





# Influence of Scarification on the Germination Capacity of Acorns Harvested from Uneven-Aged Stands of Pedunculate Oak (*Quercus robur* L.)

# Zdzisław Kaliniewicz <sup>1,\*</sup> <sup>(D)</sup> and Paweł Tylek <sup>2</sup> <sup>(D)</sup>

- <sup>1</sup> Faculty of Technical Sciences, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 11, 10-719 Olsztyn, Poland
- <sup>2</sup> Faculty of Forestry, University of Agriculture in Cracow, Al. 29 Listopada 46, 31-425 Kraków, Poland; rltylek@cyf-kr.edu.pl
- \* Correspondence: zdzislaw.kaliniewicz@uwm.edu.pl; Tel.: +48-089-523-3934

Received: 8 February 2018; Accepted: 23 February 2018; Published: 26 February 2018

**Abstract:** Scarification involves the partial removal of the seed coat on the side of the hilum, opposite the radicle, to speed up germination in acorns. The aim of this study was to determine the influence of scarification on the germination capacity of pedunculate oak acorns, selected and prepared for sowing. The diameter, length and mass of acorns were measured before and after scarification in four batches of acorns harvested from uneven-aged trees (76, 91, 131 and 161 years). The measured parameters were used to determine the correlations between acorn dimensions and mass, and to calculate the dimensional scarification index and the mass scarification index in acorns. Individual complete and scarified acorns from every batch were germinated on sand and peat substrate for 28 days. The analyzed acorns were characterized by average size and mass. Scarification and the elimination of infected acorns increased germination capacity from around 64% to around 81% on average. Acorns can be divided into size groups before scarification to obtain seed material with varied germination capacity. Larger acorns with higher germination capacity can be used for sowing in container nurseries, whereas smaller acorns with lower germination capacity can be sown in open-field nurseries.

Keywords: Quercus robur L.; seed size; scarification index; germination

# 1. Introduction

Pedunculate oak (*Quercus robur* L.) is a tree species measuring up to 40 m in height and up to 3 m in diameter at breast height. It is the main, dominant or co-dominant species in mixed-species forests, in particular in fresh mixed broadleaved forests, moist mixed broadleaved forests, fresh broadleaved forests, moist broadleaved forests, riparian forests, moist upland forests and upland forests [1]. Pedunculate oak is widely distributed throughout the European continent, excluding northern Europe and parts of Mediterranean Europe [1–5]. The species thrives in fertile and moist habitats, on loamy and sandy loam soils with high humus content and a moderately acidic or neutral pH. On nutrient-poor soils, oaks have an irregular growth pattern, they are smaller, produce twisted trunks and resemble shrubs [1,3].

Pedunculate oaks produce flowers and fruit at 40–50 years of age or even later (60–80 years) when they are grown in dense stands. Acorns or oak nuts ripen in September or October. The common name of *Quercus robur* is derived from the fact that the species produces several acorns per peduncle. The peduncle measures 5 to 12 cm in length. They are ellipsoidal in shape, and they are enclosed by woody cupules to one-third of their height. The hilum is located at the base of the acorn, and it is

covered by the cupules. Fresh and rehydrated acorns have green and, subsequently, olive-brown stripes which disappear with moisture loss. Oak trees shed acorns in October, and empty or worm-riddled acorns are usually discarded first [2]. For this reason, acorns should be harvested only after the first batch of nuts has been shed. Fresh acorns are characterized by high moisture content and high susceptibility to fungal infections. Therefore, harvested acorns should be quickly transported to a processing facility in open boxes, baskets or bags made of loose fabric of plastic mesh to enable ventilation and prevent overheating [2].

In the processing plant, acorns are cleaned, sorted, immersed in water, subjected to heat treatment, dried, dressed with fungicides and prepared for cold storage. Acorns are immersed in water to remove weakly developed, damaged, almost empty and empty nuts. They are heated to eliminate fungal spores, in particular *Ciboria batschiana* which is responsible for black rot and mummification of acorns [6,7]. Acorns are immersed in water heated to a temperature of 41 °C for 2.5 h. The moisture content of acorns should not drop below 40% by dry weight during processing. Processing temperature has to be rigorously controlled because overheating decreases the germination capacity. Acorns with moisture content higher than 45% can be dried. Acorns can be stored in non-tight containers at a temperature of around -3 °C for up to two years without loss of germination capacity [2,7].

Pedunculate oak acorns do not enter winter dormancy. However, germination is strongly suppressed, and the seed coat prevents water and air from penetrating the acorn. Germination can be enhanced through scarification, namely the partial excision of the seed coat on the side of the hilum, opposite the radicle [2,8]. In other plant species, the seed coat is also excised to promote germination. The seed coat can be punctured, scarified with sharp sand, excised or removed chemically with concentrated acid. Scarification is recommended in around 7% of tree species, including in the Persian turpentine tree [9], honey locust [10], common myrtle [11], black velvet tamarind [12], black locust [13], lebbeck tree [14], African locust bean [15], Judas tree [16], noni [17], afzelia and African teak [18].

According to Suszka et al. [2], pedunculate oak acorns are scarified by reducing their length by one-third to one-quarter, usually with the use of shears or a grinding disc. Scarification exposes the cotyledon and enables visual evaluation of acorn health. Mummified acorns are eliminated at this stage [8,19]. Researchers are currently designing a robot system that will eliminate manual sorting, increase scarification efficiency and maximize the percentage of healthy acorns in the sorted batch [8,20–24]. Automated scarification will be a highly accurate process, and the removed portion of the acorn will be minimized to guarantee the highest germination capacity.

The aim of this study was to determine the influence of acorn scarification on the germination capacity of pedunculate oak acorns, selected and prepared for sowing.

