

1 Article

2 Influence of Scarification on the Germination 3 Capacity of Acorns Harvested from Uneven-aged 4 Stands of Pedunculate Oak (*Quercus robur* L.)

5 Zdzisław Kaliniewicz ^{1,*} and Paweł Tylek²

6 ¹ Department of Heavy Duty Machines and Research Methodology, University of Warmia and Mazury in
7 Olsztyn, ul. Oczapowskiego 11, 10-719 Olsztyn, Poland; zdzislaw.kaliniewicz@uwm.edu.pl

8 ² Institute of Forest Utilization and Forest Technology, University of Agriculture in Cracow, Al. 29 Listopada
9 46, 31-425 Kraków, Poland; rtylek@cyf-kr.edu.pl

10 * Correspondence: zdzislaw.kaliniewicz@uwm.edu.pl; Tel.: +48-089-523-3934

11 **Abstract:** Scarification involves the partial removal of the seed coat on the side of the hilum,
12 opposite the radicle, to speed up germination in acorns. The aim of this study was to determine the
13 influence of scarification on the germination capacity of pedunculate oak acorns, selected and
14 prepared for sowing. The diameter, length and mass of acorns were measured before and after
15 scarification in four batches of acorns harvested from uneven-aged trees (76, 91, 131 and 161 years).
16 The measured parameters were used to determine the correlations between acorn dimensions and
17 mass, and to calculate the dimensional scarification index and the mass scarification index in
18 acorns. Scarified and non-scarified acorns from every batch were germinated on sand and peat
19 substrate for 28 days. The analyzed acorns were characterized by average size and mass.
20 Scarification decreased acorn mass by around 22% and acorn length by around 31% on average.
21 Scarification and the elimination of infected acorns increased germination capacity from around
22 64% to around 81% on average. Acorns can be divided into size groups before scarification to
23 obtain seed material with varied germination capacity. Larger acorns with higher germination
24 capacity can be used for sowing in container nurseries, whereas smaller acorns with lower
25 germination capacity can be sown in open-field nurseries.

26 **Keywords:** seeds; mass; dimensions; scarification index; germination

27

28 1. Introduction

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

38 Pedunculate oaks produce flowers and fruit at 40-50 years of age or even later (60-80 years)
39 when they are grown in dense stands. Acorns or oak nuts ripen in September or October. The
40 common name of *Quercus robur* is derived from the fact that the species produces several acorns per
41 peduncle. Acorns measure 5 to 12 cm in length. They are ellipsoidal in shape, and they are enclosed
42 by woody cupules to one-third of their height. The hilum is located at the base of the acorn, and it is
43 covered by the cupula. Fresh and rehydrated acorns have green and, subsequently, olive-brown
44 stripes which disappear with moisture loss. Oak trees shed acorns in October, and empty or
45 worm-riddled acorns are usually discarded first [2]. For this reason, acorns should be harvested only

46 after the first batch of nuts has been shed. Fresh acorns are characterized by high moisture content
47 and high susceptibility to fungal infections. Therefore, harvested acorns should be quickly
48 transported to a processing facility in open boxes, baskets or bags made of loose fabric of plastic
49 mesh to enable ventilation and prevent overheating [2].

50 In the processing plant, acorns are cleaned, sorted, immersed in water, subjected to heat
51 treatment, dried, dressed with fungicides and prepared for cold storage. Acorns are immersed in
52 water to remove weakly developed, damaged, almost empty and empty nuts. They are heated to
53 eliminate fungal spores, in particular *Ciboria batschiana* which is responsible for black rot and
54 mummification of acorns [6-7]. Acorns are immersed in water heated to a temperature of 41°C for 2.5
55 hours. The moisture content of acorns should not drop below 40% during processing. Processing
56 temperature has to be rigorously controlled because overheating decreases germination capacity.
57 Acorns with moisture content higher than 45% can be dried. Acorns can be stored in non-tight
58 containers at a temperature of around -3°C for up to two years without loss of germination capacity
59 [2,7].

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

69 According to Suszka et al. [2], pedunculate oak acorns are scarified by reducing their length by
70 one-third to one-quarter, usually with the use of shears or a grinding disc. Scarification exposes the
71 cotyledon and enables visual evaluation of acorn health. Mummified acorns are eliminated at this
72 stage [8,19]. Researchers are currently designing a robot system that will eliminate manual sorting,
73 increase scarification efficiency and maximize the percentage of healthy acorns in the sorted batch
74 [8,20-24]. Automated scarification will be a highly accurate process, and the removed portion of the
75 acorn will be minimized to guarantee the highest germination capacity.

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

78 2. Materials and Methods

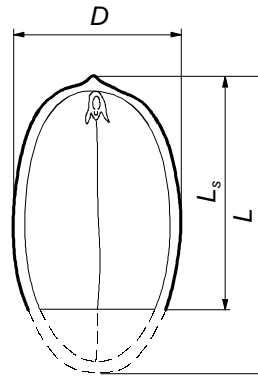
79 2.1. Sample Preparation

80 The experiment was performed on pedunculate oak acorns harvested manually in uneven-aged
81 tree stands (76, 91, 131 and 161 years) in seed zone Dbs 20, a fresh mixed broadleaved forest in
82 Szczytno municipality in north-eastern Poland. Acorns were harvested with the use of collection
83 nets between 10 and 14 October 2016. Each batch of harvested acorns was stored separately in
84 non-heated and well ventilated premises. Every day, acorns were shoveled into piles not exceeding
85 10 cm in height. When the relative moisture content of acorns reached around 42%, acorns were
86 subjected to heat treatment by immersion in water with a temperature of 41°C for 2.5 hours. After
87 the treatment, acorns were surface dried, and samples of around 2 kg each were collected from every
88 batch and refrigerated at a temperature of around 5°C. The remaining acorns were placed in plastic
89 kegs and freeze stored at a temperature of -3°C. Two samples of 96 acorns each were selected from
90 the refrigerated acorns by the survey sampling method [25]. The size of each sample corresponded
91 to the number of cells in seeding containers.

92

93 2.2. Determination of Physical Properties

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



100 **Figure 1.** Acorn dimensions: D – diameter, L , L_s – length before and after scarification.

101 The length and diameter of acorns were measured with a caliper to the nearest 0.02 mm. Acorn
 102 diameter was determined as the average of two measurements performed at the widest point,
 103 perpendicular to the longitudinal axis. Acorn mass was determined with the Hornady 1500GR
 104 Bench Scale to the nearest 0.01 g.

