – standard deviation; SC – scarified seeds; NoSC – nonscarified seeds; *nsa* – number of seedlings selected for detailed analyses; *Wpsa* – height of the aboveground part (shoot) of selected seedlings; *Dpsa* – diameter at the root neck of selected seedlings

Table 5. Mean ± SD parameters of the root system of all grown seedlings (significance level $P < 0.05$)

Variant	*Rl* (cm)	*Rsa* (cm^2)	*Rv* (cm^3)	*Rd* (mm)	*Rl*:*Rd* (cm·cm^{-3})	*Re* (pcs)	*Rc* (pcs)	*Rf* (pcs)	*Rdm* (g)
SC	1 049.4 ± 363.3	155.3 ± 50.9	1.880 ± 0.690	0.490 ± 0.092	590.2 ± 194.9	3 632.9 ± 1164.8	9 386.1 ± 3814.4	1 110.6 ± 521.0	4.85 ± 1.29
NoSC	1 027.0 ± 268.1	135.5 ± 35.6	1.470 ± 0.569	0.430 ± 0.085	767.6 ± 269.6	3 512.2 ± 1045.6	8 378.3 ± 2484.9	1 158.4 ± 414.8	4.34 ± 1.87
t-test (*P*)	$t = 0.277$ $P = 0.782$	$t = 1.784$ $P = 0.079$	$t = 2.518$ $P = 0.014$	$t = 2.548$ $P = 0.013$	$t = 0.277$ $P = 0.783$	$t = 0.432$ $P = 0.667$	$t = 1.238$ $P = 0.220$	$t = 0.400$ $P = 0.689$	$t = 0.954$ $P = 0.343$

SD – standard deviation; SC – scarified seeds; NoSC – nonscarified seeds; *Rl* – total root length; *Rsa* – total root surface area; *Rv* – total root volume; *Rd* – average root diameter; *Rl*:*Rd* – ratio of total root length to root volume; *Re* – number of root terminations; *Rc* – number of root intersections; *Rf* – root bifurcations; *Rdm* – root dry weight

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Table 6. Mean ± SD of root length *L* in the diameter ranges of the analysed root systems

Variant	*Rl* (cm) for *Rd* (mm)			*Rsa* (cm^2) for *Rd* (mm)			*Rv* (dm^3) for *Rd* (mm)		
	very fine roots	fine roots	coarse roots	very fine roots	fine roots	coarse roots	very fine roots	fine roots	coarse roots
	$Rd \leq 0.5$	$0.5 < Rd \leq 2.0$	$Rd > 2.0$	$Rd \leq 0.5$	$0.5 < Rd \leq 2.0$	$Rd > 2.0$	$Rd \leq 0.5$	$0.5 < Rd \leq 2.0$	$Rd > 2.0$
SC	856.4 ± 294.6	170.5 ± 84.2	22.2 ± 4.1	49.2 ± 18.2	44.7 ± 23.8	47.9 ± 8.9	0.310 ± 0.121	94.0 ± 30.4	9.50 ± 2.90
NoSC	874.1 ± 237.3	132.1 ± 59.4	20.5 ± 3.6	46.6 ± 12.5	33.9 ± 16.9	44.0 ± 10.3	0.280 ± 0.077	78.9 ± 25.7	8.50 ± 3.62
t-test (*P*)	$t = 0.069$ $P = 0.793$	$t = 4.348$ $P = 0.041$	$t = 2.988$ $P = 0.089$	$t = 0.422$ $P = 0.518$	$t = 4.318$ $P = 0.042$	$t = 2.671$ $P = 0.107$	$t = 1.217$ $P = 0.274$	$t = 4.525$ $P = 0.037$	$t = 1.446$ $P = 0.234$

SD – standard deviation; SC – scarified seeds; NoSC – nonscarified seeds; *Rl* – total root length; *Rd* – average root diameter; *Rsa* – total root surface area; *Rv* – total root volume

