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Variability of *Pinus sylvestris* L. seeds including seed coat colour

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Abstract. The aim of this work was to compare the variability of Scots pine *Pinus sylvestris* L. seeds collected in three forest sites in the Radomsko Forest District. This comparison was mainly based on viability and weight of 1000 seeds, which was then used to calculate seed value for each sample. Furthermore, seed size was assessed by measuring their length, width and area, using the WinSeedle software. All evaluated samples belonged to viability class I. Seeds originating from the Bąkowa Góra forest range were characterized by the highest weight, best viability and the largest size. In the next stage of our research, we performed an analysis of variability parameters taking the colour of the seed coat into consideration. For this analysis, the seeds were divided into three colour groups (fawn, brown, black) and their vitality and weight were assessed separately. The share of fawn seeds was 9.4%, brown 39.6% and black 50.9%. The weight of 1000 seeds and seed size parameters of fawn seeds were slightly higher than those of the black and brown seeds. The fawn and black seeds belonged to vitality class I, while brown seeds belonged to vitality class II. Despite the observed variability, seeds belonging to different colour groups did not show statistically significant differences in other measured parameters. The factor with the greatest impact on the seed parameters was the site of origin, which differed in their forest site type. Larger and heavier seeds came from the broadleaved forest site compared to the coniferous forest sites.

Keywords: Scots pine, seed purity, seed viability, seed weight, WinSeedle software

1. Introduction

Variability is a natural characteristic of all living organisms. It can affect an individual, a part or the whole population. Each seed lot of a particular species forms a population consisting of seeds with different individual properties. Variability is expressed bycolour, size, shape, surface, physiological properties and chemical composition (Duczmal, Tucholska 2000).

Seeds from *Pinus sylvestris* L. pine cones collected in the autumn of the second year after flowering are already physiologically mature and able to germinate, but are not suitable for long-term storage. They reach full maturity only in winter, which is why cones are collected from December to February, from the branches of cut trees, less frequently from growing trees (Załęski 1995). The seeds collected are characterised by a large degree ofcolour variation, which, however, is not related to their maturity (Cram, Lindquist 1979). The issue of the seed colour of forest trees and its relation to other traits has rarely been analysed. Studies conducted in Russia (Novikov et al. 2019; Novikov, Ivetić 2019) showed the influence of this factor on the different adaptation of seedlings in a crop. Also, different amounts of chlorogenic acid were found in different coloured seed coats, the presence of which may affect the resistance of germinating seedlings to pathogenic microorganisms (Grzywacz, Twaróg 1977). Subsequent studies showed that the colour fractions of pine seeds differed in length and sphericity coefficient (Kaliniewicz et al. 2014), as well as in the number of germinated seeds (Mukassabi et al. 2012; Kaliniewicz et al. 2013). More often, studies have investigated seed parameters as traits for separation, e.g. in the sorting process (Tylek 2004, 2012; Kaliniewicz et al. 2017), or the time required for germination (Norden et al. 2009). Demonstrating the effect of seed colour on seed parameters, including germination rates, can be used as a separation trait in currently constructed optical sorters (Tylek et al. 2021).

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The aim of the research presented in this paper was to compare the variability of Scots pine seeds obtained in stands growing in four forest habitattypes in relation to the colour of the seed coat. Viability, weight of 1000 seeds and their size were assessed in relation to the colour class distinguished (black, brown, fawn). The first research hypothesis assumed that the seeds collected from pines growing in different habitats would not differ in the proportion of seeds with a specific seed coat colour, while the second hypothesis assumed that the colour of the seed coat would not be related to seed parameters.