#### 2. Materials and Methods

#### 2.1. Sample Preparation

The experiment was performed on pedunculate oak acorns harvested manually in uneven-aged tree stands (76, 91, 131 and 161 years) in seed zone Dbs 20, a fresh mixed broadleaved forest in Szczytno municipality in north-eastern Poland. Acorns were harvested with the use of collection nets between 10 and 14 October 2016. Each batch of harvested acorns was stored separately in non-heated and well ventilated premises. Every day, acorns were shoveled into piles not exceeding 10 cm in height. When the relative moisture content of acorns reached around 42%, acorns were subjected to heat treatment by immersion in water with a temperature of 41 °C for 2.5 h. After the treatment, acorns were surface dried, and samples of around 2 kg each were collected from every batch and refrigerated at a temperature of around 5 °C. The remaining acorns were placed in plastic kegs and freeze stored at a temperature of -3 °C. Two samples of 96 acorns each were selected from the refrigerated acorns by the survey sampling method [25]. The size of each sample corresponded to the number of cells in seeding containers.

### 2.2. Determination of Physical Properties

The length *L*, diameter *D* (Figure 1) and mass *m* of every acorn were determined, and acorns from one sample in each batch were scarified by reducing their length by one-quarter to one-third with the use of shears. Acorn health was evaluated visually, and only acorns without visible symptoms of pathological changes were used in the experiment. Rejected acorns were randomly replaced with new acorns whose geometric properties and mass were determined. The length  $L_s$  and mass  $m_s$  of scarified acorns were measured.

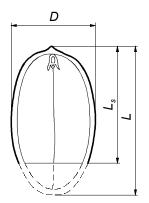


Figure 1. Acorn dimensions: *D*—diameter, *L*, *L*<sub>s</sub>—length before and after scarification.

The length and diameter of acorns were measured with a caliper to the nearest 0.02 mm. Acorn diameter was determined as the average of two measurements performed at the widest point, perpendicular to the longitudinal axis. Acorn mass was determined with the Hornady 1500GR Bench Scale (Hornady<sup>®</sup>, Grand Island, NE, USA) to the nearest 0.01 g.

The following parameters were determined in each acorn:

• arithmetic mean diameter  $D_a$  and the geometric mean diameter  $D_g$  [26]:

$$D_a = \frac{2D+L}{3} \tag{1}$$

$$D_g = \left(D^2 \times L\right)^{1/3} \tag{2}$$

• specific mass  $m_D$  [27]:

$$m_D = \frac{m}{D_g} \tag{3}$$

• shape factors  $K_1$  and  $K_2$  [26,28]:

$$K_1 = \frac{D}{L} \tag{4}$$

$$K_2 = \frac{D_g}{L} \tag{5}$$

• and in scarified acorns—the dimensional scarification index  $S_L$  and the mass scarification index  $S_m$ :

$$S_L = \frac{L - L_s}{L} \tag{6}$$

$$S_m = \frac{m - m_s}{m} \tag{7}$$

#### 2.3. Comparative Germination of Complete and Scarified Acorns

Individual acorns, whose physical parameters had been determined previously, were placed in the cell of plastic containers measuring  $51 \times 33 \times 8$  cm. Each container was composed of 96 square cells measuring  $4 \times 4$  cm. The cells were filled with sand and peat substrate (1:1) with approximate moisture content of 55%, which was compacted by twice dropping the container on the floor from a height of approximately 10 cm. Excess substrate was removed with a flat wooden slat positioned obliquely across the container, in two perpendicular motions. Acorns were pushed into the substrate with the radicle up and the upper portion of each acorn 2–3 mm below the edge of the cell, according to the method described by Tylkowski and Bujarska-Borkowska [29]. Acorns were covered with a layer of the peat substrate, and excess substrate was removed as described previously. The containers with the seeded acorns were stored indoors at a temperature of around 20 °C and were exposed to artificial light for 8 h daily. The germination test was carried out for 28 full days (from 14 November to 12 December 2016). The upper surface of the cells and the substrate were sprayed with tap water (electric conductance—0.25 mS/cm) once a day between 6 p.m. and 7 p.m. Acorns that were pushed up by the root at least 10 mm above the upper edge of the cell were regarded as germinated. Germination capacity was determined as the percentage of germinated acorns in the total number of tested acorns [2].

#### 2.4. Statistical Analysis

The physical parameters of acorns were analyzed statistically in the Statistica PL program (version 12.5, StatSoft Polska Sp. z o.o., Crakow, Poland) at a significance level of  $\alpha = 0.05$ . Differences between the measured parameters were determined by one-way ANOVA, and differences in the physical parameters of complete and scarified acorns or germinated and non-germinated acorns were determined by the Student's *t*-test for independent samples. The normality of each group was verified by the Shapiro–Wilk W-test, and the homogeneity of variance was assessed with Levene's test. Where the null hypothesis of equal population means was rejected, the significance of differences was determined by Duncan's test, and homogenous groups were identified [30].

#### 3. Results

#### 3.1. Experimental Material

The physical parameters of acorns from the analyzed batches (harvested from uneven-aged tree stands) are presented in Table 1. The average values of the measured parameters were determined in the following ranges: length—28.10–28.82 mm, diameter—16.25–16.54 mm, mass—4.35–4.87 g, arithmetic mean diameter—20.21–20.63 mm, geometric mean diameter—19.49–19.88 mm, specific mass—0.22–0.24 g mm<sup>-1</sup>, shape factor  $K_1$ —0.58, shape factor  $K_2$ —0.69–0.70. Acorns from the evaluated batches differed most significantly in length and mass. The analyzed acorns did not differ significantly in diameter, arithmetic and geometric mean diameter, specific mass and shape factors  $K_1$  and  $K_2$ . The results of the analysis revealed that the largest acorns were harvested from a 76-year-old tree stand, and the smallest acorns were harvested from a 91-year-old tree stand.

#### 3.2. Germination Capacity of Acorns

The germination capacity (Figure 2) of complete acorns was estimated in the range of 61% (batch O-91) to 66% (batches O-76 and O-161), which places the evaluated material in quality class I (germination capacity of 61–100%). Scarification and the removal of infected acorns increased germination capacity from around 77% (batch O-161) to around 86% (batch O-76), i.e., by around 16 percentage points on average. It should be noted that germination capacity was not significantly influenced by the age of the parent tree stand.

