# Cone and Seeds Variability in Seed Orchards and Seed Stands of Pinus sylvestris

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Abstract

Seeds from seed orchards and seed stands may differ in morphology and quality, both determined during the seed assessment. The study aims at the comparison of phenotypic characters of cones and seeds originating from economic seed stands and seed orchards of Scots pine from western Poland. The study was conducted in 5 seed stands and 5 clonal seed orchards, representing five Scots pine populations. The analyses included the following measurements: length and width of cones, the number of empty and full seeds in the cone and the weight of 1000 seeds. Moreover, the measurements of the length and width of the seed surface were carried out.

The analysed seed stands and orchards were characterized by high variability in terms of the size and weight of cones and the amount of seeds. An ANOVA treatment showed that seed stands and orchards significantly differed in length and width of cones and seeds. Both seeds and cones from the seed orchards were characterized by higher values of analyzed traits than ones from the economic seed stands. Differences in mass of cones and 1000 seed weigh were of low significance (p =0.022 ÷ 0.037). Significant differences between the length of seeds and seed area among all of the examined populations were demonstrated. The percentage contribution of empty seeds for all of the analysed sites of origin was not statistically

Keywords: Scots pine, population, seed orchards, variation, seeds, cones

### Introduction

Scots pine (Pinus sylvestris L.) is a Eurasian species with the widest range of distribution within the genus Pinus (Boratyński 1993). In Central Europe and Scandinavia, Scots pine is one of the most important forest tree species used for reforestation (Mikkola 1980). In the Baltic countries, there are optimal conditions for growth of Scots pine. This continental species tolerates considerable temperature fluctuations. It grows most on dry soils but occurs also in waterlogged areas and peatlands. The species thrives on deep soils, sand, loamy sand and light loam.

Scots pine seeds are collected from seed stands and seed orchards (SO). The main seed base for forestry is economic seed stands (ESSs). Such stands are characterized by favourable breeding quality, and trees forming them show full health, good quality and productivity in comparison to other tree stands of the same species and age growing in the same habitat type. ESSs are clear cut during the years of good or at least moderate seed yield. The main objective of seed production in

seed orchards, aside from facilitated seed harvesting is enhancement of the genetic quality of seeds in comparison to seeds obtained from seed stands. Selection of trees for the orchards has a phenotypic character and in few cases around the world a genotypic on the basis of offspring tests. It is expected that the offspring inherits a part of their phenotypic characters. Seed orchards are linked to the forest tree breeding programmes, which in their assumptions should play the role of seed provider. In many countries, seed orchards constitute the major source of forest reproductive material. As an instance, in Slovakia 40 % of pine seedlings used for reforestation originate from seed orchards (Gömöry et al. 2003), in Finland 60 % (Jansson et al. 2016), in Sweden over 90 %, and for spruce nearly 70 % (Stener 2015).

Scots pine cones are characterized by high variability depending on various factors such as climate soil fertility, type and age of stand, etc. (Aniszewska 2006). Type of cone scales is one of the numerous traits. Four forms of cones based on scales type are distinguished: plana, gibba, reflexa and hamata. The first three forms are found in Poland, and the highest contribution (ap-

prox. 60 %) is represented by the *gibba* form (Sokołowski 1931). Seeds of cones of the reflexa form are heaviest, while those of the plana cones are the lightest (Tylkowski 1993). Pine seeds are characterized by variable colouration. Seed colouration influences such factors as imbibition (Wrześniewski 1982) and germination (Aniszewska 2006, Udval and Batkhuu 2013), more resistant to damping-off (Rosochacka and Grzywacz 1980). Moreover, it has an impact on seedling quality. Seedlings grown from black seeds were characterized by a more intensive growth than those from lighter seeds (Udval and Batkhuu 2013 after Odgerel 2001). In Poland, 46-63 % of seeds collected in tree stands have black colour, 28-37 % brown and 9-16 % light (Grzywacz 1979). In the seed orchard of the Taborska pine 46 % seeds are dark, 42 % brown and 12 % light (Blonkowski 1993). Light colour seeds are usually weak and have less chance to germinate (Aniszewska 2006). Seed size is considered to be one of the most stable of phenotypic characters (Sorensen and Campbell 1985 after Puckridge and Donald 1967, Gallagher et al. 1975). Seeds from seed orchards and seed stands may differ not only in the genetic information and the growth potential of the seedlings yet also in morphology and quality determined during seed assessment. It is crucial for practical purposes to perform cones and seed variability studies. During processes such as collection, extraction and storage seed variability have significant meaning. We should be able to distinguish between empty and small but fully-developed seeds. This observation is crucial for gene conservation purpose and prevention of selection of seeds between maximum and minimum weights and size. This information is especially valid during seed separation, cleaning and sowing using automatic machines.

The study aimed at the comparison of phenotypic characters of cones and seeds originating from the economic seed stands and the seed orchards of Scots pine from western Poland.

#### Materials and Methods

Five economic seed stands (ESSs) and 5 clonal seed orchards were selected for the study (Table 1). The items were selected in order to obtain five pairs consisted of a stand and orchard, each representing one pine population. Cones were collected in January 2015, 30 cones from each tree representing the upper and lower portion of the crown. In the economic seed stands cones were collected from 50 trees and in the seed orchards from one randomly selected tree representing each clone. The number of clones in individual plantations is presented in Table 1. In the subsequent stage, the cones were mixed, and then from the entire collection, 100 cones were randomly selected for analyses.