2. Material and methods

The object of the study were Scots pine seeds collected from trees growing in four commercial seed stands in the Radomsko Forest District. The stands were characterised by similar tree age and location with altitude above sea level, whereas the forest site type differed (Table 1). In each stand in the winter season (XI/XII), a controlled collection of cones (from 0.3 to 1.5 tons) was conducted in three stands from standing trees and in one stand from felled trees. Seeds were hulled using the thermal method in a hulling cabinet. Average samples of 40 g were obtained from each seed lot in accordance with the procedure in force, i.e. first the primary samples were taken from several locations to form a total sample, from which the average sample was distinguished using the diagonal square method.

In the first stage of the study, a laboratory sample of approximately 20 g of seeds was separated from each average sample using the grid method, and then its purity (c, %), viability (z, %) and weight of 1000 seeds (t, g) were assessed in accordance with the qualification assessment procedure (ISTA 2012), while purity and viability classes were determined in accordance with the Polish standard PN-R-65700 (1998).

In the second stage, for each evaluated batch, 400 seeds (4×100) were taken from the pure seed fraction. Based on the colour of their seed coats, the seeds were divided into three classes: black, brown − dark brown with a hint of grey, and fawn− yellow with a hint of grey (Fig. 1). To minimise the subjectivity of the colour assessment, the classes were separated using WinSeedle Pro software (Regent Instruments Inc. 2000) and an Epson STD 1600+ scanner. First, a calibration file was created in which the individual seed coat colour classes were defined using three colour variants in each class, with a hue tolerance of ± 10% for the variant. All samples of 100 seeds were then scanned and classified based on their proportion in the colour class. Seeds with non-uniform seed coat colouration were assigned to the colour class with the highest proportion. After classification, the seeds were removed from the scanner, separately for each fraction of colouration.

For each 100 seeds assessed, the percentage share of the different colour classes of the seed coat was calculated. Seed samples were weighed with an accuracy of 0.001 g, converting the mass of a given number of seeds into the weight of 1000 seeds. Simultaneously with the colour analysis in the WinSeedle programme, the size of a single seed was determined, i.e. its length [mm], width [mm] and surface area [mm^2]. The measurement accuracy of seed size parameters was 1 pixel, which at the applied resolution of 800 dpi gives a value of 0.03175 mm. In order to determine differences in viability, previously separated and weighed seeds in seed coat colour classes were subjected to a germination test in a Jacobsen-type germinator, in accordance with the guidelines provided for the qualification assessment (ISTA 2012).

Table 1. Characteristics of Scots pine seed stands in the Radomsko Forest District, from which the seeds used for the research were derived

Forest Subdistrict Subcompartmet	Characteristic					
	stand area [ha]	trees age [years]	altidute [m]	forest site type*	geographical coordinates	
					longitude (E)	latitude (N)
Bąkowa Góra 24c	3.64	111	195	Bśw	19.54	51.70
Kruszyna 315g	3.41	133	230	BMśw	19.46	50.58
Grzebień 243c	5.81	101	215	BMw	19.28	51.00
Bąkowa Góra 52c	6.20	110	235	LMśw	19.50	51.80

*Bśw – fresh coniferous forest (fCF); BMśw – fresh mixed coniferous forest (fMCF); BMw – moist mixed coniferous forest (mMCF); LMśw – fresh mixed broadleaved forest (fMBF)

Figure 1. Scots pine seed sample, divided into three colour fractions, from the left: black, brown and fawn seeds *(photo B. Pańczyk)*

To test the significance of differences between the proportion of distinguished seed coat colour classes, a one-way-analysis of variance (fixed model) was used, while to assess the effect of the interaction "habitat × seed coat colour class" on the variation of analysed seed parameters (length, width and area), a two-way analysis of variance (fixed model) was used. For both analyses, homogeneous groups were determined by Tukey's test at a significance level of p=0.05. The statistical analysis was performed using Statistica 13.3 software (Tibco Software Inc. 2017).

