



Article Aspects of the Process of Sorting European Black Pine Seeds

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Abstract: Research Highlights: Seed separation criteria and the optimal parameters of sorting devices were described. Background and Objectives: Seeds are often sorted into fractions which are sown separately to promote uniform seed germination and seedling emergence. Therefore, the aim of this study was to determine the correlations between the basic physical properties of European black pine (Pinus nigra J.F. Arnold subsp. nigra) seeds for the needs of planning seed sorting operations. Materials and Methods: Black pine seeds were divided into 5 batches representing individual parent trees, and the physical properties (terminal velocity, thickness, width, length, angle of external friction, mass) of each seed were determined. The measured geometric parameters and seed mass were used to calculate the respective indicators for each seed. The values of the analyzed parameters were used to plan the seed separation process. *Results:* The average values of the basic physical properties of seeds were determined in the following range: Terminal velocity—8.32 to 8.73 m s⁻¹, thickness—2.24 to 2.27 mm, width—3.34 to 3.44 mm, length—5.87 to 6.08 mm, angle of external friction—28 to 32°, mass—18.8 to 20.0 mg. Seed mass was most highly correlated with terminal velocity, and it was least correlated with the angle of external friction. Conclusions: The results of this study indicate that black pine seeds should be sorted with the use of pneumatic separators or, alternatively, mesh sieves with longitudinal openings. These sorting devices separate seeds into fractions characterized by similar seed mass, which delivers both economic and environmental benefits in nursery practice.

Keywords: Pinus nigra J.F. Arnold.; dimensions; weight; correlations; separation

1. Introduction

European black pine (*Pinus nigra* J.F. Arnold) is a member of the family Pinaceae which is native to the montane regions of Southern, Central and Eastern Europe and Asia Minor [1–6]. Black pines have regular and dense crowns, and their height can reach 50 m in natural habitats [1,3,7]. Black pines are characterized by rapid growth and high resistance to windthrow disturbance. The species has an extensive root system and is tolerant to saline soils, which makes it particularly useful for reinforcing coastal sand dunes and planting along roads. Black pines thrive on dry and nutrient-deficient sandy soils. The species does not tolerate shade and has a preference for sites that are directly exposed to sunlight. Black pines are resistant to smoke, dust and gas emissions, and they are often planted in sites with high levels of industrial pollution [1,2,5,7,8].

European black pine is a monoecious and wind-pollinated coniferous species whose seeds are dispersed by wind. In individual trees, seed yields are abundant every 2–4 years. In natural open habitats, the species becomes sexually mature at 15–20 years of age. Subject to local weather conditions, flowers appear in May or June, but cones reach maturity only 18–20 months after pollination. Cones

have an average length of 8 cm, and they contain around 30–40 seeds [1,3,5,9]. In Poland and Turkey, black pine cones are harvested before their scales open, usually between November and February. Seeds are extracted, cleaned, dewinged and stored under the same conditions as Scots pine seeds [10,11]. The germination capacity of black pine seeds ranges from 15% to 99%, subject to seed batch [8,11]. Black pine seeds are stored dry. Fresh and properly stored seeds are usually ready to germinate, but in Central and Southern Europe, they are subjected to 6–7 weeks of cold stratification before sowing in forest nurseries [1,10].