Property/Indicator	Acorn Batch					
	O-76	O-91	O-131	O-161		
Length (mm)	$28.82\pm2.20^{\text{ b}}$	$28.10\pm2.36~^{a}$	$28.74\pm2.41~^{\rm ab}$	$28.49\pm2.88~^{\rm ab}$		
Diameter (mm)	$16.54\pm1.62~^{\rm a}$	$16.27\pm1.52~^{\rm a}$	$16.53\pm1.53$ $^{\rm a}$	$16.25\pm1.56$ $^{\rm a}$		
Mass (g)	$4.87\pm1.16$ <sup>b</sup>	$4.35\pm0.98~^{\rm a}$	$4.61 \pm 1.22~^{ m ab}$	$4.43\pm1.12~^{a}$		
Arithm. mean diameter (mm)	$20.63\pm1.50~^{\rm a}$	$20.21\pm1.39$ <sup>a</sup>	$20.60\pm1.53~^{\rm a}$	$20.33 \pm 1.57$ <sup>a</sup>		
Geom. mean diameter (mm)	$19.88\pm1.55$ a	$19.49\pm1.41$ a	$19.86\pm1.53$ a	$19.56\pm1.55$ a		
Specific mass (g mm $^{-1}$ )	$0.24\pm0.04$ a	$0.22\pm0.04$ a	$0.23\pm0.04$ a	$0.22\pm0.04$ a		
Shape factor $K_1$ (-)	$0.58\pm0.06$ <sup>a</sup>	$0.58\pm0.07$ $^{\mathrm{a}}$	$0.58\pm0.06$ <sup>a</sup>	$0.58\pm0.07$ $^{\mathrm{a}}$		
Shape factor $K_2$ (-)	$0.69\pm0.05$ $^{\rm a}$	$0.70\pm0.05~^{\rm a}$	$0.69\pm0.05$ $^{\rm a}$	$0.69\pm0.06~^{\rm a}$		

**Table 1.** Statistical distribution of the physical properties (mean value  $\pm$  standard deviation) of acorns and significant differences between batches.

<sup>a,b</sup>—superscript letters denote significant differences between the corresponding properties (indicators).

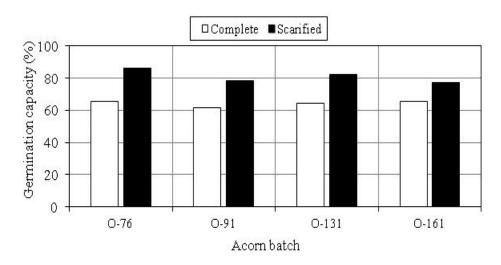


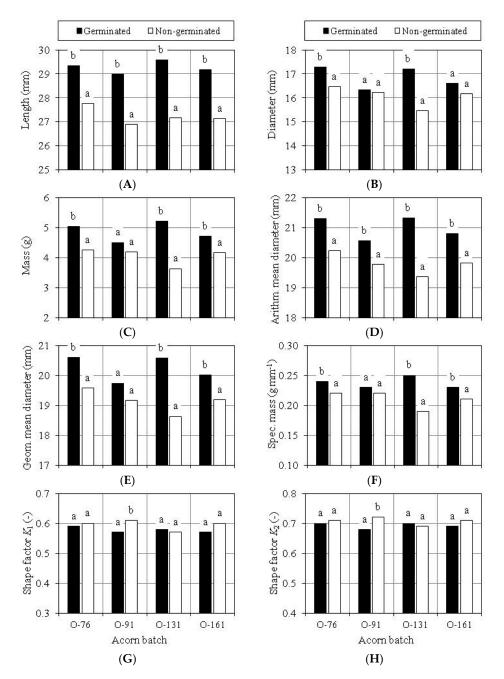
Figure 2. Germination capacity of complete and scarified pedunculate oak acorns.

The results of the Student's *t*-test for independent samples revealed that germinated and non-germinated complete acorns from four batches (Figure 3) differed mainly in average length and arithmetic mean diameter. In three batches (excluding O-91), significant differences were also observed in diameter, mass, geometric mean diameter and specific mass. Unlike in the remaining batches, germinated acorns in batch O-91 had a somewhat different shape than non-germinated acorns. These acorns were slimmer, and their average shape factor values were lower than those determined in non-germinated acorns. An analysis of the physical parameters of the evaluated acorns revealed that up to 2% of the shortest acorns can be removed from each batch without a loss of germinating complete acorns. The above will increase germination capacity by around 1.5 percentage points on average. The greatest improvement could be achieved in batch O-161 where the removal of around 15% of the shortest acorns would increase germination capacity from around 65% to around 76%. However, the above would lead to the loss of around 3% of viable acorns.

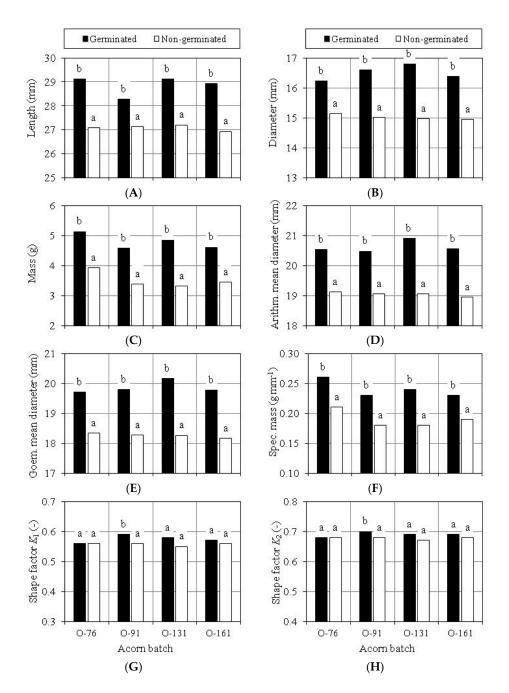
The results of the Student's t-test for independent samples revealed that germinated and non-germinated acorns from four batches of scarified material (Figure 4) differed in length, diameter, mass, arithmetic and geometric mean diameter, and specific mass. The above parameters were higher in germinated than in non-germinated acorns. In most cases (excluding batch O-91), no significant differences in shape were observed in germinated or non-germinated acorns. An analysis of the measured physical parameters revealed that the elimination of non-germinating acorns always leads to a certain loss of viable acorns.

