Correlations between Germination Capacity and Selected Properties of Black Alder (*Alnus glutinosa* Gaertn.) Achenes

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Abstract

The simplest method of improving seed quality involves the elimination of non-germinated or damaged seeds from the propagating material. The aim of this study was to determine correlations between the basic physical properties of black alder achenes and their germination capacity to generate valuable data for improving the seed material. The experiment was performed on 5 batches of achenes harvested from tree stands in 4 seed zones in 3 forest regions of north-eastern Poland. The thickness, width, length, angle of sliding friction and mass of every achene were measured. The results were used to calculate the geometric mean diameter, aspect ratio and sphericity index of black alder achenes. Achenes were germinated for 28 days, and the results were recorded daily to determine the germination rate index of every achene. The relationships between the examined parameters were determined in a t-test for independent samples and by correlation analysis. The analyzed physical parameters of achenes had a minor impact on their germination rate. The results indicate that the classification of black alder achenes based on all measured parameters would not significantly contribute to germination uniformity. The fraction of longest or widest achenes had higher potential germination capacity than unsorted achenes. Germinated achenes were generally characterized by greater thickness, width, length, mass, geometric mean diameter, aspect ratio and sphericity index, but lower angle of sliding friction in comparison with non-germinated achenes.

Keywords: dimensions, mass, angle of sliding friction, germination, correlation.

Introduction

The black alder (*Alnus glutinosa* Gaertn.) is one of the most popular forest trees in Europe which also grows in Asia and northern Africa (Suszka et al. 2000). The optimal habitats for black alder are found in the Baltic States, but the species also thrives in Poland, Belarus and Ukraine. The black alder has a preference for warm climates with good sunlight exposure. It never grows under a canopy and tolerates moderate shade only in early stages of development. The black alder has high moisture and humidity requirements, and it can be encountered in areas with stagnant water (Moricca 2002, Aguinagalde et al. 2005, Mejnartowicz 2008, Claessens et al. 2010, Jaworski 2011, Novotná and Štochlová 2012, Beatty et al. 2015, Houston Durrant et al. 2016).

The black alder grows rapidly in the first years, and its growth rate is reduced with age. In Poland, it reaches the height of 33 m, and its average lifespan is 100-120 years. The greatest increase in thickness is observed at the age circa 40 years (Mejnartowicz 2008, Jaworski 2011). The species begins to produce achenes at the age of approximately 30 years in dense stands and 20 years in open areas. Achenes are produced annually, but highly abundant yields are noted only every 2-3 years. Flowering takes place before leaf formation, at the turn of March and April. Flowers produce cones that mature in September and October and open only when the temperature drops below zero. Achenes are released between autumn and early spring (Suszka et al. 2000, Banaev and Bažant 2007, Claessens et al. 2010, Jaworski 2011, Dąbrowska and Kaszewski 2012, Houston Durrant et al. 2016).

Cones harvested at the turn of November and December are spread out in a thin layer in a well-ventilated room where they will open within several weeks. Achene release can be accelerated by storing cones in a seed extraction plant at a temperature of 27-38 °C. The released achenes can be graded in an indented cylinder in a rotary seed cleaner. Achenes released from cones have 8-9% moisture content, and they can be stored for short periods of time without additional treatment. For long-term storage that extends to years, achenes have to be dried (to 3.5% moisture content) and kept at low temperature (Aniśko et al. 2006, Chmielarz 2010, Tylkowski 2014). Alder achenes (Figure 1) are wingless, but they are edged with webbing that is filled with air, which enables achenes to float on water for longer periods of time without losing the ability to germinate (Suszka et al. 2000, Claessens et al. 2010).