3. Results

3.1. Seed variability

The share of pure seed fractions in individual laboratory samples was equal and ranged from 97.2 to 98.8%. The level of purity of the analysed seed lots was classified as high (>98%), with the exception of one sample from the Bąkowa Góra forest range, sub-compartment 52c, which was characterised by a slightly lower value of this parameter (97.2%), i.e. the average class of seed purity. The tested seeds also showed slight differentiation in terms of viability, with values ranging from 94.4 to 95.5%, which formed the same homogeneous group, and all the analysed samples belonged to the first class of seed viability (>91%). The weight of 1000 seeds differentiated the studied stands and ranged between 5.9 and 6.4 g. The highest value of this parameter was reached by seeds from the Bąkowa Góra 52c stand, whereas the lightest were those from the Grzebień 234c stand. The size of individual Scots pine seeds with respect to the three parameters (area, length, width) varied. The largest seeds were from Bąkowa Góra 52c, smaller ones were from Bąkowa Góra 24c, while the smallest, but at the same time having the same average values of the assessed parameters (the same homogeneous groups) were seeds from the stands in Kruszyna 315g and Grzebień 243c (Table 2).

3.2. Seed parameters and seed coat colour

The next stage of the study analysed the variability among the seeds depending on their seed coat colour. The propor-

Table 2. Average parameters (±SE) of Scots pine seeds from individual seed stands (a-c – homogeneous groups determined by Tukey's test, p=0.05)

Forest Subdistrict Subcompartment	Forest site type*	Seed parameters					
		purity [%]	viability [%]	weight of 1000 seeds [g]	area [mm^2]	length [mm]	width [mm]
Bąkowa Góra 24c	Bśw	98.8	94.4 ± 0.8 ^{a}	6.1 ± 0.1 ^{bc}	7.5 ± 0.1 ^{b}	4.3 ± 0.0 ^{b}	2.5 ± 0.0 ^{b}
Kruszyna 315g	BMśw	98.3	95.1 ± 0.7 ^{a}	6.2 ± 0.0 ^{ab}	7.3 ± 0.1 ^{c}	4.2 ± 0.0 ^{c}	2.5 ± 0.0 ^{b}
Grzebień 243c	BMw	98.0	94.8 ± 1.2 ^{a}	5.9 ± 0.1 ^{c}	7.3 ± 0.1 ^{c}	4.2 ± 0.0 ^{c}	2.5 ± 0.0 ^{b}
Bąkowa Góra 52c	LMśw	97.2	95.5 ± 0.8 ^{a}	6.4 ± 0.1 ^{a}	7.8 ± 0.1 ^{a}	4.4 ± 0.0 ^{a}	2.6 ± 0.0 ^{a}

*forest habitat type– see Table 1

tion of differentiated classes of seed coat colour (black, brown and fawn) differed significantly. For all the Scots pine seeds evaluated, black seeds accounted for the largest share (50.9%), followed by brown seeds (39.6%), while the lowest share was that of fawn seeds (9.5%), and the values obtained differed significantly. The black and brown seeds were on average slightly lighter than the fawn seeds (by 3.2%), but this was not a significant difference. Black and fawn seeds were found to be in the first class of viability, while brown seeds in the second class, with average values forming one homogeneous group. No clear differences were observed in the size of a single seed depending on the colour of its coat. Black and brown seeds were on average the same, with an area, length and width of 7.5 mm^2, 4.3 and 2.5 mm, respectively. Fawn seeds were slightly larger, with average values of 7.7 mm^2, 4.3 and 2.6 mm, respectively. There was no significant variation, and all trait averages formed the same homogeneous group (Table 3).

3.3. Analysis of "habitat × seed coat colour"

The share of seeds in particular colour classes at the three coniferous forest sites was equal and amounted to: an average of 53% of black seeds, 37% of brown seeds and 10% of fawn seeds, forming separate homogeneous groups. At the broadleaved forest site, the proportion of fawn seeds was slightly lower (by about 1.5%) compared to the average value for the coniferous sites. The share for the black and brown seed classes was about 46% (the same homogeneous group), comparable to the share of seeds with the same coat colour from the coniferous sites (Fig. 2).