#### 3.3. Evaluation of Scarification Treatment

The statistical distribution of scarification index values is presented in Table 2. Scarification reduced acorn length by 15% to 42% (31% on average) and decreased acorn mass by 12% to 35% (22% on average). Batch O-76 differed significantly from the remaining acorn batches in terms of the dimensional scarification index. Batch O-91 differed significantly from the remaining acorn batches in terms of the mass scarification index. The coefficient of variation of the above parameters ranged from 9.54% to 19.47%.



**Figure 3.** Significance of differences in the length (**A**), diameter (**B**), mass (**C**), arithmetic mean diameter (**D**), geometric mean diameter (**E**), specific mass (**F**), shape factor  $K_1$  (**G**) and shape factor  $K_2$  (**H**) of germinated and non-germinated complete acorns; a, b—different letters denote statistically significant differences.



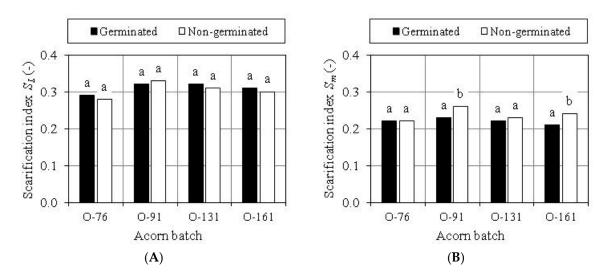
**Figure 4.** Significance of differences in the length (**A**), diameter (**B**), mass (**C**), arithmetic mean diameter (**D**), geometric mean diameter (**E**), specific mass (**F**), shape factor  $K_1$  (**G**) and shape factor  $K_2$  (**H**) of germinated and non-germinated acorns subjected to scarification; a, b—different letters denote statistically significant differences.

Scarification Index	Acorn Batch _	Value of Trait			Standard	Coefficient of Trait	
		Minimum	Maximum	Average	Deviation of Trait	Variability (%)	
Dimensional S <sub>L</sub>	O-76	0.15	0.39	0.28 <sup>a</sup>	0.046	16.18	
	O-91	0.19	0.40	0.32 <sup>b</sup>	0.041	12.69	
	O-131	0.24	0.42	0.32 <sup>b</sup>	0.031	9.77	
	O-161	0.22	0.38	0.31 <sup>b</sup>	0.030	9.54	
Mass S <sub>m</sub>	O-76	0.12	0.35	0.22 <sup>a</sup>	0.043	19.47	
	O-91	0.13	0.33	0.24 <sup>b</sup>	0.043	18.16	
	O-131	0.13	0.33	0.22 <sup>a</sup>	0.034	15.64	
	O-161	0.14	0.33	0.22 <sup>a</sup>	0.042	19.23	

**Table 2.** Statistical distribution and significant differences in the scarification index of acorns from four batches.

<sup>a,b</sup>—superscript letters denote significant differences between the corresponding properties.

The scarification index of acorns that germinated and acorns that did not germinate during the 28-day germination test is analyzed in Figure 5. No significant differences in the dimensional scarification index were found in either group in all batches, which indicates that the degree of scarification did not influence germination. In batches O-91 and O-161, minor (but statistically significant) differences were observed in the mass scarification index of germinated and non-germinated acorns, where non-germinated acorns lost more mass than germinated acorns.



**Figure 5.** Significance of differences in the dimensional (**A**) and mass (**B**) scarification index of germinated and non-germinated acorns: a, b—different letters denote statistically significant differences.

## 3.4. Germination Capacity of Scarified Acorns

The germination capacity of scarified acorns divided into three size groups based on their diameter is presented in Figure 6. The germination capacity of the smallest acorns ranged from around 33% (batch O-131) to around 73% (batch O-76). The largest acorns were characterized by the highest germination capacity in the estimated range of 89% (batch O-161) to 100% (batch O-91).

Similar relationships were noted when acorns were divided into two size groups based on their diameter (Figure 7). Germination capacity ranged from around 59% (batch O-91) to around 83% (batch O-76) in acorns measuring up to 16 mm in diameter, and it exceeded 90% in acorns with a diameter larger than 16 mm.

In most cases, the above size groups did not differ significantly in their scarification index (Figure 8). Differences in the values of the dimensional scarification index were noted only in batch O-76, and differences in the values of the mass scarification index were found only in batch O-161.

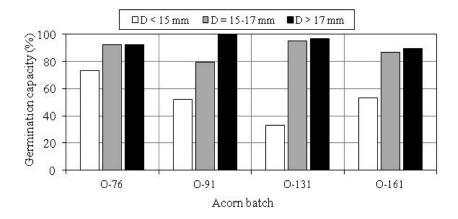


Figure 6. Germination capacity of pedunculate oak acorns divided into three size groups.

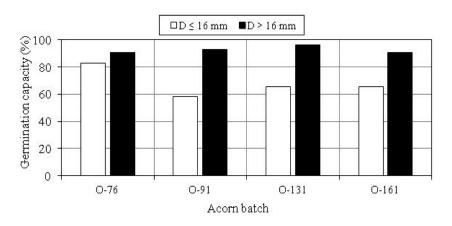
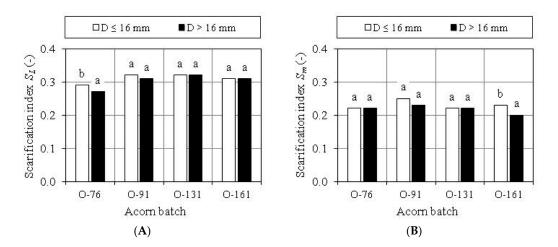


Figure 7. Germination capacity of pedunculate oak acorns divided into two size groups.



**Figure 8.** Significance of differences in the dimensional (**A**) and mass (**B**) scarification index of acorns measuring up to 16 mm and more than 16 mm in diameter: a, b—different letters denote statistically significant differences.

The germination capacity of the analyzed size groups relative to the values of the dimensional scarification index is presented in Table 3. These groups differed significantly in their germination

capacity which ranged from 0% to even 100%. The scarification index and germination capacity were not directly correlated within the analyzed range of values of the dimensional scarification index. In the group of acorns measuring up to 16 mm in diameter, germination capacity exceeded 90% in acorns with a scarification index of 0.31 to 0.35 (batch O-76) and in acorns with a scarification index higher than 0.35 (batches O-131 and O-161). Acorns measuring more than 16 mm in diameter were characterized by significantly higher germination capacity which did not drop below 84% regardless of the value of the scarification index.