Figure 1. Black alder achenes

The germination capacity of black alder achenes ranges from 35% to 66% with an average of 45%. It is largely determined by weather conditions during flowering and the formation of flower primordia in the previous year (Suszka et al. 2000, Bodył 2007, Chmielarz 2010). Studies of seeds from other plant species (Schopfer et al. 2001, Lynikiene et al. 2006, Kornarzyński and Pietruszewski 2008, Domoradzki and Korpal 2009, Grzesik et al. 2012, Pourakbar and Hatami 2012) demonstrated that germination efficiency can be improved through chemical, physical and physiological treatments, including seed dressing, pelleting, conditioning, irradiation with light of different wavelength and intensity, and exposure to a constant or variable electromagnetic field. The simplest and the most effective method is the removal of non-germinated and damaged seeds from the propagating material. This can be achieved with the involvement of seed cleaning and sorting equipment which is used to prepare seeds for storage. Black alder achenes and the seeds of other tree species are first separated with the use of highly efficient mesh sieves adjusted to different seed/achene sizes. If the results of separation are unsatisfactory, other devices can be used to separate seeds/achenes based on e.g. their frictional properties. Effective separation processes, in particular those that rely on traditional seed graders, require in-depth knowledge about correlations between the physical properties and germination capacity of seeds.

The aim of this study was to determine correlations between the basic geometric and frictional parameters of black alder achenes and their germination capacity. The results can be used to improve the effectiveness of seed separation processes to obtain achenes of the highest quality.

Materials and Methods

Sample preparation

The experimental material comprised 5 batches of black alder achenes harvested in 2012 from 4 seed zones in 3 forest regions in north-eastern Poland. The analyzed batches were harvested from the following tree stands:

a) registration No. MP/1/9383/05, region of origin: 103, municipality: Górowo Iławeckie, geographic location: 20°19'E, 54°19'N, forest habitat: alder forest, age: 75 years (batch A1),

b) registration No. MP/1/11841/05, region of origin: 251, municipality: Kętrzyn, geographic location: 21°29'E, 54°07'N, forest habitat: alder forest, age: 88 years (batch A2),

c) registration No. MP/1/45687/06, region of origin: 103, municipality: Lelkowo, geographic location: 20°16'E, 54°20'N, forest habitat: fresh broadleaved forest, age: 95 years (batch A3),

d) registration No. MP/1/10869/05, region of origin: 401, municipality: Czarnia, geographic location: 21°11'E, 53°19'N, forest habitat: alder forest, age: 108 years (batch A4),

e) registration No. MP/1/12322/05, region of origin: 157, municipality: Iława, geographic location: 19°36'E, 53°42'N, forest habitat: alder forest, age: unknown (batch A5).

Achenes were removed from cones in the Seed Extraction Plant in Jedwabno. All seedlots were processed in the same way. Freshly collected cones were spread out to dry on shelves in a well-ventilated room, at ambient temperature, for 2 days. Then each batch of cones was placed in an extraction chamber, and a temperature of around 40 °C was maintained for 2 days. Small and larger size impurities were separated from achenes using pneumatic sieves. Achenes were placed in air-tight containers in a cold store at a temperature of around –6 °C. Initial samples of achenes, weighing approximately 0.1 kg, were collected.

Analytical subsamples collected from every initial sample of achenes were divided by halving (Załęski 1995), and one half was randomly selected for successive halving. The above procedure was repeated to produce samples of around 100 achenes each. The analyzed achene samples had the following sizes: A1 – 101, A2 – 96, A3 – 101, A4 – 100 and A5 – 100. The remaining achenes were sampled to determine their moisture content in the Radwag MAX 50/WH drying oven with a weighing scale (Radwag Radom, Poland). The moisture content of the analyzed achenes ranged from 7.2% to 7.6%.

Physical properties and germination

The length, *L*, and width, *W*, of achenes were determined with the use of the MWM 2325 laboratory microscope (PZO Warszawa, Poland) to the nearest 0.02 mm. Achene thickness, *T*, was determined with a dial thickness gauge to the nearest 0.01 mm. The above measurements were conducted according to the methods described by Kaliniewicz et al. (2016).