Black pines do not regenerate easily in natural habitats, which can be attributed mainly to the short life span of seeds in soil as well as the fact that black pine seeds are eagerly consumed by birds, rodents and ants. Seedlings are also often damaged by rodents and deer [2,12,13]. To address these problems, seeds should be produced under controlled conditions. Seeds of all plant species should be characterized by the highest quality that are characterized by similar germination rates to ensure effective restocking. Seeds are separated immediately after extraction and dewinging to remove impurities and eliminate empty and damaged seeds. The smallest seeds are generally characterized by the lowest germination capacity, and they are often removed together with impurities. This treatment improves germination capacity, but variations in seed germination and seedling growth rates can be observed. For this reason, seeds should be divided into fractions based on a trait that promotes even seedling development and prevents seedling dominance [14]. According to many authors, the average seed mass in a given fraction significantly affects germination in a seed batch [15–21]. The simplest solution would be to divide seeds into fractions based on their mass. However, this task is not easy to accomplish in practice. Seed batches contain seeds that differ considerably in mass and dimensions, and they cannot be effectively separated with the use of vibratory or vibratory-pneumatic cleaners [22]. Therefore, physical parameters that are strongly correlated with seed mass have to be taken into account when designing the process of seed separation and sorting. If seeds are separated based on a selected trait, fractions characterized by similar seed mass can be obtained, thus promoting more even seedling emergence and development. Such seedlings are more uniform in size, which facilitates nursery operations. It should be noted, however, that seedlings developing from only one fraction must not be supplied to customers because such a practice could contribute to the narrowing of genetic diversity. Each lot of planting material should contain seedlings that developed from seeds of different fractions, mixed in the right proportions. In view of the above, an appropriate method of dividing seeds into fractions prior to sowing in an important consideration. The characteristic features of the processed seeds, including variations in their physical properties and the mutual interactions between these attributes, have to be thoroughly investigated before the sorting process is initiated [22,23].

The aim of this study was to determine the correlations between the basic physical properties of European black pine seeds for planning the process of dividing seeds into fractions prior to sowing.

2. Materials and Methods

2.1. Sample Preparation

The basic physical properties of European black pine (*Pinus nigra* J.F. Arnold subsp. *nigra*—syn. *Pinus nigra* J.F. Arnold var. *austriaca*) seeds (Figure 1) were examined. Seeds were obtained in 2016 from 5 selected parent trees aged 66 to 72 years. The sampled trees of similar height grew on calcareous soil in a mixed forest in the municipality of Leba, Szczenurze Forest District. All cones within the crown were harvested in the second half of November and stored. In January, seeds were extracted in the L-78 screening unit. The extraction process lasted 8 h. A constant temperature of 35–40 °C was maintained during the first 4 h, after which it was increased in steps of 5 °C until the achievement of 55–60 °C in the final stage of the extraction process. The extracted seeds were dewinged in a drum dewinger (SOB), and dewinged seeds were separated in a pneumatic sieve separator (SNS) to remove impurities and empty and damaged seeds. Seeds were placed in plastic bags and refrigerated at a constant temperature of 4 °C. In March 2017, preliminary samples of around 200 g each were obtained from the harvested seeds.

Analytical samples of around 100 seeds each were acquired by halving the preliminary samples [10]. Each seed batch was divided into two portions, and one of the two portions was selected randomly for further division until the required number of seeds was achieved in the final sample. The final samples for analyses had the following size: BP-66—115 seeds, BP-68—112 seeds, BP-70—117 seeds, BP-71—112 seeds and BP-72—110 seeds (BP-xx—black pine-age of the stand). The remaining seeds were used to determine the moisture content of seeds using a halogen moisture analyzer fitted with a MAX 5-/WH halogen lamp (Radwag, Radom, Poland). The moisture content of seeds in the analyzed batches was similar at 5.8%–6.2%.



Figure 1. Black pine seeds.

2.2. Physical Properties

The following measuring instruments were used in the first stage of the study: Petkus K-293 pneumatic classifier (Petkus Technologie GmbH, Wutha-Farnroda, Germany), MWM 2325 laboratory microscope (PZO, Warsaw, Poland), a self-designed clock thickness gauge, a steel friction plate (surface roughness— $Ra = 0.48 \mu m$) positioned on a horizontal plane with an adjustable tilt angle, and WAA 100/C/2 laboratory scale (Radwag, Radom, Poland). These instruments were used to determine the following seed parameters: terminal velocity v to the nearest 0.11 m s⁻¹, length L and width W to the nearest 0.02 mm, thickness T to the nearest 0.01 mm, angle of external friction γ to the nearest 1°, and mass m to the nearest 0.1 mg, respectively. The measuring procedures were described previously by Kaliniewicz et al. [24] and Kaliniewicz and Poznański [25]. The angle of external friction was determined based on the mean value of two initial measurements where the seeds were positioned with the longitudinal axis parallel and perpendicular to the direction of movement on the steel friction plate.