105 The following parameters were determined in each acorn:

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

$$D_a = \frac{2D + L}{3} \quad (1)$$

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

- 107 • specific mass m_D [27]:

$$m_D = \frac{m}{D_g} \quad (3)$$

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

$$K_1 = \frac{D}{L} \quad (4)$$

$$K_2 = \frac{D_g}{L} \quad (5)$$

- 109 • and in scarified acorns – the dimensional scarification index S_L and the mass scarification index
 110 S_m :

$$S_L = \frac{L - L_s}{L} \quad (6)$$

$$S_m = \frac{m - m_s}{m} \quad (7)$$

111 2.3. Germination Test

112 Individual acorns were placed in plastic containers measuring 51×33×8 cm. Each container was
 113 composed of 96 square cells measuring 4×4 cm. The cells were filled with sand and peat substrate
 114 (1:1) which was compacted by twice dropping the container to the floor from a height of
 115 approximately 10 cm. Excess substrate was removed with a flat wooden slat positioned obliquely
 116 across the container, in two perpendicular motions. Acorns were pushed into the substrate with the
 117 radicle up and the upper portion of each acorn 2-3 mm below the edge of the cell, according to the
 118 method described by Tylkowski and Bujarska-Borkowska [29]. Acorns were covered with a layer of
 119 the peat substrate, and excess substrate was removed as previously. The containers with the seeded
 120 acorns were stored indoors at a temperature of around 20°C and were exposed to artificial light for 8
 121 h daily. The germination test was carried out for 28 full days (from 14 November to 12 December
 122 2016). Each day, acorns were sprayed with water between 6 p.m. and 7 p.m. Acorns that were
 123 pushed up by the root at least 10 mm above the upper edge of the cell were regarded as germinated.
 124 Germination capacity was determined as the percentage of germinated acorns in the total number of
 125 tested acorns [2].

126 2.4. Statistical Analysis

127 The physical parameters of acorns were analyzed statistically in the Statistica PL program (v.
 128 12.5) at a significance level of $\alpha=0.05$. Differences between the measured parameters were
 129 determined by one-way ANOVA, and differences in the physical parameters of scarified and
 130 non-scarified acorns or germinated and non-germinated acorns were determined by the Student's
 131 t-test for independent samples. The normality of each group was verified by the Shapiro-Wilk
 132 W-test, and the homogeneity of variance was assessed with Levene's test. Where the null hypothesis
 133 of equal population means was rejected, the significance of differences was determined by Duncan's
 134 test, and homogenous groups were identified [30] (Rabiej 2012).

135 3. Results

136 3.1. Experimental Material

137 The physical parameters of acorns from the analyzed batches (harvested from uneven-aged tree
 138 stands) are presented in Table 1. The average values of the measured parameters were determined in
 139 the following ranges: length – 28.10-28.82 mm, diameter – 16.25-16.54 mm, mass – 4.35-4.87 g,
 140 arithmetic mean diameter – 20.21-20.63 mm, geometric mean diameter – 19.49-19.88 mm, specific
 141 mass – 0.22-0.24 g mm⁻¹, shape factor K_1 – 0.58, shape factor K_2 – 0.69-0.70. Acorns from the evaluated
 142 batches differed most significantly in length and mass. The analyzed acorns did not differ
 143 significantly in diameter, arithmetic and geometric mean diameter, specific mass and shape factors
 144 K_1 and K_2 . The results of the analysis revealed that the largest acorns were harvested from a
 145 76-year-old tree stand, and the smallest acorns – from a 91-year-old tree stand.

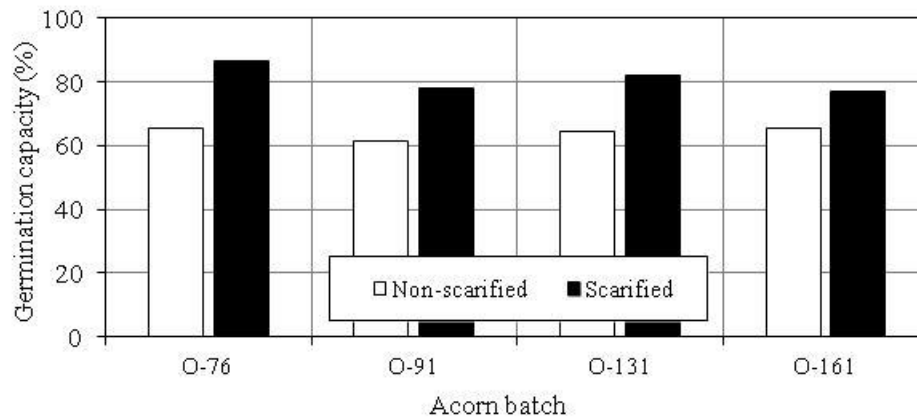
146 **Table 1.** Statistical distribution of the physical properties (mean value ± standard deviation) of acorn
 147 and significant differences between batches.

Property / indicator	Acorn batch			
	O-76	O-91	O-131	O-161
Length (mm)	28.82±2.20 ^b	28.10±2.36 ^a	28.74±2.41 ^{ab}	28.49±2.88 ^{ab}
Diameter (mm)	16.54±1.62 ^a	16.27±1.52 ^a	16.53±1.53 ^a	16.25±1.56 ^a
Mass (g)	4.87±1.16 ^b	4.35±0.98 ^a	4.61±1.22 ^{ab}	4.43±1.12 ^a
Arithm. mean diameter (mm)	20.63±1.50 ^a	20.21±1.39 ^a	20.60±1.53 ^a	20.33±1.57 ^a
Geom. mean diameter (mm)	19.88±1.55 ^a	19.49±1.41 ^a	19.86±1.53 ^a	19.56±1.55 ^a
Specific mass (g mm ⁻¹)	0.24±0.04 ^a	0.22±0.04 ^a	0.23±0.04 ^a	0.22±0.04 ^a
Shape factor K_1 (–)	0.58±0.06 ^a	0.58±0.07 ^a	0.58±0.06 ^a	0.58±0.07 ^a
Shape factor K_2 (–)	0.69±0.05 ^a	0.70±0.05 ^a	0.69±0.05 ^a	0.69±0.06 ^a

148 a, b – superscript letters denote significant differences between the corresponding properties (indicators).