Achene mass, *m*, was determined on the WAA 100/C/2 laboratory scale (Radwag, Radom, Poland) to the nearest 0.1 mg. The angle of sliding friction, *ć*, was measured to the nearest 1° on a steel friction plate (GPS – *Ra*=0.46 µm) positioned on a horizontal plane with an adjustable angle of inclination. Achenes were placed on the friction plate with the longitudinal axis parallel to the direction of movement. The angle of the friction plate was modified until achenes were set in motion, and the result was read from the angle indicator on the device.

A germination test was carried out in 2013 by placing black alder achenes on moistened filtered paper in a container covered with a glass pane. Water loss was supplemented daily with a sprinkler, and filter paper was kept constantly moist. Achenes were germinated at the recommended temperature of 22±1 °C (De Atrib et al. 2007) under exposure to natural light. Germination progress was evaluated daily between 6 p.m. and 7 p.m. Achenes that produced a sprout with a minimum length of 50% achene length were classified as germinated. Observations were continued for 28 days (from 20 October to 17 November). Germinative energy, V_g, and germination capacity, C_g, were determined after 14 and 28 days of germination as the percentage ratio of the number of achenes that germinated in a given period to total number of achenes subjected to the germination test (Suszka et al. 2000).

At the second stage of the study, the physical parameters of achenes and their germination efficiency were used to calculate:

a) geometric mean diameter, *D*, aspect ratio, *R*, and sphericity index, *Φ* (Mohsenin 1986):

$$D = (T \times W \times L)^{1/3} \qquad (1)$$

$$R = \frac{W}{L} \times 100 \qquad (2)$$

$$\Phi = \frac{(T \times W \times L)^{1/3}}{L} \times 100 \qquad (3)$$

b) germination rate index, Wg (Kaliniewicz et al. 2015):

$$W_g = \frac{t_o + 1 + t_n}{t_o + 1} \qquad (4)$$

where: t_n is the time required to produce a healthy germ (days), t_o is the duration of germination test (days).

Statistical analysis

The results of measurements and calculations were processed statistically with the aid of Statistica software package, v. 12.5. The differences in the physical properties of germinated and non-germinated achenes were determined in the *t*-test for independent samples, and the relationships between those variables were determined in a linear correlation analysis (Rabiej 2012). Calculations were performed at a significance level of 0.05.

Results

Experimental material

The standard error of the estimate in the mean values of the evaluated physical properties of black alder achenes did not exceed:

– for achene thickness:	0.02 mm,
– for achene width and length:	0.01 mm,
– for angle of static friction:	2.8°,
– for achene mass:	0.1 mg.

The physical properties of achenes are presented in Table 1. The only physical property that did not differ significantly between the analyzed batches was achene thickness. Locally significant differences were noted in the remaining cases. The average angle of sliding friction was relatively large in the range of 62.8° to 67.37°, and achene mass ranged from 0.85 to 1.26 mg. Batch A5 was composed of the smallest achenes with the smallest geometric mean diameter that differed significantly from the remaining batches. The average aspect ratio of black alder achenes ranged from 79.67% (A3) to 86.47% (A4). The average sphericity index of achenes extended from 58.61% (A1) to 60.90% (A5). The average germination rate index was determined in the range of 0.08 (A5) to 0.35 (A1 and A4).

Table 1. Variations in the physical properties of black alder achenes with an indication of statistically significant differences