In the second stage of the study, the measured parameters were used to calculate the main aspect ratios (*T*/*W*, *T*/*L* and *W*/*L*), geometric mean diameter *D*, sphericity index Φ and specific mass m_D of each seed [22,26,27].

Seeds were divided into three nearly identically sized groups based on their mass, and the boundaries of weight ranges were rounded off to the nearest milligram: small seeds (group 1—seeds lighter than 16 mg), medium-sized seeds (group 2—seeds weighing 16–22 mg) and large seeds (group 3—seeds heavier than 22 mg).

2.3. Statistical Analysis

Data were processed with the use of descriptive statistics, analysis of variance, correlation analysis and regression analysis in Statistica PL v. 12.5 (StatSoft Poland Ltd., Cracow, Poland) at a significance level of $\alpha = 0.05$. Normality was assessed by the Shapiro-Wilk test, and the homogeneity of variance was determined by Levene's test. The variations in the properties of each seed batch and the calculated indicators were determined by one-way analysis of variance (ANOVA). The strength and direction of the correlations between the examined seed properties were determined by calculating Pearson's correlation coefficient [28].

3. Results and Discussion

3.1. Experimental Material

The physical properties of each batch of European black pine seeds are presented in Table 1. The analyzed samples were composed of 110 to 117 seeds, and the standard error of the estimate of the measured attributes did not exceed:

- for terminal velocity—0.3 m s⁻¹,
- for basic dimensions (thickness, width and length)-0.1 mm,
- for the angle of external friction—1°,
- for seed mass—1.4 mg.

Table 1. Mean values of the physical properties of black pine seeds and the significance of differences.

Property/Indicator			Seed Batch		
	BP-66	BP-68	BP-70	BP-71	BP-72
Terminal velocity v (m s ⁻¹)	8.32 ^a	8.41 ^{a,b}	8.64 ^{a,b}	8.73 ^b	8.67 ^{a,b}
Thickness T (mm)	2.25 ^a	2.24 ^a	2.24 ^a	2.26 ^a	2.27 ^a
Width W (mm)	3.44 ^b	3.38 ^{a,b}	3.40 ^{a,b}	3.34 ^a	3.34 ^a
Length <i>L</i> (mm)	6.04 ^b	6.00 ^{a,b}	6.08 ^b	5.87 ^a	6.07 ^b
Angle of external friction γ (°)	32 ^c	31 ^c	32 ^c	30 ^b	28 ^a
Mass m (mg)	19.5 ^a	18.8 ^a	19.4 ^a	18.8 ^a	20.0 ^a
Geometric mean diameter <i>D</i> (mm)	3.60 ^a	3.56 ^a	3.58 ^a	3.53 ^a	3.58 ^a
Aspect ratio T/W (%)	65.9 ^a	66.5 ^a	66.1 ^a	68.6 ^b	68.6 ^b
Aspect ratio T/L (%)	37.6 ^a	37.5 ^a	37.0 ^a	39.0 ^b	37.8 ^a
Aspect ratio W/L (%)	57.3 ^b	56.7 ^{a,b}	56.3 ^{a,b}	57.2 ^b	55.4 ^a
Sphericity index Φ (%)	59.8 ^{a,b}	59.6 ^{a,b}	59.2 ^a	60.5 ^b	59.2 ^a
Specific mass m_D (g m ⁻¹)	5.34 ^a	5.22 ^a	5.35 ^a	5.25 ^a	5.50 ^a

^{a,b,c}—superscript letters denote significant differences between the corresponding properties. BP-xx—black pine-age of the stand.