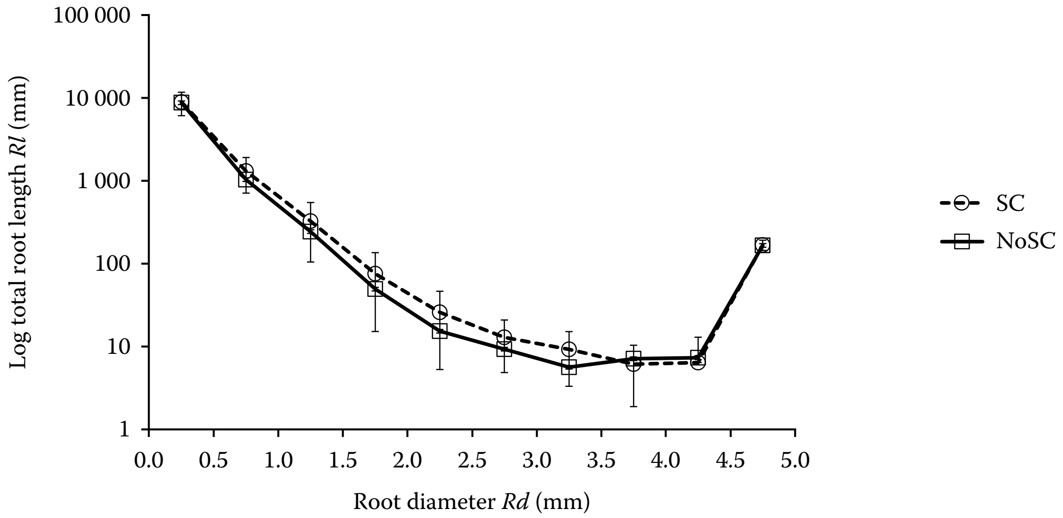

Figure 3. Root length *Rl* in the root ball depending on the average root diameter *D*

SC – scarified seeds; NoSC – nonscarified seeds

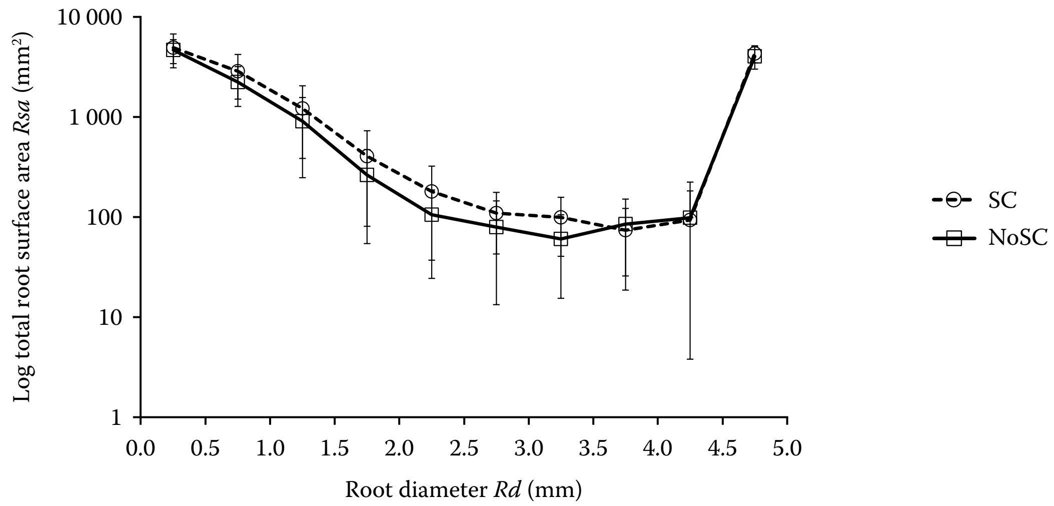

Figure 4. Root surface area *Rsa* in the root ball as a function of average root diameter *D*

SC – scarified seeds; NoSC – nonscarified seeds

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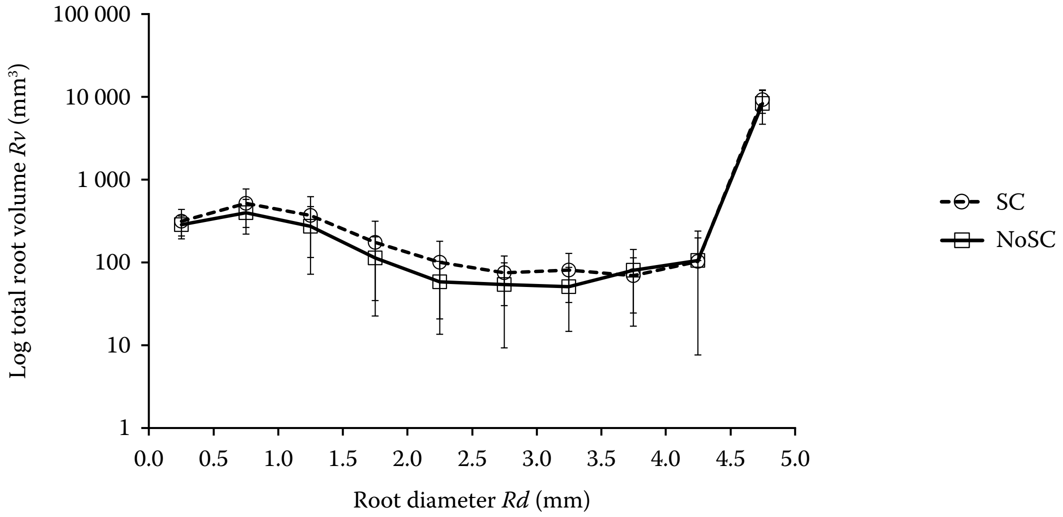