Physical property/ parameter	Seed batch				
	A1 *x±SD*	A2 *x±SD*	A3 *x±SD*	A4 *x±SD*	A5 *x±SD*
T [mm]	0.67±0.11^{a}	0.67±0.11^{a}	0.65±0.11^{a}	0.66±0.10^{a}	0.64±0.10^{a}
W [mm]	2.23±0.31^{a}	2.26±0.43^{a}	2.20±0.30^{a}	2.22±0.33^{a}	2.01±0.33^{b}
L [mm]	2.72±0.34^{a}	2.75±0.42^{a}	2.80±0.41^{a}	2.60±0.36^{b}	2.40±0.32^{c}
γ [°]	66.64±13.74^{a}	62.80±10.37^{b}	64.10±11.68^{ab}	67.33±13.04^{a}	67.37±12.93^{a}
m [mg]	1.12±0.44^{bc}	1.20±0.46^{ab}	1.07±0.41^{c}	1.26±0.38^{a}	0.85±0.39^{d}
D [mm]	1.59±0.16^{a}	1.60±0.21^{a}	1.58±0.18^{a}	1.55±0.16^{a}	1.45±0.15^{b}
R [%]	82.68±12.48^{ab}	83.06±15.02^{ab}	79.67±11.42^{b}	86.47±14.99^{a}	84.44±13.88^{a}
Φ [%]	58.61±5.20^{b}	58.78±6.21^{b}	56.89±5.20^{c}	60.09±5.31^{ab}	60.90±5.78^{a}
W_g [-]	0.35±0.24^{a}	0.17±0.19^{b}	0.30±0.21^{a}	0.35±0.22^{a}	0.08±0.12^{c}

Notes: x±*SD* is the mean value ± standard deviation of trait; a, b, c, d are different letters denote statistically significant differences in the values of achene property / parameter

Achene quality

The germination test (Figure 2) revealed that black alder achenes began to sprout after around 12 days. The lowest germinative energy, V_g, was noted in batch A5 (around 1%) and the highest one was noted in batch A1 (around 47%). The number of germinated achenes increased with time during the 24-day germination test. The germination capacity, C_g, of achenes from the analyzed batches was determined at: A1 – 76.24%, A2 – 54.17%, A3 – 80.2%, A4 – 79% and A5 – 32%.

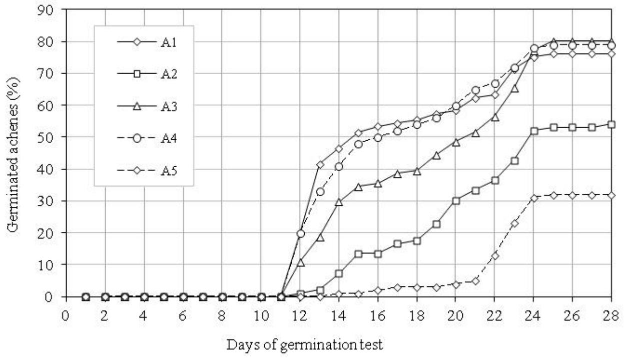

Figure 2. Germination curves of black alder achenes

Physical properties of germinated and non-germinated achenes

The results of a *t*-test for independent samples from 5 batches of black alder achenes (Figure 3) revealed an absence of significant differences in most parameters of germinated and non-germinated achenes. The greatest variations were observed in achene mass, but statistically significant differences were noted only between batches A3 and A5.

An analysis of linear correlations between the physical parameters of black alder achenes (Table 2) demonstrated that mass was most highly correlated with geometric mean diameter and, subject to the evaluated batch, achene thickness (A1, A2 and A5), achene width (A4 and all batches) or achene length (A3). However, a detailed analysis revealed that the quality of plant propagation material would not be significantly improved if achenes were graded based on their physical properties. All achene fractions had a high percentage of germinated achenes; therefore, the separation process would lead to a substantial loss of viable achenes without eliminating non-germinated achenes from the sorted material. A certain improvement in germination capacity (by around 3 percentage points) could be achieved by