The average terminal velocity of black pine seeds ranged from 8.32 to 8.73 m s⁻¹. In this respect, the analyzed seeds resembled pine nuts [29] and Morinda spruce seeds [27]. The average values of basic seed parameters were determined in the following range: Thickness-2.24 to 2.27 mm, width—3.34 to 3.44 mm, and length—5.87 to 6.08. The analyzed seeds were somewhat smaller than those harvested in Spain [30,31] and Turkey [32], but their dimensions were similar to those given in the literature [1,5,10,33]. In terms of thickness, black pine seeds resemble Morinda spruce seeds [27], grand fir seeds [34] and Sierra white fir seeds [35]; and in terms of width—Korean fir seeds [35]. The length of the examined seeds is similar to that determined in the seeds of small-leaved lime [36], western juniper, Utah and slash pine [34]. Black pine seeds most closely resemble Aleppo pine seeds in terms of dimensions [37] as well as average mass [37,38]. Seed mass in the analyzed batches ranged from 18.8 to 20.0 mg. According to the literature [12,31,33,39], the mass of the examined seeds is consistent with the average values for the species. Similar seed mass was noted in Weymouth pine seeds [40], common ivy seeds [41], black locust seeds [42] and Morinda spruce seeds [29]. The angle of external friction ranged from 28° to 32°, and it was highly similar to that reported in Scots pine seeds [43], common hornbeam seeds [42] and the seeds of selected spruce [27] and fir species [35]. The aspect ratios and the sphericity index of the evaluated seeds were similar to those noted in Scots pine seeds [43,44], sand pine seeds [34], Japanese larch seeds [34], and the seeds of selected spruce [27,34,45] and fir species [34,35]. In terms of specific mass, black pine seeds are highly similar to Morinda spruce seeds [27] and grand fir seeds [35]. Only local significant differences in seed parameters were noted between batches only randomly, and none of the examined batches differed in all physical attributes from the remaining batches. The highest number of significant differences were observed between batches BP-66 and BP-71 (6 out of

12 comparison), whereas no significant differences were noted between batches BP-66, BP-68 and BP-70. All batches were highly similar in terms of seed thickness, mass, geometric mean diameter and specific mass. Due to minor variations in the examined batches, black pine seeds were pooled into a single experimental group for further analyses.

3.2. Correlations between Basic Physical Properties

The linear correlation analysis (Table 2) revealed the strongest correlations between seed mass and specific mass (r = 0.979) and the weakest correlations between seed length and the angle of external friction. The relatively weak correlations between the basic physical properties of black pine seeds point to considerable variations in the overall shape of the examined material. Practical significance, where the value of the correlation coefficient is minimum 0.4, was observed only between seed length and width. As noted in the Introduction, the germination capacity can be assessed based on seed mass or specific mass. Seed mass was most strongly correlated with terminal velocity, which indicates that black pine seeds should be sorted mainly with the use of pneumatic separators. Similar values of the correlation was also determined between seed mass and seed thickness (r = 0.615), which suggests that black pine seeds could also be sorted with the use of mesh sieves with longitudinal openings. Similar values of the correlation coefficient between seed mass and seed thickness were reported in common beech and mountain ash seeds [42] and in the seeds of selected spruce and fir species [27,35].

Property	Т	W	L	γ	т	m _D
υ	0.400 *	0.229 *	0.223 *	-0.287 *	0.760 *	0.814 *
Т	1	0.385 *	0.359 *	-0.254 *	0.615 *	0.522 *
W		1	0.494 *	0.036	0.495 *	0.366 *
L			1	-0.004	0.569 *	0.455 *
γ				1	-0.196 *	-0.204 *
m					1	0.979 *

Table 2. Coefficients of linear correlation between selected physical properties of black pine seeds.

v—terminal velocity, *T*—thickness, *W*—width, *L*—length, γ —angle of external friction, *m*—mass, *m*_D—specific mass; *—mean significant correlation at the 0.05.