Figure 5. Root volume *Rv* in the root ball as a function of average root diameter *D*

SC – scarified seeds; NoSC – nonscarified seeds

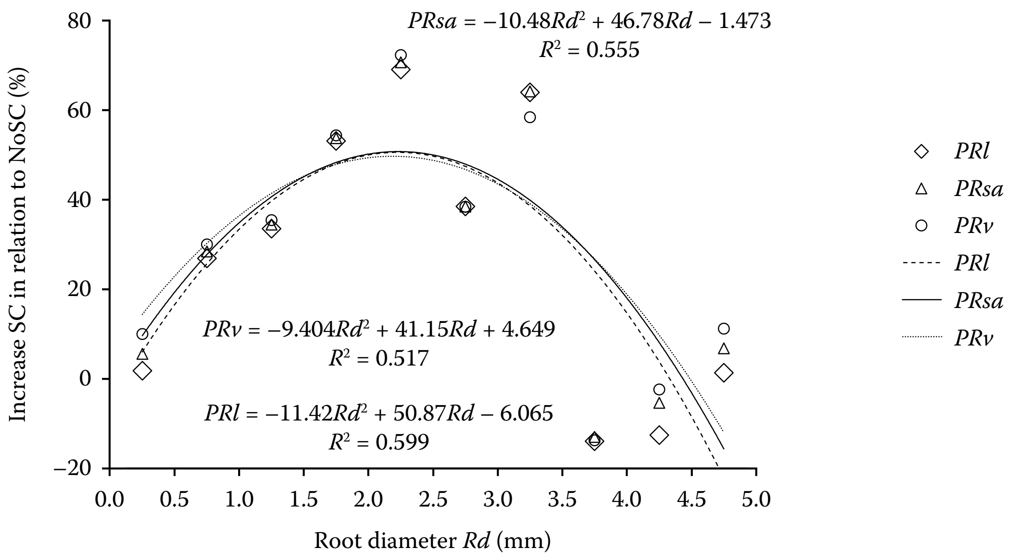

Figure 6. Increase in *PRl* length, *PRsa* area, and *PRv* volume of roots in the root ball as a function of average root diameter *D*

SC – scarified seeds; NoSC – nonscarified seeds; *Rd* – root diameter; *PRl* – percentage increase of root length in SC variant compared to NoSC variant; *PRsa* – percentage increase of root area in SC variant compared to NoSC variant; *PRv* – percentage increase of root volume in SC variant compared to NoSC variant

DISCUSSION

Acorn scarification resulted in a higher number of germinated seeds and increased emergence. There were 34 more seedlings germinated from scarified (SC) seeds out of 200 sown, with a success rate 11.5% higher for the SC variant compared to the NoSC variant. The increment in the number of seedlings obtained was 16.7%, confirming results from Giertych and Suszka (2011) for sessile oak, where the difference reached 20%, and from Skrzyszewska et al. (2019) for pedunculate oak, showing a difference of 14–18%, depending on the sowing date. Research confirms that based on the analysis of the topography of acorn mummification lesions, determined visually after scarification in the experiment, it is possible to significantly increase field germination capacity by pre-sowing rejection of necrotic seeds (Jabłoński et al. 2016). Unfortunately, such separation is usually associated with the loss of sev-

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eral percent of acorns wrongly eliminated during the separation stage. This occurs due to an incorrect assessment of the distribution of necrotic foci on the transverse section of the seed. The worker performing scarification examines only the cotyledon area inside the acorn, which can lead to two types of errors. Firstly, acorns with partially damaged cotyledons may be classified as rotten, even though, in fact, as spoiled seeds, they have a high germination potential. Secondly, acorns without necrotic lesions in the cotyledon area may be classified as healthy, although mummification of the invisible embryonic root will not allow germination in the nursery. Notably, the assessment of acorns stored for a certain period, such as 2 years, may also introduce additional errors due to changes in cotyledon colour. However, despite the above limitations, the introduction of automated seed separation processes using computerised image recognition techniques and the elimination of subjective perception of seed health by forest nursery staff could result in the optical properties of acorns undergoing scarification becoming an innovative resolution feature with high efficiency and high application potential (Bubliński et al. 2017). This is important because correlations with physical properties, known as resolution traits, among which aerodynamic traits, geometric traits, density, coefficient of friction, surface texture, and mechanical properties are the most important, are used to separate seeds with expected biological traits from the mixture (Kaliniewicz, Tylek 2019). Research on pedunculate oak acorns has shown that only their density and mass (to a minimal extent), aerodynamic, and mechanical properties depend on their viability. However, it is not possible to separate only healthy seeds using these methods; it is only possible to separate the class of healthy seeds with slightly spoiled seeds and separate them from the remaining (definitely spoiled) seeds (Tylek 2012).