Acorn Batch	Size Group	Germination Capacity of Acorns with Dimensional Scarification Index S <sub>L</sub> :					
Acom Daten	once onoup	≤0.25	0.26-0.30	0.31-0.35	>0.35		
	$D \le 16 \text{ mm}$	60.0	83.3	92.7	75.0		
O-76	<i>D</i> > 16 mm	100.0	84.2	92.2	-		
	Total	88.2	83.6	92.3	75.0		
O-91	$D \le 16 \text{ mm}$	0	63.6	57.9	60.0		
	<i>D</i> > 16 mm	100.0	88.9	95.8	88.9		
	Total	80.0	79.3	79.1	73.7		
	$D \le 16 \text{ mm}$	-	50.0	70.4	100.0		
O-131	<i>D</i> > 16 mm	100.0	94.4	96.3	100.0		
	Total	100.0	76.7	81.8	100.0		
O-161	$D \le 16 \text{ mm}$	33.3	64.7	64.0	100.0		
	<i>D</i> > 16 mm	-	81.0	100.0	100.0		
	Total	33.3	73.2	78.6	100.0		

**Table 3.** Germination capacity of acorns from different size groups relative to their dimensional scarification index.

Similar results were noted in an analysis of the mass scarification index (Table 4). The germination capacity of different size groups ranged from 0% to 100%, and germination capacity was not directly correlated with the scarification index. In most acorns measuring more than 16 mm in diameter (excluding acorns from batch O-161 with a scarification index of 0.26 to 0.30), germination capacity exceeded 80%, and it reached 100% in 11 out of 19 cases. In acorns measuring up to 16 mm in diameter, germination capacity was highest when the scarification index was below 0.15 (batches O-91 and O-131), 0.16–0.20 (batch O-131) and above 0.30 (batch O-76).

**Table 4.** Germination capacity of acorns from different size groups relative to their mass scarification index.

Acorn Batch	Size Group	Germination Capacity of Acorns with Mass Scarification Index $S_m$ :					
		$\leq$ 0.15	0.16-0.20	0.21-0.25	0.26-0.30	>0.30	
O-76	$D \le 16 \text{ mm}$	66.6	82.3	82.6	88.9	100.0	
	<i>D</i> > 16 mm	100.0	100.0	81.0	100.0	100.0	
	Total	83.3	90.3	81.8	91.7	100.0	
O-91	$D \le 16 \text{ mm}$	100.0	66.7	66.7	42.9	50.0	
	<i>D</i> > 16 mm	100.0	92.3	96.4	90.0	66.7	
	Total	100.0	87.5	84.8	62.5	57.1	
O-131	$D \le 16 \text{ mm}$	0	93.3	40.0	63.6	-	
	<i>D</i> > 16 mm	100.0	100.0	93.1	100.0	100.0	
	Total	50.0	97.1	75.0	73.3	100.0	
O-161	$D \le 16 \text{ mm}$	100.0	50.0	79.2	57.1	0	
	<i>D</i> > 16 mm	100.0	100.0	81.3	75.0	-	
	Total	100.0	82.8	80.0	61.1	0	

#### 4. Discussion

An analysis of the physical parameters of acorns harvested from uneven-aged tree stands revealed that the largest acorns were harvested from 76-year-old trees and the smallest acorns were harvested from 91-year-old trees. Despite significant differences in length and mass, the acorns from the above batches were characterized by similar diameter and shape. Acorns were harvested from tree stands in the same geographical location; therefore, differences in acorn size can probably be attributed to genetic variations which significantly influence the physical properties of seeds [31–33]. In the current study, the age of the parent tree stand (76 to 161 years) did not exert a significant influence on the physical parameters of acorns. Different results were reported by Kaliniewicz et al. [34] in Scotts pine where the physical dimensions and mass of seeds decreased with the age of parent trees. Similar trends were noted by Suszka et al. [2] based on long-term observations of tree stands rather than a comparison of the physical properties of acorns harvested from uneven-aged tree stands where genetic variations could play a key role. The significant influence of tree age on the physical parameters of seeds was also noted in a study of Norway spruce, but the nature of the observed changes was difficult to describe due to the disrupting influence of genetic factors [35]. In the present experiment, the dimensions and mass of pedunculate oak acorns were within the range of values reported by Suszka et al. [2], Nikolić and Orlović [36] and Tylkowski and Bujarska-Borkowska [29]. The evaluated acorns were somewhat smaller than those harvested in southern Poland [8,37] and in Serbia [38]. Seed size and mass generally decrease in northern regions of the globe [39-41].

In terms of germination capacity, acorns from uneven-aged tree stands were within the lower range of values in quality class I (61.5–65.6%). Thermal treatment was effective in preventing fungal diseases and acorn mummification, but failed to eliminate already infected and partially damaged acorns from the processed batches. According to Tylek [37] and Tylek et al. [42], small and large acorns are equally susceptible to fungal infections; therefore, they cannot be effectively separated based on their geometric parameters or shape. The results of the present study indicate that germination capacity can be somewhat improved (by around 1.5 percentage points) by eliminating around 2% of the shortest acorns from each batch. Scarification was a more effective treatment which increased germination capacity from around 64% to around 81%. Similar results were reported by Giertych and Suszka [19]. During scarification, the seed coat and cotyledons are partially removed, which improves water penetration and aeration, thus accelerating germination. Symptoms of disease are also more visible in scarified acorns which can be removed from the batch. Unlike the seeds of other forest trees [43], pedunculate oak acorns cannot be sorted effectively based on physical parameters; therefore, the optical parameters of acorn cross-sections could be used as an innovative selection trait. Optical parameters cannot be reliably evaluated by the naked eye, which is why an automated scarification device with a vision system has been developed [8,24] to identify early symptoms of disease, eliminate damaged acorns and increase germination capacity by up to 10% relative to manually processed material. However, evaluations of acorn health can be compromised by two types of errors. Firstly, acorns with normally developed cotyledons are often classified as healthy despite the presence of necrotic changes in the radicle, which are not visible to the evaluator. Secondly, acorns with damaged cotyledons can be classified as unfit for sowing even when the radicle is healthy and potentially capable of germinating. Nonetheless, the germination capacity of acorns is influenced mainly by the severity of pathological changes and effective removal of non-viable acorns. Some batches contain up to several dozen percent of damaged acorns [6].