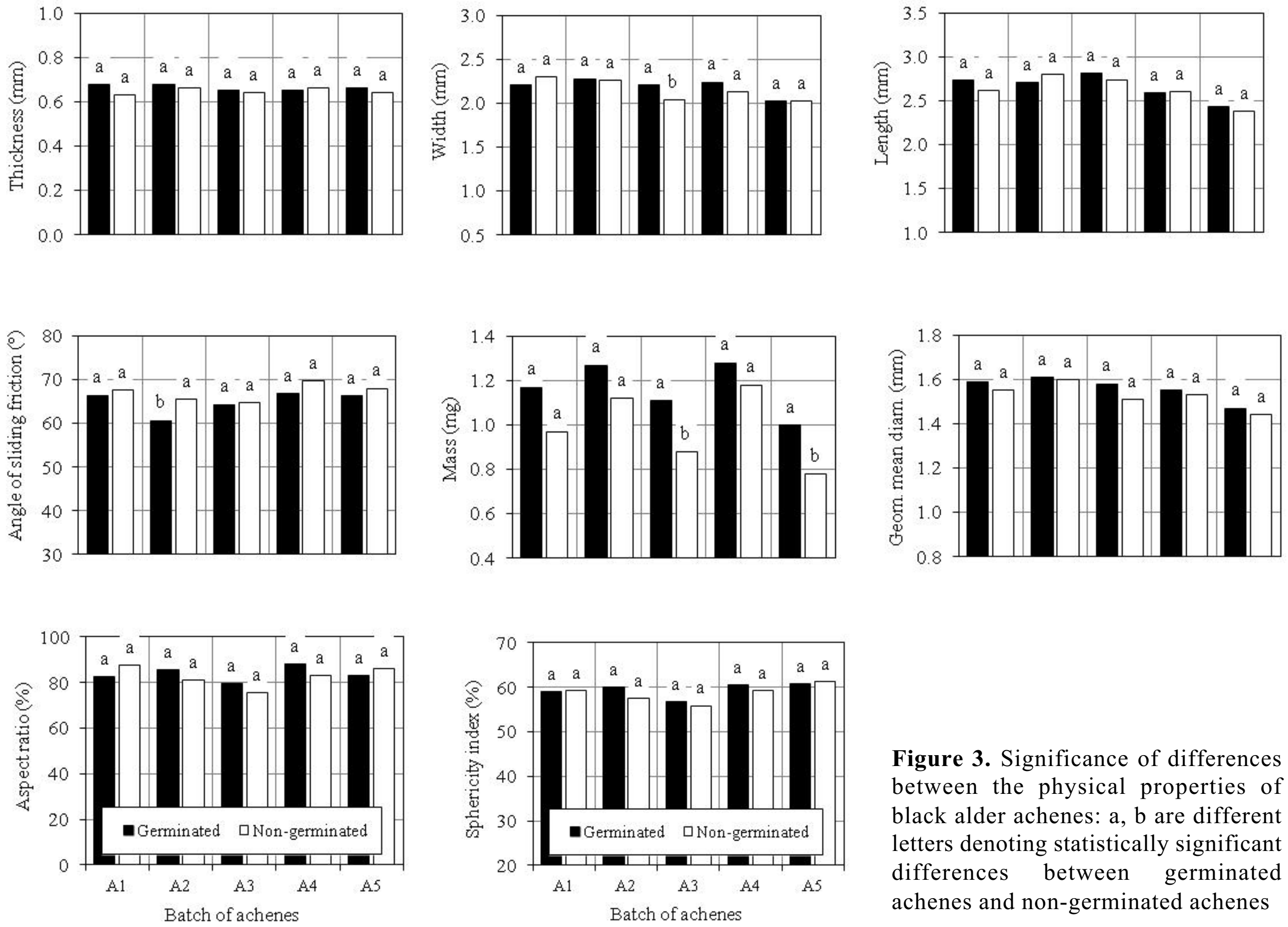

Figure 3. Significance of differences between the physical properties of black alder achenes: a, b are different letters denoting statistically significant differences between germinated achenes and non-germinated achenes

Table 2. Coefficients of linear correlation between the evaluated properties of black alder achenes