Not only did the scarification process affect the number of acorns germinating, but the seedlings grown at the same time from scarified seeds were 15.3% taller and had a larger diameter at the root neck by 12.5%. This differs from the result reported by Giertych and Suszka (2011) for sessile oak, where seedlings from scarified variants were shorter than those grown from untreated acorns. Similarly, saplings of pedunculate oak grown from scarified acorns were shorter, but their diameters at the root neck were slightly higher (Skrzyszewska et al. 2019). Importantly, seedlings from scarified seeds showed less variability, with a coefficient of variation of 7.7% for height and 5.9% for diameter at the root neck in the SC variant, compared to 13.2% and 10.6%, respectively, in the NoSC variant. This reduction in variability in seedling parameters due to scarification was also confirmed in the study by Skrzyszewska et al. (2019). Seedlings in the SC variant had leaves with a smaller total area by 17.3% and an average area per leaf smaller by 8% due to their smaller average length dimensions by 4.1% and width by 5.6%. Nevertheless, they had more leaves by 6.5% and a higher dry weight by 18.4% compared to the NoSC variant. A negative correlation between leaf length and dry mass was also shown by Ponton et al. 2004.

Acorn scarification affected the root system parameters of the seedlings. In the SC variant, the seedlings exhibited a significantly higher total root volume of 27.5% and an average root diameter of 13.3% compared to the NoSC variant. For other root traits of the seedlings, although the averages were higher in the SC variant, the differences compared to the NoSC variant were not statistically significant.

When considering root systems within individual diameter ranges, there was a statistically significant increase in total length (*Rl*) by 29.1% (38.4 cm), area (*Rsa*) by 33.0% (11.1 cm^2), and volume (*Rv*) by 19.1% (15.10 cm^3) of roots in the diameter range of $0.5 < D \leq 2.0$ (fine roots) in the SC variant. In the other root diameter intervals, the parameters were higher in the SC variant compared to the NoSC variant, but these differences were not statistically significant. Thus, a positive effect was observed for roots within the diameter range relevant to plant growth (Nicola 1998).

The highest relative increase in root length, area, and volume as a function of root diameter, determined between the SC and NoSC variants, followed a parabolic trend, with the maximum increase observed in the root diameter range of 2.0 mm to 2.5 mm, reaching 65%. Such growth increments in roots, necessary for seedling growth, undoubtedly provide better development potential for the seedlings after they are planted on the crop (Kormanek et al. 2015a, b).

There are numerous negative factors that affect the growth of forest tree seedlings' root systems. The most commonly mentioned include excessive substrate compaction (Kormanek 2013; Kormanek et al. 2013a, b, 2015a, b; Kormanek, Gołąb 2021; Pająk et al. 2022a, b), excessive moisture (Metcalfe et al. 2008; Xinyue 2022), unfavourable tempera-

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tures (Kaspar, Bland 1992; García-Tejera 2016), and substrate chemistry (Pawlik, Šamonil 2018). In contrast, there is little information on factors that positively affect root systems. The positive changes shown in the experiment regarding the number of seedlings grown and the improvement of root parameters due to scarification indicate that this is a beneficial process that stimulates important root parameters for seedling growth.

The demonstrated difference in root system structure positively affects the biometric parameters of the seedlings. The more developed the root system, the better the biometric parameters of the seedlings, mainly shoot height and diameter at the root neck. Research indicates that this improvement is linked to better uptake of elements from the substrate following fertilisation, as indicated by studies on pine (Pająk et al. 2022c) and beech (Pająk et al. 2022d) plants.

CONCLUSION

Based on the analysis of the experimental results, it was found that pre-sowing scarification of pedunculate oak (*Q. robur* L.) seeds influenced an increase in the number of germinated seeds and raised seedlings, as well as an increase in the length and diameter at the root neck of seedling shoots raised from scarified seeds. These parameters were less differentiated among the raised seedlings. Seedlings grown from scarified seeds were also characterised by an increase in the number of leaves with a higher unit weight, which was smaller. The root systems of seedlings grown from scarified seeds exhibited higher average diameters and total root volumes. Additionally, they showed greater total root length, area, and volume in diameter intervals of $0.5 < D \leq 2.5$ mm.

The effect of seed scarification on the grown seedlings has been confirmed. Scarification positively affects the quantity and quality of the seedlings grown, as illustrated by their biometric parameters. Therefore, the use of this process in container nurseries is justified.

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