Seed batches for sowing should contain both small and large acorns to preserve the genetic diversity of the future generations [2]. Gradual removal of small acorns can lead to the elimination of acorns produced by old trees, which are best adapted to a given habitat, local soil and weather conditions. For this reason, the quality of seed material can be more effectively improved through scarification than through the elimination of the shortest acorns—a procedure that induces only a minor increase in germination capacity (around 1.5 percentage points in the analyzed case).

According to Tylkowski and Bujarska-Borkowski [29], pedunculate oak acorns should be sorted based on size before planting. Acorn mass is positively correlated with seedling size [44–49]; therefore, similarly sized acorns should be planted separately to promote even emergence of seedlings. The results of the present study also demonstrate that acorns should be sorted into size groups before scarification and sowing. Germination capacity decreased with a decrease in acorn diameter, which implies that the seeding rate of acorns from different size groups should be adjusted accordingly to obtain the required number of seedlings. Larger acorns with a higher germination capacity (>90%) should be used mainly in container nurseries, whereas smaller acorns should be sown in open-field nurseries. The seeding rate should be determined based on the germination capacity of acorns. Acorns are easy to separate with the use of conventional sorting devices, and mesh sieves with longitudinal openings are particularly recommended for separating acorns into size groups based on their diameter.

Acorns with partially excised seed cover and cotyledons germinate faster [2,8,19,50]. This is a particularly important consideration in container nurseries where the growth cycle is relatively short and where polyethylene tents are used several times during the growing season. In the current study, the variations in the values of the dimensional scarification index (0.15 to 0.42) and the mass scarification index (0.12 to 0.35) did not influence the germination capacity of differently sized acorns. Shi et al. [51] reported the best results where acorns were reduced in length by one-third to one-half. In the cited study, scarification increased fertilizer absorption by oak acorns and seedlings grown in a nursery. According to Giertych and Suszka [19] and Tadeusiewicz et al. [8], the reduction in acorn mass during scarification should not exceed 20%. More extensive scarification increases the accuracy of health assessments, but it also compromises seedling growth. The presence of intact nutrient reserves in acorns promotes embryonic development, increases seedling resistance to adverse environmental factors and improves the morphological parameters of developing plants [19,50,52].

#### 5. Conclusions

The results of this study indicate that the age of parent pedunculate oak trees (76 to 161 years) generally does not influence the physical parameters or the germination capacity of acorns. The germination capacity of complete acorns ranged from 61.5% to 65.6%. Up to 2% of the shortest acorns can be removed from the processed batch without the loss of germinating acorns.

Scarification and the elimination of acorns with symptoms of disease are the most effective methods of improving the quality of pedunculate oak acorns for sowing. The above treatments increased the germination capacity of acorns from around 64% to around 81%. Germination capacity was not correlated with the dimensional scarification index (15% to 42%) or the mass scarification index (12% to 35%).

The germination capacity of scarified acorns was correlated with their diameter. Acorns should be sorted into size groups before scarification and sowing to promote even seedling emergence. The germination capacity of acorns with the largest diameter (e.g., above 16 mm) exceeds 90%, and these acorns are recommended for sowing in container nurseries. Acorns with the smallest diameter (up to 16 mm) are characterized by lower germination capacity (around 58% to 83%), and they are more suited for sowing in open-field nurseries where the seeding rate should be determined based on the germination capacity of a given acorn batch.

**Acknowledgments:** The authors would like to thank Anna Prusik, a student of the University of Warmia and Mazury in Olsztyn majoring in Agricultural and Forest Engineering, for determining the physical parameters of acorns and conducting acorn germination tests as part of her Master's degree research.

**Author Contributions:** Z.K. conceived and designed the experiment; Z.K. and P.T. analyzed the data; Z.K. contributed materials/analysis tools; Z.K. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