149 3.2. Germination Capacity of Acorns

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



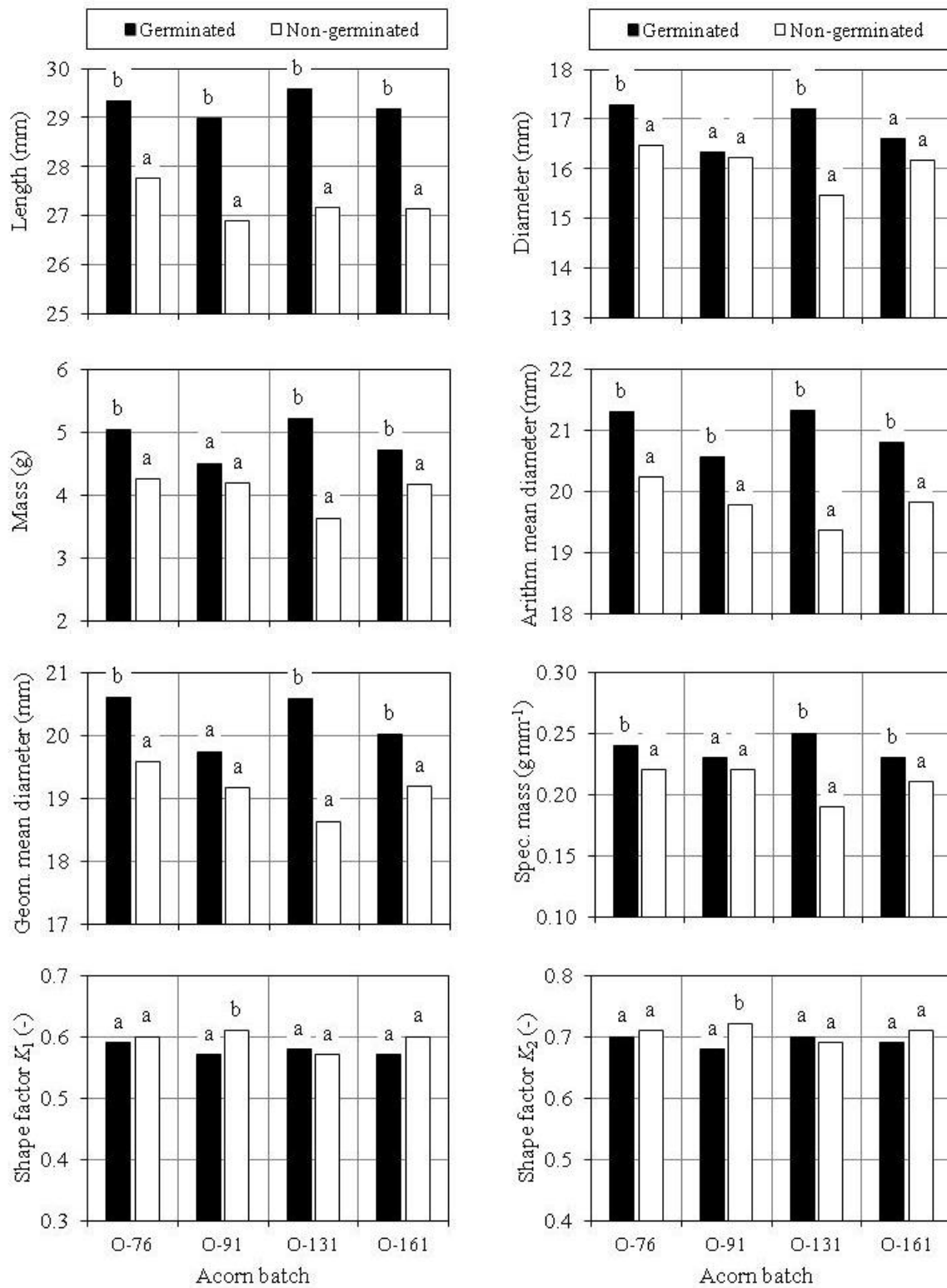
156 **Figure 2.** Germination capacity of scarified and non-scarified pedunculate oak acorns.

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

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

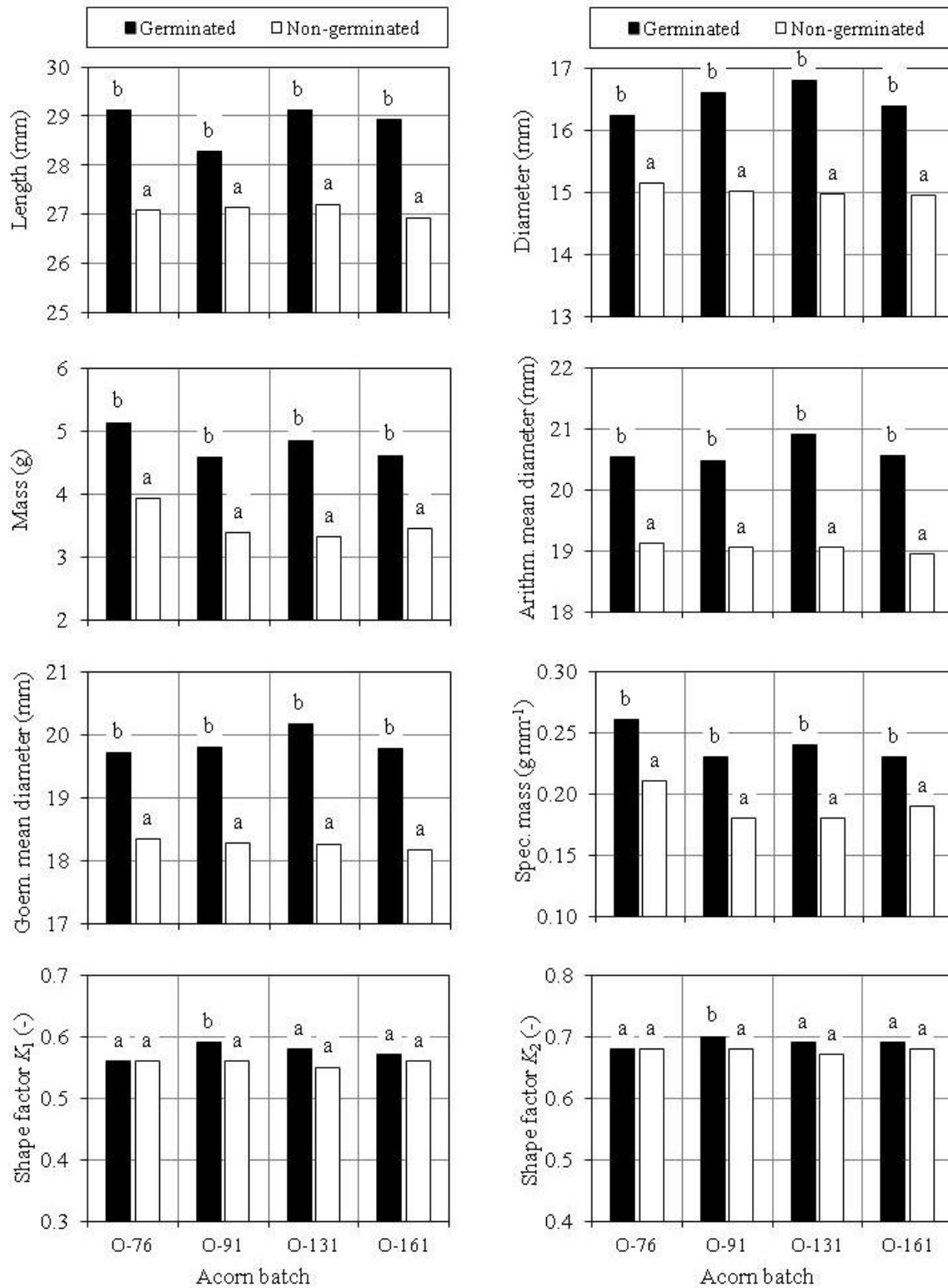
177 3.3. Evaluation of Scarification Treatment

178 The statistical distribution of scarification index values is presented in Table 2. Scarification
 179 reduced acorn length by 15% to 42% (31% on average) and decreased acorn mass by 12% to 35%
 180 (22% on average). Batch O-76 differed significantly from the remaining acorn batches in terms of the
 181 dimensional scarification index. Batch O-91 differed significantly from the remaining acorn batches
 182 in terms of the mass scarification index. The coefficient of variation of the above parameters ranged
 183 from 9.54% to 19.47%.