In assessing the interaction effect for the fawn seeds from the fresh coniferous forest site, a significantly higher viability was observed in comparison to the brown seeds. Black-coated seeds obtained from pines growing in the fresh mixed broadleaved forest site were found to have a viability comparable to that of the fawn seeds (the same homogeneous group). Seed viability did not differ significantly in the remaining cases of interaction between seed coat colour class and site. The result obtained for the weight of 1000 seeds differed. As with viability, the weight of 1000 fawn seeds from the fresh coniferous forest site was significantly higher than that of brown seeds. The fawn and brown seeds collected from pines in the fresh mixed broadleaved forest were comparable to the fawn seeds in the fresh coniferous forest site. Seeds in all colour classes did not differ significantly between the moist mixed coniferous and fresh mixed coniferous forest (Fig. 3).

Analysis of the effect of site on seed size parameters in seed coat colour classes showed no directional change relating to site richness or moisture content. Fawn seeds were characterised on average by the largest size parameters, i.e. length, width and area, irrespective of forest site type, with the exception of those collected in the fresh mixed coniferous forest, but this was not a significant difference with respect to the other two classes. Brown and black seeds obtained similar size parameters under varied habitat conditions. The seed material obtained from the stand in the moist mixed coniferous forest showed significant differences (different homogeneous groups) between the fraction of fawn seeds and

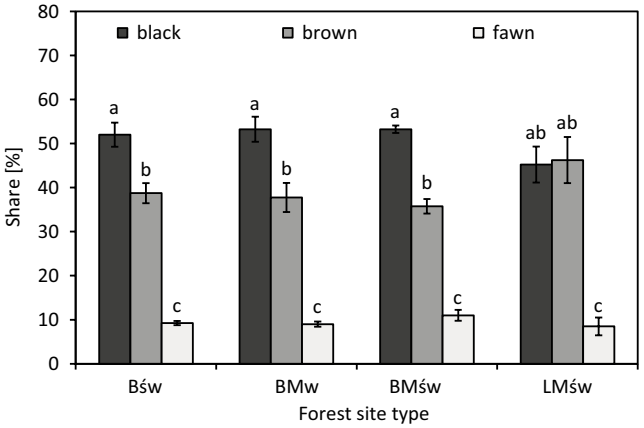

Figure 2. Share (± SE) of Scots pine seeds in seed coat colour classes and forest site types; a-c – homogeneous groups determined by Tukey's test (p=0.05), type of forest site – see Tab. 1

Table 3. Share and average parameters of Scots pine seeds in seed coat colour classes (a-c – homogeneous groups determined by Tukey's test, p=0.05)

Seed coat colour	Share [%]	Seed parameter				
		viability [%]	weight of 1000 seeds [g]	area [mm^2]	length [mm]	width [mm]
Black	50.9 ± 1.5 ^{a}	92.6 ± 1.0 ^{a}	6.2 ± 0.1 ^{a}	7.5 ± 0.0 ^{a}	4.3 ± 0.0 ^{a}	2.5 ± 0.0 ^{a}
Brown	39.6 ± 1.8 ^{b}	90.7 ± 1.2 ^{a}	6.2 ± 0.1 ^{a}	7.5 ± 0.0 ^{a}	4.3 ± 0.0 ^{a}	2.5 ± 0.0 ^{a}
Fawn	9.5 ± 0.6 ^{c}	91.8 ± 2.1 ^{a}	6.4 ± 0.2 ^{a}	7.7 ± 0.1 ^{a}	4.3 ± 0.0 ^{a}	2.6 ± 0.0 ^{a}

the others (seed area), between fawn and black seeds (seed length) and between fawn and brown seeds (seed width). The largest and significant differences in seed size parameters were found for the fawn seed fraction. Fawn seeds from the moist mixed coniferous forest (mMCF) were characterised by significantly smaller parameters (separate homogeneous groups) compared to fawn seeds collected from pines growing in the other forest site types (Fig. 4).