## References

- 1. Jaworski, A. Hodowla lasu. Tom III. Charakterystyka hodowlana drzew i krzewów leśnych (Silviculture. Volume 3. Breeding Characteristics of Forest Trees and Shrubs); PWRiL: Warszawa, Poland, 2011; pp. 242–266. ISBN 9788309010760. (In Polish)
- Suszka, B.; Muller, C.; Bonnet-Masimber, M. Nasiona leśnych drzew liściastych od zbioru do siewu (Seeds of Deciduous Forest Trees—From Harvest to Sowing); Wydawnictwo Naukowe PWN: Warszawa-Poznań, Poland, 2000; pp. 1–274. ISBN 8301133430. (In Polish)
- 3. Rodriguez-Campos, A.; Diaz-Maroto, I.J.; Barcala-Perez, E.; Vila-Lameiro, P. Comparison of the autoecology of *Quercus robur* L. and *Q. petraea* (Mattuschka) Liebl. Stands in the Northwest of the Iberian Peninsula. *Ann. For. Res.* **2010**, *53*, 7–25.
- 4. Brus, D.J.; Hengeveld, G.M.; Walvoort, D.J.J.; Goedhart, P.W.; Heidema, A.H.; Nabuurs, G.J.; Gunia, K. Statistical mapping of tree species over Europe. *Eur. J. For. Res.* **2012**, *131*, 145–157. [CrossRef]
- Peguero-Pina, J.J.; Sisó, S.; Sancho-Knapik, D.; Díaz-Espejo, A.; Flexas, J.; Galmés, J.; Gil-Pelegrín, E. Leaf morphological and physiological adaptations of a deciduous oak (*Quercus faginea* Lam.) to the Mediterranean climate: A comparison with a closely related temperate species (*Quercus robur* L.). *Tree Phys.* 2016, 36, 287–299. [CrossRef] [PubMed]
- 6. Knudsen, I.M.B.; Thomsen, K.A.; Jensen, B.; Poulsen, K.M. Effect of hot water treatment, biocontrol agents, disinfectants and a fungicide on storability of English oak acorns and control of the pathogen, Ciboria batschiana. *For. Pathol.* **2004**, *1*, 47–64. [CrossRef]
- 7. Schröder, T.; Kehr, R.; Procházková, Z.; Sutherland, J.R. Practical methods for estimating the infection rate of *Quercus robur* acorn seedlots by *Ciboria batschiana*. *For. Pathol.* **2004**, *34*, 187–196. [CrossRef]
- Tadeusiewicz, R.; Tylek, P.; Adamczyk, F.; Kiełbasa, P.; Jabłoński, M.; Pawlik, P.; Piłat, A.; Walczyk, J.; Szczepaniak, J.; Juliszewski, T.; et al. Automation of the acorn scarification process as a contribution to sustainable forest management. Case study: Common oak. *Sustainability* 2017, *9*, 2276. [CrossRef]
- Chebouti-Meziou, N.; Merabet, A.; Chebouti, Y.; Bissaad, F.Z.; Behidj-Benyounes, N.; Doumandji, S. Effect of cold and scarification on seeds germination of *Pistacia atlantica* L. for rapid multiplication. *Pak. J. Bot.* 2014, 46, 441–446.
- 10. Asl, M.B.; Sharivivash, R.; Rahbari, A. Effect of different treatments on seed germination of honey locust (*Gleditschia triacanthos*). *Mod. Appl. Sci.* **2011**, *5*, 200–204.
- 11. Ciccarelli, D.; Andreucci, A.C.; Pagni, A.M.; Garbari, F. The role of the elaiosome in the germination of seeds of *Myrtus communis* L. (Myrtaceae). *Atti Soc. Tosc. Sci. Nat.* **2004**, *111*, 143–146.
- 12. Nwaoguala, C.N.C.; Osaigbovo, A.U. Enhancing seedling production of black velvet tamarind (*Dialium guineense* Willd). J. Appl. Nat. Sci. 2009, 1, 36–40.
- 13. Tylkowski, T.; Grupa, R. Effectiveness of pre-sowing scarification methods of black locust seeds. *Sylwan* **2010**, *154*, 33–40.
- 14. Missanjo, E.; Maya, C.; Kapira, D.; Banda, H.; Kamanga-Thole, G. Effect of seed size and pretreatment methods on germination of *Albizia lebbeck. ISRN Bot.* **2013**. [CrossRef]
- 15. Aliero, B.L. Effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of seeds of African locust bean tree, *Parkia biglobosa. Afric. J. Biotechnol.* **2004**, *3*, 179–181. [CrossRef]
- 16. Pipinis, E.; Milios, E.; Smiris, P.; Gioumousidis, C. Effect of acid scarification and cold moist stratification on the germination of *Cercis siliquastrum* L. seeds. *Turk. J. Agric. For.* **2011**, *35*, 259–264. [CrossRef]
- 17. Nelson, S. Noni seed handling and seedling production. Fruit. Nut. 2005, 10, 1-4.
- 18. Botsheleng, B.; Mathowa, T.; Mojeremane, W. Effects of pre-treatments methods on the germination of pod mahogany (*Afzelia quanzensis*) and mukusi (*Baikiaea plurijuga*) seeds. *Int. J. Innov. Res. Sci.* **2014**, *3*, 8108–8113.
- 19. Giertych, M.J.; Suszka, J. Consequences of cutting off distal ends of cotyledons of *Quercus robur* acorns before sowing. *Ann. For. Sci.* 2011, *68*, 433–442. [CrossRef]
- 20. Jabłoński, M.; Tylek, P.; Walczyk, J.; Tadeusiewicz, R.; Piłat, A. Colour-based binary discrimination of scarified *Quercus robur* acorn under varying illumination. *Sensors* **2016**, *16*, 1319. [CrossRef] [PubMed]
- Grabska-Chrząstowska, J.; Kwiecień, J.; Drożdż, M.; Bubliński, Z.; Tadeusiewicz, R.; Szczepaniak, J.; Walczyk, J.; Tylek, P. Comparison of selected classification methods in automated oak seed sorting. *J. Res. Appl. Agric. Eng.* 2017, 62, 31–33.