184
185

Figure 3. Significance of differences in the physical parameters of germinated and non-germinated acorns not subjected to scarification; a, b – different letters denote statistically significant differences.



186
187

Figure 4. Significance of differences in the physical parameters of germinated and non-germinated acorns subjected to scarification; a, b – different letters denote statistically significant differences.

188

189
190**Table 2.** Statistical distribution and significant differences in the scarification index of acorns from four batches.

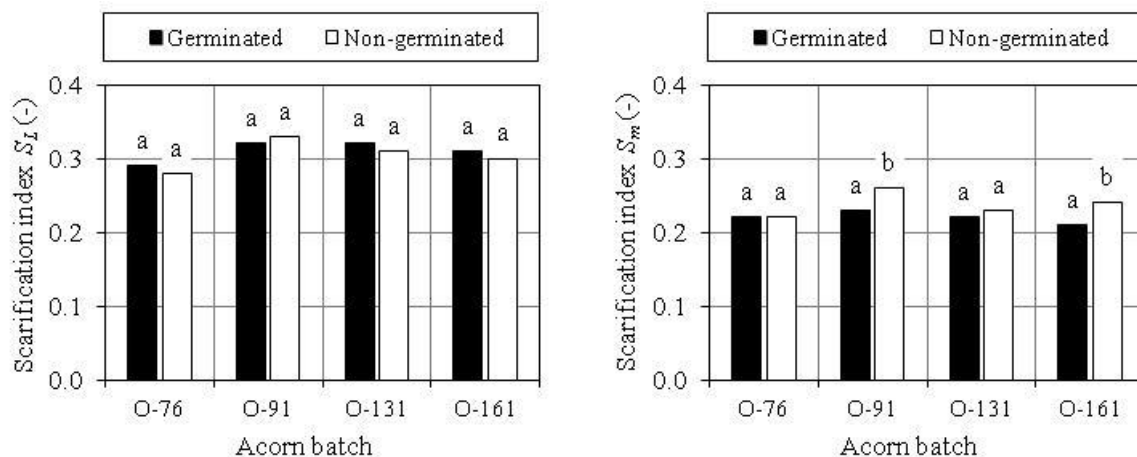
Scarification index	Acorn batch	Value of trait			Standard deviation of trait	Coefficient of trait variability (%)
		minimum	maximum	average		
Dimensional S_L	O-76	0.15	0.39	0.28 ^a	0.046	16.18
	O-91	0.19	0.40	0.32 ^b	0.041	12.69
	O-131	0.24	0.42	0.32 ^b	0.031	9.77
	O-161	0.22	0.38	0.31 ^b	0.030	9.54
Mass S_m	O-76	0.12	0.35	0.22 ^a	0.043	19.47
	O-91	0.13	0.33	0.24 ^b	0.043	18.16
	O-131	0.13	0.33	0.22 ^a	0.034	15.64
	O-161	0.14	0.33	0.22 ^a	0.042	19.23

191

a, b – superscript letters denote significant differences between the corresponding properties.

192
193
194
195
196
197

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.

198
199**Figure 5.** Significance of differences in the scarification index of germinated and non-germinated acorns: a, b – different letters denote statistically significant differences.

200

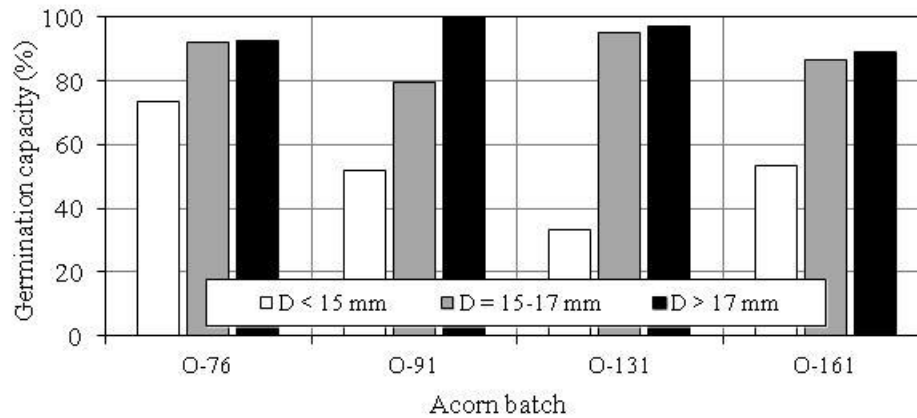
3.4. Germination Capacity of Scarified Acorns

201
202
203
204
205
206
207
208
209
210
211

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).

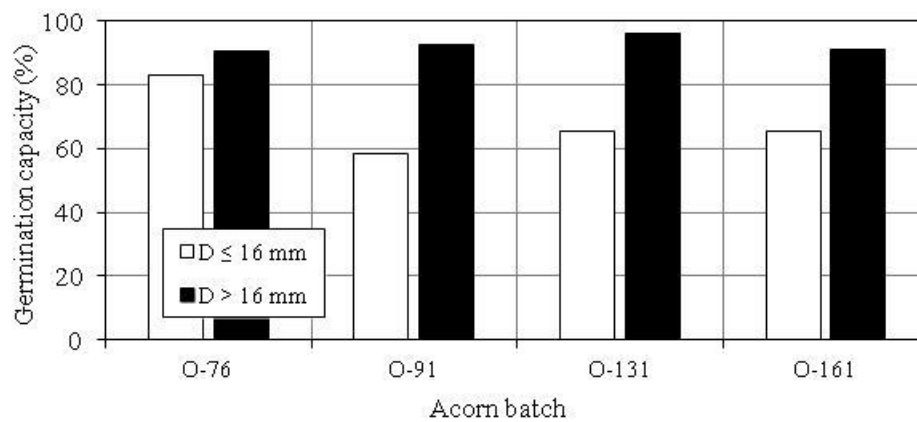
Similar relationships were noted when acorns were divided into two size groups based on their diameter (Figure 7). Germination capacity ranged from around 59% (batch O-91) to around 83% (batch O-76) in acorns measuring up to 16 mm in diameter, and it exceeded 90% in acorns with a diameter larger than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