4. Discussion

The analysis showed that the assessed Scots pine seeds were of a very good quality. Three seed samples were characterised by a high level of purity (above 98%) and one by an average level. In all samples, the seeds germinated at a similar level and all of them could be classified as viability class I. The weight of 1000 seeds ranged from 5.9 g to 6.4 g and did not differ significantly from the average value de-

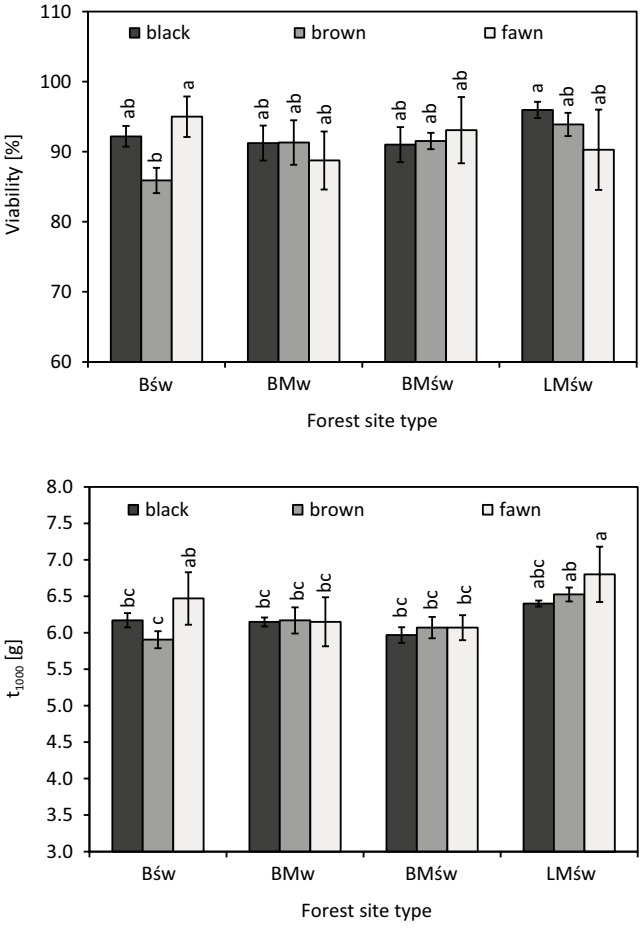

Figure 3. Viability (± SE) and weight of 1000 seeds – t_{1000} (± SE) of Scots pine seeds in seed coat colour classes and forest site types; a-c – homogeneous groups determined by Tukey's test (p=0.05), type of forest site – see Tab. 1