Batch	Property	T	W	L	γ	m	D	R	Φ
A1	W	**0.297**	1						
	L	**0.279**	**0.229**	1					
	γ	-0.141	0.030	**0.209**	1				
	m	**0.564**	**0.540**	**0.353**	**-0.205**	1			
	D	**0.749**	**0.688**	**0.696**	0.038	**0.678**	1		
	R	-0.005	**0.571**	**-0.631**	-0.141	0.151	-0.046	1	
	Φ	**0.379**	**0.380**	**-0.659**	**-0.242**	**0.223**	0.050	**0.871**	1
	W_g	0.091	-0.143	0.134	0.100	0.186	0.039	-0.172	-0.094
A2	W	**0.374**	1						
	L	**0.268**	**0.434**	1					
	γ	**-0.327**	-0.157	-0.026	1				
	m	**0.554**	**0.498**	**0.422**	**-0.230**	1			
	D	**0.714**	**0.828**	**0.722**	**-0.236**	**0.650**	1		
	R	0.137	**0.653**	**-0.386**	-0.129	0.161	**0.231**	1	
	Φ	**0.449**	**0.361**	**-0.572**	**-0.223**	0.171	0.146	**0.842**	1
	W_g	0.040	-0.031	-0.114	-0.154	0.152	-0.049	0.101	0.152
A3	W	**0.284**	1						
	L	**0.342**	**0.419**	1					
	γ	**-0.427**	-0.159	**-0.213**	1				
	m	**0.589**	**0.562**	**0.607**	**-0.323**	1			
	D	**0.739**	**0.774**	**0.730**	**-0.368**	**0.775**	1		
	R	-0.014	**0.613**	**-0.448**	0.012	0.027	0.126	1	
	Φ	**0.425**	**0.366**	**-0.515**	-0.180	0.097	**0.199**	**0.817**	1
	W_g	0.003	0.181	0.100	0.074	0.178	0.126	0.073	-0.012
A4	W	0.063	1						
	L	**0.392**	0.157	1					
	γ	**-0.296**	-0.013	-0.096	1				
	m	0.167	**0.483**	**0.326**	**-0.241**	1			
	D	**0.700**	**0.613**	**0.738**	-0.190	**0.483**	1		
	R	**-0.249**	**0.655**	**-0.615**	0.052	0.094	-0.083	1	
	Φ	0.143	**0.416**	**-0.694**	-0.070	-0.010	-0.048	**0.861**	1
	W_g	0.019	0.195	0.015	-0.174	0.172	0.116	0.165	0.130
A5	W	0.126	1						
	L	0.166	**0.301**	1					
	γ	**-0.378**	-0.141	-0.055	1				
	m	**0.583**	**0.466**	**0.473**	**-0.303**	1			
	D	**0.608**	**0.756**	**0.662**	**-0.273**	**0.735**	1		
	R	-0.011	**0.710**	**-0.438**	-0.057	0.058	**0.214**	1	
	Φ	**0.409**	**0.399**	**-0.613**	-0.182	0.111	0.170	**0.826**	1
	W_g	0.102	-0.036	0.075	-0.012	**0.256**	0.065	-0.109	-0.058
Total	W	**0.246**	1						
	L	**0.289**	**0.360**	1					
	γ	**-0.309**	**-0.102**	-0.068	1				
	m	**0.492**	**0.536**	**0.455**	**-0.253**	1			
	D	**0.692**	**0.757**	**0.732**	**-0.217**	**0.679**	1		
	R	-0.024	**0.604**	**-0.503**	-0.035	**0.103**	0.069	1	
	Φ	**0.352**	**0.330**	**-0.627**	**-0.147**	**0.097**	0.054	**0.843**	1
	W_g	0.050	**0.105**	**0.133**	-0.015	**0.255**	**0.131**	0.004	-0.031

Note: Values in bold denote statistically significant correlations.

eliminating around 10% of the thinnest and shortest achenes from batch A5.

Despite considerable similarities in the evaluated parameters (absence of statistically significant differences in most cases), germinated achenes were generally characterized by greater thickness, width, length, mass, geometric mean diameter, aspect ratio and sphericity index, and by a lower angle of sliding friction in comparison with non-germinated achenes.