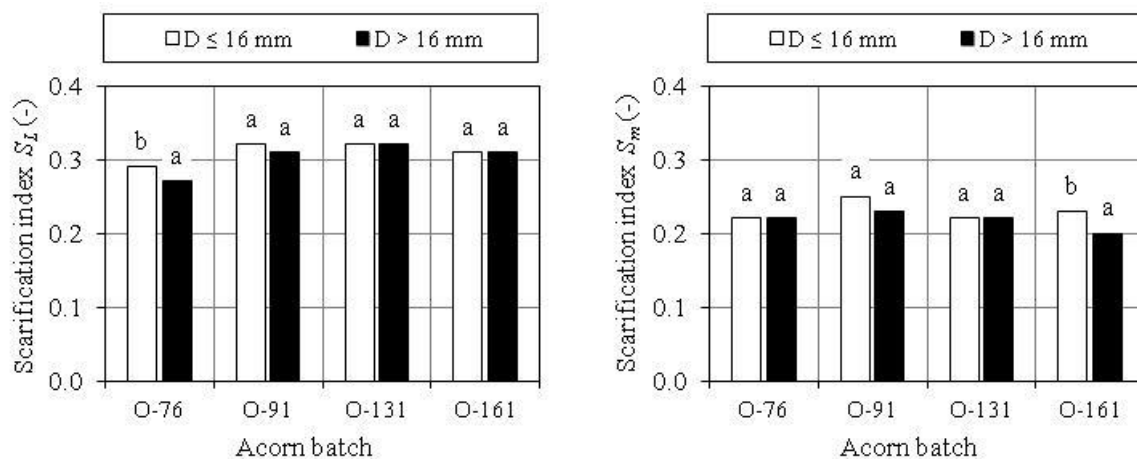
212

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



213

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



214

Figure 8. Significance of differences in the scarification index of acorns measuring up to 16 mm and more than 16 mm in diameter: a, b – different letters denote statistically significant differences.

215

216

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

217

218

219

220

221

222 higher than 0.35 (batches O-131 and O-161). Acorns measuring more than 16 mm in diameter were
 223 characterized by significantly higher germination capacity which did not drop below 84% regardless
 224 of the value of the scarification index.

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

Acorn batch	Size group	Germination capacity of acorns with dimensional scarification index S_L :			
		≤ 0.25	0.26-0.30	0.31-0.35	> 0.35
O-76	$D \leq 16$ mm	60.0	83.3	92.7	75.0
	$D > 16$ mm	100.0	84.2	92.2	-
	Total	88.2	83.6	92.3	75.0
O-91	$D \leq 16$ mm	0	63.6	57.9	60.0
	$D > 16$ mm	100.0	88.9	95.8	88.9
	Total	80.0	79.3	79.1	73.7
O-131	$D \leq 16$ mm	-	50.0	70.4	100.0
	$D > 16$ mm	100.0	94.4	96.3	100.0
	Total	100.0	76.7	81.8	100.0
O-161	$D \leq 16$ mm	33.3	64.7	64.0	100.0
	$D > 16$ mm	-	81.0	100.0	100.0
	Total	33.3	73.2	78.6	100.0

227
 228 Similar results were noted in an analysis of the mass scarification index (Table 4). The
 229 germination capacity of different size groups ranged from 0% to 100%, and germination capacity
 230 was not directly correlated with the scarification index. In most acorns measuring more than 16 mm
 231 in diameter (excluding acorns from batch O-161 with a scarification index of 0.26 to 0.30),
 232 germination capacity exceeded 80%, and it reached 100% in 11 out of 19 cases. In acorns measuring
 233 up to 16 mm in diameter, germination capacity was highest when the scarification index was below
 234 0.15 (batches O-91 and O-131), 0.16-0.20 (batch O-131) and above 0.30 (batch O-76).

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

Acorn batch	Size group	Germination capacity of acorns with mass scarification index S_m :				
		≤ 0.15	0.16-0.20	0.21-0.25	0.26-0.30	> 0.30
O-76	$D \leq 16$ mm	66.6	82.3	82.6	88.9	100.0
	$D > 16$ mm	100.0	100.0	81.0	100.0	100.0
	Total	83.3	90.3	81.8	91.7	100.0
O-91	$D \leq 16$ mm	100.0	66.7	66.7	42.9	50.0
	$D > 16$ mm	100.0	92.3	96.4	90.0	66.7
	Total	100.0	87.5	84.8	62.5	57.1
O-131	$D \leq 16$ mm	0	93.3	40.0	63.6	-
	$D > 16$ mm	100.0	100.0	93.1	100.0	100.0
	Total	50.0	97.1	75.0	73.3	100.0
O-161	$D \leq 16$ mm	100.0	50.0	79.2	57.1	0
	$D > 16$ mm	100.0	100.0	81.3	75.0	-
	Total	100.0	82.8	80.0	61.1	0

237

238 4. Discussion

239 An analysis of the physical parameters of acorns harvested from uneven-aged tree stands
 240 revealed that the largest acorns were harvested from 76-year-old trees and the smallest acorns – from
 241 91-year-old trees. Despite significant differences in length and mass, the acorns from the above
 242 batches were characterized by similar diameter and shape. Acorns were harvested from tree stands
 243 in the same geographical location, therefore, differences in acorn size can probably be attributed to

244 genetic variations which significantly influence the physical properties of seeds [31-33]. In the
245 current study, the age of the parent tree stand (76 to 161 years) did not exert a significant influence
246 on the physical parameters of acorns. Different results were reported by Kaliniewicz et al. [34] in
247 Scots pine where the physical dimensions and mass of seeds decreased with the age of parent trees.
248 Similar trends were noted by Suszka et al. [2] based on long-term observations of tree stands rather
249 than a comparison of the physical properties of acorns harvested from uneven-aged tree stands
250 where genetic variations could play a key role. The significant influence of tree age on the physical
251 parameters of seeds was also noted in a study of Norway spruce, but the nature of the observed
252 changes was difficult to describe due to the disrupting influence of genetic factors [35]. In the present
253 experiment, the dimensions and mass of pedunculate oak acorns were within the range of values
254 reported by Suszka et al. [2], Nikolić and Orlović [36] and Tylkowski and Bujarska-Borkowska [29].
255 The evaluated acorns were somewhat smaller than those harvested in southern Poland [8,37] and in
256 Serbia [38]. Seed size and mass generally decrease in northern regions of the globe [39-41].