Significant correlations (0.05) were observed in 19 out of 21 comparisons, but the correlation coefficient was practically significant in only 10 cases. Practical significance was not achieved in any case only in the correlations between the angle of external friction and the remaining seed properties. These observations suggest that the frictional properties of the seeds of forest trees can be used as secondary rather than primary distinguishing features in seed separation processes. Similar conclusions were formulated by Tylek [46] and Kaliniewicz et al. [27,35,42–44].

3.3. Recommendations for Seed Separation

The germination efficiency of a seed fraction is considerably affected by average seed mass, but the largest seeds do not always germinate at the fastest rate. Seed mass is determined by genetic and environmental factors as well as weather conditions [32,39,47–51], which suggests that seed batches should not be combined due to considerable differences in average seed mass. In nursery practice, the smallest seed fraction containing empty and malformed seeds is often discarded. Thus, different seed batches should not be combined before separation to eliminate the risk of removing high-quality seeds which are naturally smaller in size. The analyzed batches of black pine seeds were harvested in the

same region from similarly aged trees, and their physical properties were highly similar; therefore, the obtained results can be analyzed jointly. However, each seed batch is separated individually in nursery practice.

Larger seeds have a higher germination potential because they are more abundant in nutrient reserves and are more tolerant to drought stress [19–21,39]. Nursing practice indicates that different seed fractions should be sown separately to promote uniform seed germination and seedling growth, which facilitates the planning of nursery treatments [14]. In the current study, seed mass was most highly correlated with terminal velocity, which indicates that this parameter should play the key role in the seed separation process, as previously postulated by Grochowicz [22]. Black pine seeds were sorted into three mass fractions based on terminal velocity (Table 3), which increased the variation in seed mass in the lightest fraction, but decreased the difference in seed mass in the remaining fractions relative to unsorted material. As a result, the estimated variation in seed mass decreased from approximately 32% to approximately 22% and 18%, respectively. A somewhat smaller decrease in the coefficient of variation of seed mass was noted in all three fractions when seeds were separated based on their thickness. Black pine seeds were least effectively divided into fractions based on their angle of external friction, as demonstrated in the previous analysis.

Seed Fraction	Percentage (%)	Coefficient of Variation (%) of Seed Mass		
Seed Flaction		Fraction	Total	
I ($v < 8 \text{ m s}^{-1}$)	28.6	40.6		
II ($v = 8-9 \text{ m s}^{-1}$)	44.5	21.6		
III ($v > 9 \text{ m s}^{-1}$)	26.9	18.1		
I ($T \le 2.15 \text{ mm}$)	35.0	30.9		
II ($T = 2.16 - 2.40 \text{ mm}$)	43.1	28.2		
III ($T > 2.40 \text{ mm}$)	21.9	19.8		
I ($W \le 3.15 \text{ mm}$)	28.3	35.8	32.2	
II ($W = 3.16 - 3.50 \text{ mm}$)	36.6	27.4		
III ($W > 3.50 \text{ mm}$)	35.1	25.7		
I ($L \le 5.75 \text{ mm}$)	35.0	32.4		
II ($L = 5.76-6.40 \text{ mm}$)	36.4	26.6		
III ($L > 6.40 \text{ mm}$)	28.6	24.5		
I (γ < 29°)	32.3	28.8		
II ($\gamma = 29-32^{\circ}$)	37.1	30.8		
III ($\gamma > 32^{\circ}$)	30.6	36.3		

Table 3. Coefficient of variation of seed mass in three seed fractions.

Based on the adopted method of dividing seeds into size groups, small seeds (group 1) accounted for 27.7%, medium-sized seeds (group 2)—for 38.2%, and large seeds (group 3)—for 34.1% of the examined material.