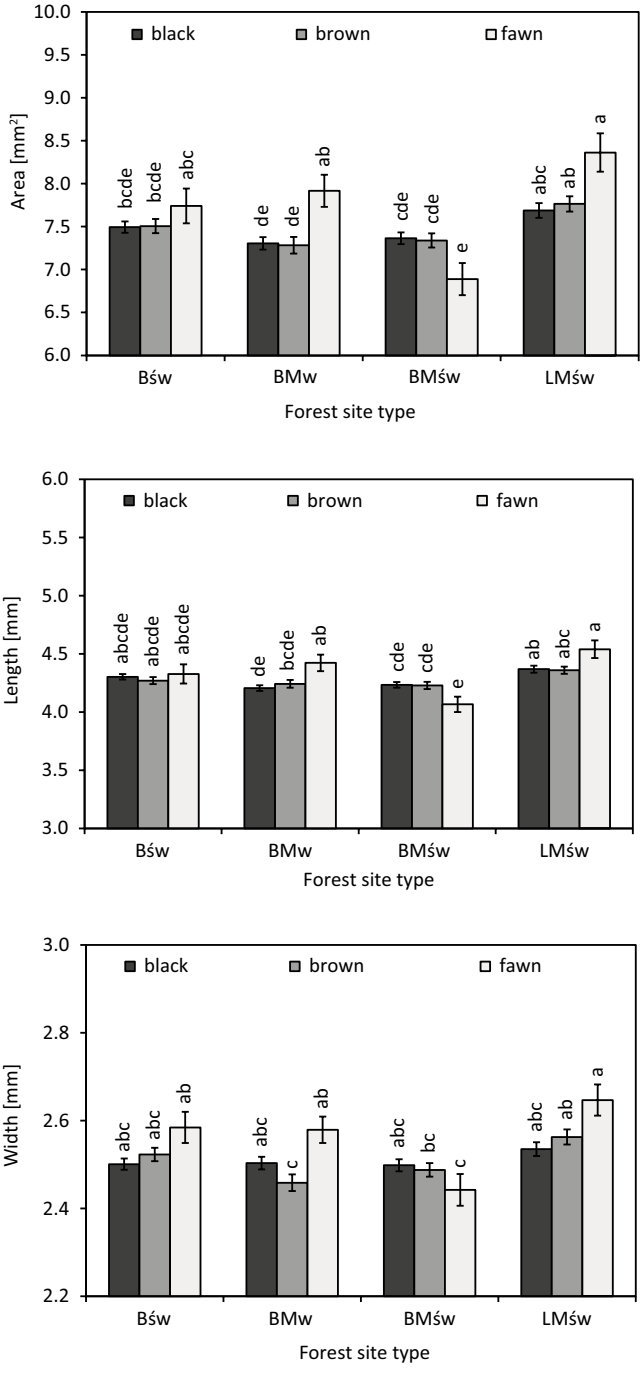

Figure 4. Average parameters (± SE) of Scots pine seeds in colour classes of seed coat and in forest site types, a-e – homogeneous groups determined by Tukey's test (p=0.05), type of forest site – see Tab. 1

termined for pine trees growing in Poland, which is about 6.2 g (Załęski, Bodył 2005). A slightly lower value of this parameter, amounting to 5.8 g, was obtained by Aniszewska (2006), who showed that seed weight is related to cone shape. The heaviest seeds are obtained from elongated cones, while the lightest – from egg-shaped cones, with a difference in weight exceeding 20%.

Each stand from which the seeds were collected grows in a different habitat, varying in moisture level and richness. The lowest and the highest viability values were represented by seeds originating from one forest district (Bąkowa Góra), but collected from stands growing in different habitat conditions. The highest viability was represented by seeds originating from the fresh mixed broadleaved forest (fMBF) habitat and the lowest by seeds from fresh coniferous forest (fCF), but the differences were not statistically significant. Seeds collected in the stand growing in the fMBF habitat were characterised by a significantly higher mass of 1000 seeds, compared with those collected in the mMCF and fCF habitats. In the case of seed parameters (area, length and width) the highest mean values were also obtained for seeds from the fMBF habitat, but for each feature the mean values formed the same homogeneous group, which indicates a lack of significant differences. Thus, one could see the influence of habitat on the formation of seed parameters, which is confirmed by the results of the study on Scots pine seeds covering 10 stands in central and eastern Europe (Köbölkuti, Höhn 2018). Although in the presented study higher values of seed parameters were obtained for the broadleaved forest habitat and lower values for all coniferous habitats, the consideration of only one broadleaved forest habitat does not authorise a more general conclusion in this respect.