- 22. Pawlik, P.; Jabłoński, M.; Bubliński, Z.; Tadeusiewicz, R.; Walczyk, J.; Tylek, P.; Juliszewski, T.; Adamczyk, F. Use of Harris detector for determination of orientation of acorns in the process of automated scarification. *J. Res. Appl. Agric. Eng.* **2017**, *62*, 163–165.
- 23. Przybyło, J.; Jabłoński, M.; Pociecha, D.; Tadeusiewicz, R.; Piłat, A.; Walczyk, J.; Kiełbasa, P.; Szczepaniak, J.; Adamczyk, F. Application of model-based design in algorithms' prototyping for experimental acorn scarification rig. *J. Res. Appl. Agric. Eng.* **2017**, *62*, 166–170.
- 24. Tadeusiewicz, R.; Tylek, P.; Adamczyk, F.; Kiełbasa, P.; Jabłoński, M.; Bubliński, Z.; Grabska-Chrząstowska, J.; Kaliniewicz, Z.; Walczyk, J.; Szczepaniak, J.; et al. Assessment of selected parameters of the automatic scarification device as an example of a device for sustainable forest management. *Sustainability* **2017**, *9*, 2370. [CrossRef]
- 25. Greń, J. Statystyka matematyczna. Modele i zadania (Mathematical Statistics. Models and Tasks); PWN: Warszawa, Poland, 1984; pp. 237–280. ISBN 8301036990. (In Polish)
- 26. Mohsenin, N.N. *Physical Properties of Plant and Animal Materials;* Gordon and Breach Science Public: New York, NY, USA, 1986; pp. 1–891. ISBN 9780677213705.
- Kaliniewicz, Z.; Tylek, P.; Markowski, P.; Anders, A.; Rawa, T.; Jóźwiak, K.; Fura, S. Correlations between the germination capacity and selected physical properties of Scots pine (Pinus sylvestris L.) seeds. *Balt. For.* 2013, 19, 201–211.
- 28. Grochowicz, J. *Maszyny do czyszczenia i sortowania nasion (Seed Cleaning and Sorting Machines);* Akademia Rolnicza: Lublin, Poland, 1994; pp. 25–28. ISBN 839016129X. (In Polish)
- 29. Tylkowski, T.; Bujarska-Borkowska, B. Effect of acorn size and sowing depth on *Quercus robur* and *Q. petraea* seedling emergence and height. *Sylwan* **2011**, *155*, 159–170. (In Polish)
- 30. Rabiej, M. *Statystyka z programem Statistica (Statisctics in Statistica Software);* Helion: Gliwice, Poland, 2012; pp. 1–344. ISBN 9788324641109. (In Polish)
- 31. García-Mozo, H.; Gómez-Casero, M.T.; Domínguez, E.; Galán, C. Influence of pollen emission and weather-related factors on variations in holm-oak (*Quercus ilex* subsp. *ballota*) acorn production. *Environ. Exp. Bot.* **2007**, *61*, 35–40. [CrossRef]
- 32. Koenig, W.D.; Knops, J.M.H.; Carmen, W.J.; Sage, D. No trade-off between seed size and number in the valley oak *Quercus lobata*. *Am. Nat.* **2009**, *173*, 682–688. [CrossRef] [PubMed]
- 33. Alejano, R.; Vázquez-Piqué, J.; Carevic, F.; Fernández, M. Do ecological and silvicultural factors influence acorn mass in Holm Oak (southwestern Spain)? *Agroforest Syst.* **2011**, *83*, 25–39. [CrossRef]
- 34. Kaliniewicz, Z.; Rawa, T.; Tylek, P.; Markowski, P.; Anders, A.; Fura, S. The effect of the age of Scots pine (*Pinus sylvestris* L.) stands on the physical properties of seeds and the operating parameters of cleaning machines. *Tech. Sci.* **2013**, *16*, 63–72.
- 35. Kaliniewicz, Z.; Markowski, P.; Anders, A.; Tylek, P.; Krzysiak, Z.; Fura, S. Influence of the age of parent stand on selected physical properties of Norway spruce seeds. *Sylwan* **2017**, *161*, 548–557. (In Polish)
- Nikolić, N.P.; Orlović, S. Genotypic variability of morphological characteristics of English oak (*Quercus robur* L.) acorn. *Zb. Matice Srp. Prir. Nauk.* 2002, 102, 53–58. [CrossRef]
- 37. Tylek, P. Size and shape as separation properties of pedunculate oak seeds (*Quercus robur* L.). *Acta Agrophys.* **2012**, *19*, 673–687. (In Polish)
- 38. Rakić, S.; Povrenović, D.; Tešević, V.; Simić, M.; Maletić, R. Oak acorn, polyphenols and antioxidant activity in functional food. *J. Food Eng.* **2006**, *74*, 416–423. [CrossRef]
- 39. Aizen, M.A.; Patterson, W.A. Acorn size and geographical range in the North American oaks (*Quercus* L.). *J. Biogeogr.* **1990**, *17*, 327–332. [CrossRef]
- 40. Oleksyn, J.; Reich, P.B.; Tjoelker, M.G.; Chalupka, W. Biogeographic differences in shoot elongation pattern among European Scots pine populations. *For. Ecol. Manag.* **2001**, *148*, 207–220. [CrossRef]
- 41. Moles, A.T.; Westoby, M. Latitude, seed predation and seed mass. J. Biogeogr. 2003, 30, 105–128. [CrossRef]
- Tylek, P.; Kaliniewicz, Z.; Kiełbasa, P.; Zagrobelny, T. Mass and density as separation criteria of pedunculate oak (*Quercus robur* L.) seeds. *Electron. J. Pol. Agric. Univ. Ser. For.* 2015, *18*. Available online: http://www.ejpau.media.pl/volume18/issue4/art-05.html (accessed on 5 February 2018).
- 43. Kaliniewicz, Z.; Tylek, P.; Anders, A.; Markowski, P.; Rawa, T.; Ołdakowski, M.; Wąsowski, L. An analysis of the physical properties of selected deciduous tree species. *Balt. For.* **2016**, *22*, 169–174.
- 44. Kleinschmit, J. Intraspecific variation of growth and adaptive traits in European oak species. *Ann. For. Sci.* **1993**, *50*, 166–185. [CrossRef]

- 45. Kormanik, P.P.; Sung, S.S.; Kormanik, T.L.; Schlarbaum, S.E.; Zarnoch, S.J. Effect of acorn size on development of northern red oak 1-0 seedlings. *Can. J. For. Res.* **1998**, *28*, 1805–1813. [CrossRef]
- Quero, J.L.; Villar, R.; Marañón, T.; Zamora, R.; Poorter, L. Seed-mass effects in four Mediterranean *Quercus* species (Fagaceae) growing in contrasting light environments. *Am. J. Bot.* 2007, *94*, 1795–1803. [CrossRef] [PubMed]
- 47. Tilki, F. Influence of acorn size and storage duration on moisture content, germination and survival of *Quercus petraea* (Mattuschka). *J. Environ. Biol.* **2010**, *31*, 325–328. [PubMed]
- 48. Yi, X.; Zhang, J.; Wang, Z. Large and small acorns contribute equally to early-stage oak seedlings: A multiple species study. *Eur. J. For. Res.* 2015, 134, 1019–1026. [CrossRef]
- Llanderal-Mendoza, J.; Gugger, P.F.; Oyama, K.; Uribe-Salas, D.; González-Rodríguez, A. Climatic determinants of acorn size and germination percentage of Quercus rucosa (Fagaceae) along a latitudinal gradient in Mexico. *Bot. Sci.* 2017, 95, 37–45. [CrossRef]
- 50. García-Cebrián, F.; Esteso-Martínez, J.; Gil-Pelegrín, E. Influence of cotyledon removal on early seedling growth in *Quercus robur* L. *Ann. For. Sci.* **2003**, *60*, 69–73. [CrossRef]
- Shi, W.; Bloomberg, M.; Li, G.; Su, S.; Jia, L. Combined effects of cotyledon excision and nursery fertilization on root growth, nutrient status and outplanting performance of *Quercus variabilis* container seedlings. *PLoS ONE* 2017, 12, e0177002. [CrossRef] [PubMed]
- 52. Hou, X.; Yi, X.; Yang, Y.; Liu, W. Acorn germination and seedling survival of *Q. variabilis*: Effects of cotyledon excision. *Ann. For. Sci.* **2010**, *67*. [CrossRef]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).