257 In terms of germination capacity, acorns from uneven-aged tree stands were within the lower
258 range of values in quality class I (61.5-65.6%). Thermal treatment was effective in preventing fungal
259 diseases and acorn mummification, but failed to eliminate already infected and partially damaged
260 acorns from the processed batches. According to Tylek [37] and Tylek et al. [42], small and large
261 acorns are equally susceptible to fungal infections; therefore, they cannot be effectively separated
262 based on their geometric parameters or shape. The results of the present study indicate that
263 germination capacity can be somewhat improved (by around 1.5 percentage points) by eliminating
264 around 2% of the shortest acorns from each batch. Scarification was a more effective treatment which
265 increased germination capacity from around 64% to around 81%. Similar results were reported by
266 Giertych and Suszka [19]. During scarification, the seed coat and cotyledons are partially removed,
267 which improves water penetration and aeration, thus accelerating germination. Symptoms of
268 disease are also more visible in scarified acorns which can be removed from the batch. Unlike the
269 seeds of other forest trees [43], pedunculate oak acorns cannot be sorted effectively based on
270 physical parameters; therefore, the optical parameters of acorn cross-sections could be used as an
271 innovative selection trait. Optical parameters cannot be reliably evaluated with a naked eye, which is
272 why an automated scarification device with a vision system has been developed [8,24] to identify
273 early symptoms of disease, eliminate damaged acorns and increase germination capacity by up to
274 10% relative to manually processed material. However, evaluations of acorn health can be
275 compromised by two types of errors. Firstly, acorns with normally developed cotyledons are often
276 classified as healthy despite the presence of necrotic changes in the radicle, which are not visible to
277 the evaluator. Secondly, acorns with damaged cotyledons can be classified as unfit for sowing even
278 when the radicle is healthy and potentially capable of germinating. Nonetheless, the germination
279 capacity of acorns is influenced mainly by the severity of pathological changes and effective removal
280 of non-viable acorns. Some batches contain up to several dozen percent of damaged acorns [6].

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

287 According to Tylkowski and Bujarska-Borkowska [29], pedunculate oak acorns should be sorted
288 based on size before planting. Acorn mass is positively correlated with seedling size [44-49],
289 therefore, similarly sized acorns should be planted separately to promote even emergence of
290 seedlings. The results of the present study also demonstrate that acorns should be sorted into size
291 groups before scarification and sowing. Germination capacity decreased with a decrease in acorn
292 diameter, which implies that the seeding rate of acorns from different size groups should be
293 adjusted accordingly to obtain the required number of seedlings. Larger acorns with a higher
294 germination capacity (> 90%) should be used mainly in container nurseries, whereas smaller acorns
295 should be sown in open-field nurseries. Seeding rate should be determined based on the

296 germination capacity of acorns. Acorns are easy to separate with the use of conventional sorting
297 devices, and mesh sieves with longitudinal openings are particularly recommended for separating
298 acorns into size groups based on their diameter.

299 Acorns with partially excised seed cover and cotyledons germinate faster [2,8,19,50]. This is a
300 particularly important consideration in container nurseries where the growth cycle is relatively short
301 and where polyethylene tents are used several times during the growing season. In the current
302 study, the variations in the values of the dimensional scarification index (0.15 to 0.42) and the mass
303 scarification index (0.12 to 0.35) did not influence the germination capacity of differently sized
304 acorns. Shi et al. [51] reported the best results where acorns were reduced in length by one-third to
305 one-half. In the cited study, scarification increased fertilizer absorption by oak acorns and seedlings
306 grown in a nursery. According to Giertych and Suszka [19] and Tadeusiewicz et al. [8], the reduction
307 in acorn mass during scarification should not exceed 20%. More extensive scarification increases the
308 accuracy of health assessments, but it also compromises seedling growth. The presence of intact
309 nutrient reserves in acorns promotes embryonic development, increases seedling resistance to
310 adverse environmental factors and improves the morphological parameters of developing plants
311 [19,50] (Hou et al. 2010).

312 5. Conclusions

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

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

322 The germination capacity of scarified acorns was correlated with their diameter. Acorns should
323 be sorted into size groups before scarification and sowing to promote even seedling emergence. The
324 germination capacity of acorns with the largest diameter (e.g. above 16 mm) exceeds 90%, and these
325 acorns are recommended for sowing in container nurseries. Acorns with the smallest diameter (up to
326 16 mm) are characterized by lower germination capacity (around 58% to 83%), and they are more
327 suited for sowing in open-field nurseries where seeding rate should be determined based on the
328 germination capacity of a given acorn batch.

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

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

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

335 References

- 336 1. Jaworski, A. *Hodowla lasu. Tom III. Charakterystyka hodowlana drzew i krzewów leśnych [Silviculture. Volume 3.*
337 *Breeding characteristics of forest trees and shrubs]*. Publisher: PWRiL, Warszawa, 2011, pp. 242–266, ISBN
338 9788309010760 (in Polish).
- 339 2. Suszka, B.; Muller, C.; Bonnet-Masimber, M. *Nasiona leśnych drzew liściastych od zbioru do siewu [Seeds of*
340 *deciduous forest trees – from harvest to sowing]*. Publisher: Wydawnictwo Naukowe PWN,
341 Warszawa-Poznań, 2000, pp. 1–274, ISBN 8301133430 (in Polish).
- 342 3. Rodriguez-Campos, A.; Diaz-Maroto, I.J.; Barcala-Perez, E.; Vila-Lameiro P. Comparison of the
343 autoecology of *Quercus robur* L. and *Q. petraea* (Mattuschka) Liebl. Stands in the Northwest of the Iberian
344 Peninsula. *Ann. For. Res.* **2010**, *53*, 7–25.