The histograms presenting the distribution of seed fractions sorted based on terminal velocity and thickness are shown in Figure 2. Based on the above attributes, black pine seeds can be divided into fractions with varied proportions of differently-sized seeds. Seeds are separated into three fractions when the airflow rate is approximately 7.2 and 9.3 m s⁻¹, and the fraction with the lowest terminal velocity contains approximately 54% of small seeds and only 1% of medium-sized seeds. The fraction with the highest terminal velocity contains approximately 1% of small seeds. As previously demonstrated by the performed calculations, seeds are sorted into less homogeneous mass fractions when they are sorted based on thickness. Black pine seeds can be divided into the following fractions with the use of a double mesh sieve separator with longitudinal openings (2.15 and 2.45 mm):

- fraction I (thinnest seeds)—approximately 61% of small seeds, 41% of medium-sized seeds, and 5% of large seeds from the entire batch,
- fraction II (medium-thick seeds)—approximately 36% of small seeds, 52% of medium-sized seeds, and 54% of large seeds from the entire batch,
- fraction III (thickest seeds)—approximately 3% of small seeds, 7% of medium-sized seeds, and 41% of large seeds from the entire batch.



(b)

Figure 2. Distribution of mass-weighted terminal velocity (a) and thickness (b) of black pine seeds.

These findings indicate that black pine seeds intended for sowing are more effectively sorted with the use of pneumatic separators than mesh sieves with longitudinal openings. This is consistent with nursery practice where pneumatic separators are the preferred equipment for sorting the seeds of forest trees and shrubs [10]. Pneumatic separators are particularly recommended for separating black locust, small-leaved lime, European beech, rowan, European hornbeam and grey alder seeds, as well as the seeds of many coniferous tree species [27,35,44,52,53].

A two-stage sorting process (Figure 3) involving a pneumatic separator, followed by a mesh sieve, could be even more effective in diving seeds into homogeneous fractions. The airflow rate in different channels of the pneumatic separator should be adjusted to ensure that approximately 12%

of the seeds with the lowest terminal velocity and approximately 12% of the seeds with the highest terminal velocity are separated from the batch, and the remaining material should be directed to the mesh sieve. When two mesh sieves with longitudinal openings measuring \neq 2.0 mm and \neq 2.3 mm are used, seeds are separated into three fractions containing around 9%, 57% and 34% of the sorted seeds. The fractions with the lowest terminal velocity and the thinnest seeds as well as the fractions with the highest terminal velocity and the thickest seeds can be combined. As a result, seeds are separated into three fractions 18%, 44% and 38% of the sorted seeds (Table 4). Fraction I will comprise around 55% of small seeds, around 7% of medium-sized seeds and only 1% of large seeds from the entire seed material. In turn, fraction III will contain approximately 78% of large seeds, approximately 27% of medium-sized seeds and only 4% of small seeds. Specific seed mass will be more uniform in the resulting seed fractions; however, each fraction should be sown separately.



Figure 3. Process of sorting black pine seeds.

Table 4. Process of sorting black pine seeds into fractions with a pneumatic separator and a mesh sieve.

Seed Fraction	Percentage (%)	Coefficient of Variation (%) of Seed Mass	Proportion (%) of Seeds		
			Group 1	Group 2	Group 3
I	18.1	33.8	54.8	6.9	0.5
II	43.9	24.0	41.4	65.8	21.8
III	38.0	15.4	3.8	27.3	77.7

The application of different seed fractions will deliver the greatest economic and environmental benefits. The appropriate seeding rates should be selected in conventional and container nurseries to promote the rational use of space, minimize the loss of viable seeds, and facilitate the transfer of the entire gene pool to the next generation.

4. Conclusions

European black pine seeds harvested from similarly-aged trees in the same region are characterized by minor variations in basic physical parameters. No significant differences were found in seed thickness, mass, geometric mean diameter and specific mass. Black pine seeds are highly similar to Aleppo pine seeds in terms of average dimensions, average mass and shape factors.

Seed mass and specific mass are mostly highly correlated with terminal velocity, followed by seed thickness, length and width. Seed mass is least correlated with the angle of external friction.

Black pine seeds intended for sowing are most effectively separated into homogeneous mass fractions with the use of pneumatic separators or, alternatively, mesh sieves with longitudinal openings. Seed fractions characterized by similar mass should be sown separately to promote uniform germination. Black pine seeds can be separated into uniform mass fractions even more effectively when both devices are used in combination rather than separately.

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