A linear correlation analysis (Table 2) revealed that the germination rate index was not bound by significant relationships with the remaining achene properties. The germination rate index was most highly correlated with mass, in particular in batch A5 (the correlation coefficient of 0.256 was statistically significant).

The structure of the germination rate index before and after achenes were separated into 3 fractions (containing nearly identical numbers of achenes) based on the length and width is presented in Figure 4. The separated fractions of black alder achenes differed mainly in the percentage of non-germinated and most rapidly germinating achenes. In the fraction containing the largest achenes, the percentage of non-germinated achenes clearly decreased, whereas the percentage of rapidly germinating achenes increased in comparison with unsorted material. The above effect was exacerbated when achenes were graded based on their length. The share of non-germinated achenes decreased by around 24 percentage points and the share of rapidly germinating achenes increased by around 13 percentage points relative to unsorted material. Those changes lowered the quality of the fraction comprising the smallest achenes, where the share of non-germinated achenes increased by around 15% and the share of rapidly germinating achenes decreased by around 10% relative to unsorted material.

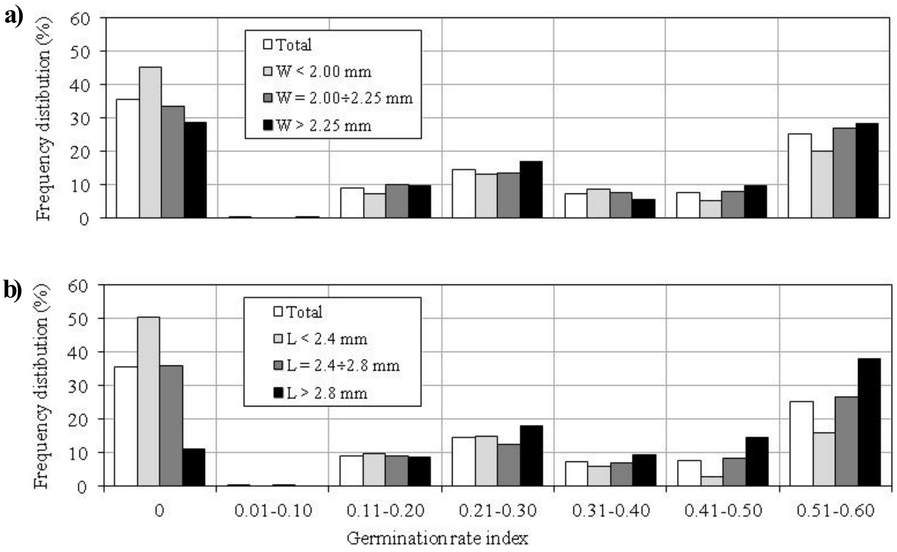

Figure 4. Distribution of the germination rate index of black alder achenes based on achene width, *a*, and achene length, *b*

Discussion

Significant differences in the traits of black alder achenes could be attributed to differences in the geographic location of maternal tree stands and soil types, factors that can considerably influence the size and mass of seeds (Załęski 1995, Oleksyn et al. 1998, 2001, Karlsson and Örlander 2002, Sivacioğlu 2010). The smallest achenes were noted in batch A5. Their average thickness, length and width were similar to those reported by Kaliniewicz and Trojanowski (2011) in achenes harvested from 46-year-old trees. The average value of the angle of sliding friction, similar to that noted by Kaliniewicz and Trojanowski (2011), could be attributed to the specific morphology of black alder achenes. The achenes have webbed edges (Suszka et al. 2000, Claessens et al. 2010) that increase their dimensions and keep achene mass low, which is why they do not slide easily on various surfaces. According to Suszka et al. (2000), the average achene weight ranges from 0.7 to 1.5 mg. In this study, achenes weighed from 0.85 to 1.26 mg in all analyzed batches. Similar results were reported by Aguinagalde et al. (2005), Bodył (2007), De Atrip and O'Reilly (2007), O'Reilly and De Atrip (2007), Kaliniewicz and Trojanowski (2011) and Tylkowski (2014). In terms of their aspect ratio, black alder achenes are similar to coriander seeds (Coşkuner and Karababa 2007), and with regard to their average sphericity index – to African breadfruit (Omobuwajo et al. 1999), African star apple (Oyelade et al. 2005) and the seeds of selected wheat varieties (Khazaei and Ghanbari 2010, Kalkan and Kara 2011).