- 345 4. Brus, D.J.; Hengeveld, G.M.; Walvoort, D.J.J.; Goedhart, P.W.; Heidema, A.H.; Nabuurs, G.J.; Gunia, K.
346 Statistical mapping of tree species over Europe. *Eur. J. For. Res.* **2012**, *131*, 145–157.
347 <http://dx.doi.org/10.1007/s10342-011-0513-5>
- 348 5. Peguero-Pina, J.J.; Sisó, S.; Sancho-Knapik, D.; Díaz-Espejo, A.; Flexas, J.; Galmés, J.; Gil-Pelegrín, E. Leaf
349 morphological and physiological adaptations of a deciduous oak (*Quercus faginea* Lam.) to the
350 Mediterranean climate: A comparison with a closely related temperate species (*Quercus robur* L.). *Tree*
351 *Phys.* **2016**, *36*, 287–299. <http://dx.doi.org/10.1093/treephys/tpv107>
- 352 6. Knudsen, I.M.B.; Thomsen, K.A.; Jensen, B.; Poulsen, K.M. Effect of hot water treatment, biocontrol agents,
353 disinfectants and a fungicide on storability of English oak acorns and control of the pathogen, *Ciboria*
354 *batschiana*. *For. Pathol.* **2004**, *1*, 47–64. <http://dx.doi.org/10.1046/j.1439-0329.2003.00348.x>
- 355 7. Schröder, T.; Kehr, R.; Procházková, Z.; Sutherland, J.R. Practical methods for estimating the infection rate
356 of *Quercus robur* acorn seedlots by *Ciboria batschiana*. *For. Pathol.* **2004**, *34*, 187–196.
357 <http://dx.doi.org/10.1111/j.1439-0329.2004.00357.x>
- 358 8. Tadeusiewicz, R.; Tylek, P.; Adamczyk, F.; Kiełbasa, P.; Jabłoński, M.; Pawlik, P.; Piłat, A.; Walczyk, J.;
359 Szczepaniak, J.; Juliszewski, T.; Szaroleta, M. Automation of the acorn scarification process as a
360 contribution to sustainable forest management. Case study: Common oak. *Sustainability* **2017**, *9*, 2276.
361 <http://dx.doi.org/10.3390/su9122276>
- 362 9. Chebouti-Meziou, N.; Merabet, A.; Chebouti, Y.; Bissaad, F.Z.; Behidj-Benyounes, N.; Doumandji, S. Effect
363 of cold and scarification on seeds germination of *Pistacia atlantica* L. for rapid multiplication. *Pak. J. Bot.*
364 **2014**, *46*(2), 441–446.
- 365 10. Asl, M.B.; Sharivivash, R.; Rahbari, A. Effect of different treatments on seed germination of honey locust
366 (*Gleditsia triacanthos*). *Modern Applied Science* **2011**, *5*(1), 200–204.
- 367 11. Ciccarelli, D.; Andreucci, A.C.; Pagni, A.M.; Garbari, F. The role of the elaiosome in the germination of
368 seeds of *Myrtus communis* L. (Myrtaceae). *Atti Soc. Tosc. Sci. nat., Mem., Serie B* **2004**, *111*, 143–146.
- 369 12. Nwaoguala, C.N.C.; Osaigbovo, A.U. Enhancing seedling production of black velvet tamarind (*Dialium*
370 *guineense* Willd). *Journal of Applied and Natural Science* **2009**, *1*(1), 36–40.
- 371 13. Tylkowski, T.; Grupa, R. Effectiveness of pre-sowing scarification methods of black locust seeds. *Sylvan*
372 **2010**, *154*(1), 33–40 (in Polish).
- 373 14. Missanjo, E.; Maya, C.; Kapira, D.; Banda, H.; Kamanga-Thole, G. Effect of seed size and pretreatment
374 methods on germination of *Albizia lebbek*. *ISRN Botany* **2013**, Article ID 969026, 4 pages.
375 <http://dx.doi.org/10.1155/2013/969026>
- 376 15. Aliero, B.L. Effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of
377 seeds of African locust bean tree, *Parkia biglobosa*. *Afric. J. Biotechnol.* **2004**, *3*(3), 179–181.
378 <http://dx.doi.org/10.5897/AJB2004.000-2031>
- 379 16. Pipinis, E.; Milios, E.; Smiris, P.; Gioumousidis, C. Effect of acid scarification and cold moist stratification
380 on the germination of *Cercis siliquastrum* L. seeds. *Turk. J. Agric. For.* **2011**, *35*, 259–264.
381 <http://dx.doi.org/10.3906/tar-1003-848>
- 382 17. Nelson, S. Noni seed handling and seedling production. *Fruits and Nuts* **2005**, *10*, 1–4.
- 383 18. Botsheleng, B.; Mathowa, T.; Mojeremane, W. Effects of pre-treatments methods on the germination of pod
384 mahogany (*Azalia quanzensis*) and mukusi (*Baikiaea plurijuga*) seeds. *International Journal of Innovative*
385 *Research in Science* **2014**, *3*(1), 8108–8113.
- 386 19. Giertych, M.J.; Suszka, J. Consequences of cutting off distal ends of cotyledons of *Quercus robur* acorns
387 before sowing. *Ann. Forest Sci.* **2011**, *68*(2), 433–442. DOI: 10.1007/s13595-011-0038-6
- 388 20. Jabłoński, M.; Tylek, P.; Walczyk, J.; Tadeusiewicz, R.; Piłat, A. Colour-based binary discrimination of
389 scarified *Quercus robur* acorn under varying illumination. *Sensors* **2016**, *16*, 1319.
390 <http://dx.doi.org/10.3390/s16081319>
- 391 21. Grabska-Chrzastowska, J.; Kwiecień, J.; Drożdż, M.; Bublński, Z.; Tadeusiewicz, R.; Szczepaniak, J.;
392 Walczyk, J.; Tylek, P. Comparison of selected classification methods in automated oak seed sorting. *J. Res.*
393 *Appl. Agric. Engng* **2017**, *62*(1), 31–33.
- 394 22. Pawlik, P.; Jabłoński, M.; Bublński, Z.; Tadeusiewicz, R.; Walczyk, J.; Tylek, P.; Juliszewski, T.; Adamczyk,
395 F. Use of Harris detector for determination of orientation of acorns in the process of automated scarification. *J. Res.*
396 *Appl. Agric. Engng* **2017**, *62*(1), 163–165.