Scots pine seeds are most often black, brown and fawn coloured (Załęski 1995). In the present study, the highest share was found for black seeds (50.9%), a smaller share for brown seeds (39.6%), whereas fawn seeds had the smallest share (9.4%). A similar average share of the same seed coat colour classes was found by Grzywacz and Twaróg (1977). Different values were obtained by Udval and Batkhuu (2013), with the proportion of black seeds being about 45% and fawn seeds about 24%, as well as by Kaliniewicz et al. (2013) with 40 and 21%, respectively. Aniszewska (2006) obtained an even lower mean share of black seeds at 37% and a much higher share of fawn seeds at 29%. The cited author noted, however, that the share of seeds with a given seed coat colour depends on the shape of the cone, and the highest share of black seeds (40%) occurs in egg-shaped cones. The variation in the proportion of particular colour fractions may be influenced by the visual classification of seeds used by individual researchers or by the origin of the seeds used in the analyses. According to Čerepnin (1980), Scots pine growing in moist habitats produces a greater number of darker seeds compared to dry habitats, where a higher proportion of light seeds are present. Such a result was not observed in the present study, but a similar relationship was obtained for habitat richness. More black seeds were found in poorer sites – coniferous forests (>52%) compared to richer sites – the broadleaved forest (ca. 45%).

The weight of 1000 seeds was slightly related to their colour. Fawn seeds were slightly heavier than black and brown seeds, only by about 2.7%, confirming the result obtained by Grzywacz and Twaróg (1977). Similarly to the weight, the viability of seeds of different coat colours was quite similar, with a difference of only 1.9%. Despite such a small difference, black and fawn seeds were classified as viability class I, while brown seeds as class II. Different results were obtained in a study conducted in Mongolia, in which the germination of black seeds was the highest at 92.3%, lower for brown seeds at 81.2% and the lowest for fawn seeds at 60.7% (Udval, Batkhuu 2013). Thus, using the Polish classification (Załęski et al. 2000), the germination of black seeds can be classified as viability class I, brown seeds as class II, while fawn seeds as non-class material. Coinciding with the result obtained by Udvala and Batkhuu (2013) was the result of the analysis of Scots pine seeds collected in populations growing in England, which also showed lower weight and poorer germination of lighter seeds (Mukassabi et al. 2012).

In the study presented here, fawn seeds were characterised on average by the highest values of size parameters (length, width and area). When analysing the simultaneous effect of habitat and seed coat colour, no directional change was observed for the former factor. A significant difference, indicated by the fact that the averages belonged to separate homogeneous groups, was obtained for the moist mixed coniferous forest between the fraction of fawn and other seeds (for seed area), fawn and black seeds (for seed length), and fawn and brown seeds (for seed width). The highest differences in seed size parameters were obtained for the fawn fraction in the case of the moist mixed coniferous forest (mMCF) and fresh mixed broadleaved forest (fMBF).

5. Summary and conclusions

All seed samples from the stands of the Radomsko Forest District were characterized by a high level of purity (>98%), with the exception of seeds from sub-compartment 52c of the Bąkowa Góra forest range, which showed an average level of purity (97.2%). No statistical difference was found in the case of seed viability and all samples were classified to class I.

The weight of 1000 seeds depended on the site where the seed sample was collected, i.e. the habitat where the stand grew, but it did not differ significantly from the average for Poland, which is 6.2 g.

The average proportion of black seeds was the highest and amounted to 50.9%, brown seeds amounted to 39.6% and fawn seeds – 9.5%. Individual lots differed significantly in the percentage share of seeds with a specific seed coat colour, only the fresh mixed broadleaved forest had an equal share of black and brown seeds. Therefore, the hypothesis of the lack of a site effect on the proportion of particular seed coat colour classes was rejected.

The parameters of fawn seeds (weight, length, width, area) were slightly higher than those of brown and black seeds, but this difference was not statistically significant, which confirms the research hypothesis of no effect of seed coat colour on size parameters. However, the analysis of these parameters taking into account the interaction of the colour of the seed coat and the site where the seeds were collected (habitat) indicated significantly lower values of fawn seed parameters from the fresh mixed coniferous forest in comparison to such seeds collected from the other sites.

Conflict of interest

The authors declare the absence of potential conflicts of interest.

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J.B. – study concept, statistical analysis of the results, literature review, graphics preparation, manuscript writing, manuscript editing; K.S. – analysis of the results, literature review, manuscript editing; B.P. – conducting the research, analysis of the results, manuscript writing.

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