According to Janson and Załęski (1998) and Suszka et al. (2000), black alder achenes belong to the group of easily germinating seeds. Despite the above, germination is initiated only several days after achene imbibition, and in this study, achenes began to sprout after around 12 days of continuous water supply (Figure 2). The above can probably be attributed to the presence of webbed edges which enable the achenes to float and become dispersed by waterways across considerable distances, but which also block water from entering the achene. According to O'Reilly and De Atrip (2007), the optimal moisture content for the germination of black alder achenes is around 30%. The initial moisture content of achenes is estimated at 7%, which implies that several days of imbibition are needed to initiate sprouting. Three batches (A1, A3 and A4) had higher germination capacity and one batch (A5) had lower germination capacity than that reported by Suszka et al. (2000), Bodył (2007) and Gosling et al. (2009). The low germination capacity of A5 achenes could result from a fungal infection (e.g. with *Phytophthora alni)* which – as reported by Suszka et al. (2000), and Haque and Diez (2012) – considerably lowers the quality of seeds. The low viability of A5 achenes could also be affected by unspecified weather conditions during their formation and development, and by tree stand age which was probably advanced.

Germinated achenes were heavier than non-germinated achenes (Figure 3), which is consistent with the results reported for the seeds of Scots pine, Norway spruce and European larch (Kaliniewicz et al. 2012a, 2012b). Despite the above, achenes are difficult to sort based on their mass only. Vibratory separators or pneumatic vibratory separators can be used (Grochowicz 1994), but the separation process is only effective when seeds have similar size but differ in mass or when seeds have similar mass but differ in size. The separation process may not be feasible if achenes have unique structure and relatively low mass and differ in both mass and size.

The correlations between mass and other physical parameters of black alder achenes need to be identified to increase separation efficiency.

Germination rate index increased with a rise in achene mass, which means that larger achenes germinated faster. Similar observations were made in studies of *Dipterocarpus macrocarpus* (Shankar 2006), European larch (Kaliniewicz et al. 2012a) and Norway spruce (Kaliniewicz et al. 2012b), whereas a reverse relationship was reported for seeds of tropical trees (Norden et al. 2009). According to many authors (Mikola 1980, Sabor 1984, Castro 1999, Khan 2004, Parker et al. 2006, Upadhaya et al. 2007, Wu and Du 2007, Castro et al. 2008, Buraczyk 2010), seed mass significantly influences sprout development and seedling growth. For this reason, seed material should be sorted before sowing in nurseries to promote uniform germination and to produce evenly-sized seedlings. In this study, achene mass was also most highly correlated with the germination rate index, but as mentioned before, achenes are difficult to sort based on this trait only. The parameters correlated with the germination rate index, which potentially could be used in the separation process, are the width and length of black alder achenes. However, a detailed analysis revealed that the separation of black alder achenes based on their dimensions would not contribute to germination uniformity because every sorted fraction would contain both early and late germinating achenes.

Conclusions

1. Black alder achenes removed from cones in the extraction chamber have lower moisture content, which is conducive to long-term storage but adversely affects germination rate. Viable achenes begin to germinate only after around 12 days of imbibition.

2. The results of this study indicate that germinated and non-germinated black alder achenes cannot be effectively separated. The quality of black alder achenes with relatively low germination capacity could be slightly improved by eliminating around 10% of the thinnest and shortest achenes from the propagating material.

3. The classification of black alder achenes based on their length or width only slightly improves seedling emergence uniformity when each fraction is sown separately. The largest achenes can be expected to germinate faster, and they could be used in container nursery production.

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