- 397 23. Przybyło, J.; Jabłoński, M.; Pociecha, D.; Tadeusiewicz, R.; Piłat, A.; Walczyk, J.; Kielbasa, P.; Szczepaniak,
398 J.; Adamczyk, F. Application of model-based design in algorithms' prototyping for experimental acorn
399 scarification rig. *J. Res. Appl. Agric. Engng* **2017**, *62*(1), 166–170.
- 400 24. Tadeusiewicz, R.; Tylek, P.; Adamczyk, F.; Kielbasa, P.; Jabłoński, M.; Bubliski, Z.;
401 Grabska-Chrzastowska, J.; Kaliniewicz, Z.; Walczyk, J.; Szczepaniak, J.; Juliszewski, T.; Szaroleta, M..
402 Assessment of selected parameters of the automatic scarification device as an example of a device for
403 sustainable forest management. *Sustainability* **2017**, *9*, 2370. <http://dx.doi.org/10.3390/su9122370>
- 404 25. Greń, J. *Statystyka matematyczna. Modele i zadania [Mathematical statistics. Models and tasks]*. Publisher: PWN,
405 Warszawa, 1984, pp.237-280, ISBN 8301036990 (in Polish).
- 406 26. Mohsenin, N.N. *Physical properties of plant and animal materials*. Publisher: Gordon and Breach Science
407 Public, New York, 1986, pp. 1–891, ISBN 9780677213705.
- 408 27. Kaliniewicz, Z.; Tylek, P.; Markowski, P.; Anders, A.; Rawa, T.; Józwiak, K.; Fura, S. Correlations between
409 the germination capacity and selected physical properties of Scots pine (*Pinus sylvestris* L.) seeds. *Baltic*
410 *Forestry* **2013**, *19*(2), 201–211.
- 411 28. Grochowicz, J. *Maszyny do czyszczenia i sortowania nasion [Seed cleaning and sorting machines]*. Publisher:
412 Akademia Rolnicza, Lublin, 1994, pp. 25-28, ISBN 839016129X (in Polish).
- 413 29. Tylkowski, T.; Bujarska-Borkowska, B. Effect of acorn size and sowing depth on *Quercus robur* and *Q.*
414 *petraea* seedling emergence and height. *Sylvan* **2011**, *155*(3), 159–170 (in Polish).
- 415 30. Rabiej, M. *Statystyka z programem Statistica [Statistics in Statistica software]*. Publisher: Helion, Gliwice, 2012,
416 pp. 1–344, ISBN 9788324641109 (in Polish).
- 417 31. García-Mozo, H.; Gómez-Casero, M.T.; Domínguez, E.; Galán, C. Influence of pollen emission and
418 weather-related factors on variations in holm-oak (*Quercus ilex* subsp. *ballota*) acorn production. *Environ.*
419 *Exp. Bot.* **2007**, *61*(1), 35–40. <http://dx.doi.org/10.1016/j.envexpbot.2007.02.009>
- 420 32. Koenig, W.D.; Knops, J.M.H.; Carmen, W.J.; Sage, D. No trade-off between seed size and number in the
421 valley oak *Quercus lobata*. *Am. Nat.* **2009**, *173*(5), 682–688. <http://dx.doi.org/10.1086/597605>
- 422 33. Alejano, R.; Vázquez-Piqué, J.; Carevic, F.; Fernández, M. Do ecological and silvicultural factors influence
423 acorn mass in Holm Oak (southwestern Spain)? *Agroforest Syst.* **2011**, *83*(1), 25–39.
424 <http://dx.doi.org/10.1007/s10457-011-9369-4>
- 425 34. Kaliniewicz, Z.; Rawa, T.; Tylek, P.; Markowski, P.; Anders, A.; Fura, S. The effect of the age of Scots pine
426 (*Pinus sylvestris* L.) stands on the physical properties of seeds and the operating parameters of cleaning
427 machines. *Technical Sciences* **2013**, *16*(1), 63–72.
- 428 35. Kaliniewicz, Z.; Markowski, P.; Anders, A.; Tylek, P.; Krzysiak, Z.; Fura, S. Influence of the age of parent
429 stand on selected physical properties of Norway spruce seeds. *Sylvan* **2017**, *161*(7), 548–557 (in Polish).
- 430 36. Nikolić, N.P.; Orlović, S. Genotypic variability of morphological characteristics of English oak (*Quercus*
431 *robur* L.) acorn. *Zbornik Matice srpske za prirodne nauke* **2002**, *102*, 53–58. DOI: 10.2298/ZMSPN0201053N
- 432 37. Tylek, P. Size and shape as separation properties of pedunculate oak seeds (*Quercus robur* L.). *Acta*
433 *Agrophys.* **2012**, *19*(3), 673–687 (in Polish).
- 434 38. Rakić, S.; Povrenović, D.; Tešević, V.; Simić, M.; Maletić, R. Oak acorn, polyphenols and antioxidant
435 activity in functional food. *J. Food Eng.* **2006**, *74*(3), 416–423.
436 <http://dx.doi.org/10.1016/j.jfoodeng.2005.03.057>
- 437 39. Aizen, M.A.; Patterson, W.A. Acorn size and geographical range in the North American oaks (*Quercus* L.).
438 *J. Biogeogr.* **1990**, *17*(3), 327–332. <http://dx.doi.org/10.2307/2845128>
- 439 40. Oleksyn, J.; Reich, P.B.; Tjoelker, M.G.; Chalupka, W. Biogeographic differences in shoot elongation
440 pattern among European Scots pine populations. *For. Ecol. Manage.* **2001**, *148*, 207–220.
441 [http://dx.doi.org/10.1016/S0378-1127\(00\)00537-5](http://dx.doi.org/10.1016/S0378-1127(00)00537-5)
- 442 41. Moles, A.T.; Westoby, M. Latitude, seed predation and seed mass. *J. Biogeogr.* **2003**, *30*(1), 105–128.
443 <http://dx.doi.org/10.1046/j.1365-2699.2003.00781.x>
- 444 42. Tylek, P.; Kaliniewicz, Z.; Kielbasa, P.; Zagrobelny, T. Mass and density as separation criteria of
445 pedunculate oak (*Quercus robur* L.) seeds. *Electron. J. Pol. Agric. Univ. Ser. For.* **2015**, *18*(4), #5. Available
446 online: <http://www.ejpau.media.pl/volume18/issue4/art-05.html> (accessed on 5 February 2018).
- 447 43. Kaliniewicz, Z.; Tylek, P.; Anders, A.; Markowski, P.; Rawa, T.; Ołdakowski, M.; Wąsowski, L. An analysis
448 of the physical properties of seeds of selected deciduous tree species. *Balt. For.* **2016**, *22*(1), 169–174.
- 449 44. Kleinschmit, J. Intraspecific variation of growth and adaptive traits in European oak species. *Ann. For. Sci.*
450 **1993**, *50*(Suppl. 1), 166–185. <http://dx.doi.org/10.1051/forest:19930716>

- 451 45. Kormanik, P.P.; Sung, S.S.; Kormanik, T.L.; Schlarbaum, S.E.; Zarnoch, S.J. Effect of acorn size on
452 development of northern red oak 1-0 seedlings. *Can. J. For. Res.* **1998**, *28*(12), 1805–1813.
453 <http://dx.doi.org/10.1139/x98-152>
- 454 46. Quero, J.L.; Villar, R.; Marañón, T.; Zamora, R.; Poorter, L. Seed-mass effects in four Mediterranean
455 *Quercus* species (Fagaceae) growing in contrasting light environments. *Am. J. Bot.* **2007**, *94*(11), 1795–1803.
456 <http://dx.doi.org/10.3732/ajb.94.11.1795>
- 457 47. Tilki, F. Influence of acorn size and storage duration on moisture content, germination and survival of
458 *Quercus petraea* (Mattuschka). *J. Environ. Biol.* **2010**, *31*, 325–328.
- 459 48. Yi, X.; Zhang, J.; Wang, Z. Large and small acorns contribute equally to early-stage oak seedlings: a
460 multiple species study. *Eur. J. Forest Res.* **2015**, *134*(6), 1019–1026. <https://doi.org/10.1007/s10342-015-0906-y>
- 461 49. Llanderal-Mendoza, J.; Gugger, P.F.; Oyama, K.; Uribe-Salas, D.; González-Rodríguez, A. Climatic
462 determinants of acorn size and germination percentage of *Quercus rucosa* (Fagaceae) along a latitudinal
463 gradient in Mexico. *Bot. Sci.* **2017**, *95*(1), 37–45. <http://dx.doi.org/10.17129/botsci.640>
- 464 50. García-Cebrián, F.; Esteso-Martínez, J.; Gil-Pelegrín, E. Influence of cotyledon removal on early seedling
465 growth in *Quercus robur* L. *Ann. For. Sci.* **2003**, *60*(1), 69–73. <http://dx.doi.org/10.1051/forest:2002075>
- 466 51. Shi, W.; Bloomberg, M.; Li, G.; Su, S.; Jia, L. Combined effects of cotyledon excision and nursery
467 fertilization on root growth, nutrient status and outplanting performance of *Quercus variabilis* container
468 seedlings. *PLoS ONE* **2017**, *12*(5), e0177002. <https://doi.org/10.1371/journal.pone.0177002>
- 469 52. Hou, X.; Yi, X.; Yang, Y.; Liu, W. Acorn germination and seedling survival of *Q. variabilis*: effects of
470 cotyledon excision. *Ann. For. Sci.* **2010**, *67*(7), 711p1–711p7. <http://dx.doi.org/10.1051/forest/2010036>