**Table 1.** Studied Scots pine populations

Population	Object (Forest District)	Type of seed base/number of clones	Location (compartment)	Date of planting	Regional Directorate of State Forest
Bolewice	Bolewice ESS	economic seed stand	285 a	1919	Szczecin
Dolewice	Międzyrzecz SO	clonal seed orchard / 66	Rokitno	2001	Szczecin
Goleniów	Goleniów ESS	economic seed stand	110 d	1900	Szczecin
	Nowogard SO	clonal seed orchard / 58	Radosław	1983	Szczecin
Gubin	Gubin ESS	economic seed stand	31 h	1902	Zielona Góra
	Gubin SO	clonal seed orchard / 51	Sękowice (Suchodół)	1999	Zielona Góra
Bory Dolnośląskie	Węgliniec ESS	economic seed stand	465 b	1905	Wrocław
	Oborniki Śl. SO	clonal seed orchard / 54	Prusice	1988	Wrocław
Rychtal	Syców ESS	economic seed stand	77 h	1912	Poznań
	Syców SO	clonal seed orchard / 64	Międzybórz	1994	Poznań

Within the analyses, the following measurements were performed: length, width, type of scale (apophisis), fresh and dry weight of cone (for 10 cones).

At the next step, the seeds were removed from the cones by placing in a dry air oven at 105 °C for 24 hours. Then, the seeds were extracted and the total number of seeds per cone, as well as the weight of 1,000 seeds was determined (3 repeats × 100 seeds). Empty seeds were identified manually by pressing on the seed. Moreover, the length, width and surface of 50 seeds were determined using the WinSEEDLE software package (Regent 2019). The one-way ANOVA was used to test significance of differences among mean values of cones and seeds parameters of the analysed origins.

#### Results

The analysed stands and seed orchards were characterized by high variability in terms of the size and weight of cones and the amount of seeds (Table 2). The length of cones ranged from 3.88 to 5.03 cm and the width from 1.83 to 2.44 cm. The mean cone length was 4.67 cm for seed orchards and 4.21 cm for ESSs. The mean cone width was 2.01 cm for ESSs and 2.23 cm for seed orchards. The analysed seed orchards and stands were characterized by significant variability of cone length and width (p < 0.001) (Table 3).

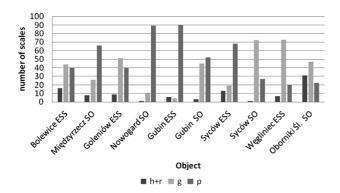
The cones differed in terms of scale shape (Figure 1). On the basis of the  $\chi^2$  test it was determined, that cones originating from Bory Dolnośląskie are characterized by the greatest scale variability. This population is characterized by the dominance of gibba scales, whereas *plana* scales dominate in the remaining populations.

Table 2. Detailed data of the cones and seeds of Scots pine of different origin. Different letters denote significant differences between seed sources at p < 0.05 (Tukey test)

Object	Mean for one fresh cone		Dry mass of	Mean seed number per cone		Proportion of empty	1000 -seed		
	Lengh [mm]	Width [mm]	Weight [g]	cone [g]	Full	Empty	Total	seeds [%]	weight [g]
Bolewice ESS	42.82±5.2 ab	19.95±0.2 cd	6.6	5.36	12.9	5.8	18.7	31.0	5.2±0.03 c
Międzyrzecz SO	49.52±0.3 f	23.09±0.3 e	11.4	8.71	32.0	4.3	36.3	11.8	7.6±0.02 a
Goleniów ESS	45.99±6.0 c	21.81±0.3 a	10.2	8.22	31.2	5.7	36.9	15.4	8.2±0.01 ab
Nowogard SO	44.53±0.3 abc	21.16±0.3 ab	10.0	8.21	25.5	8.8	34.3	25.6	7.7±0.02 a
Gubin ESS	42.32±0.4 a	19.76±0.2 cd	6.6	5.28	23.8	3.5	27.3	12.2	5.9±0.03 c
Gubin SO	50.34±0.3 f	24.48±0.3 g	11.8	8.88	34.3	3.4	37.7	9.0	7.8±0.01 a
Węgliniec ESS	38.86±0.2 e	18.35±0.2 f	5.6	4.56	25.2	5.5	30.7	17.9	5.4±0.02 c
Oborniki Śl. SO	44.57±0.3 abc	22.09±0.3 e	9.6	6.52	26.7	7.9	34.6	22.8	9.1±0.01 d
Syców ESS	40.71±0.3 de	20.95±0.2 b	6.2	4.95	13.9	11.6	25.5	45.4	7.9±0.03 ab
Syców SO	44.69±0.3 bcd	21.11±0.3 ab	8.0	6.26	27.4	4.8	32.2	14.9	7.6±0.02 a

Table 3. Analysis of variance of Scots pine cones: significance (P) of impact of Objects and Type of seed source on length and width of the cones

		<i>P</i>		
Source of variation	DF	Length of cones	Width of cones	
Objects	9	<0.001	<0.001	
Type of seed source: stands vs. seed orchards	1	<0.001	<0.001	



**Figure 1.** Type of cone scales (h+r - hamata + reflexa;g - gibba; p - plana)Result of  $\chi^2$  test: significance level for populations,  $\alpha = 0.001$ ; for types of sources,  $\alpha = 0.794$ 

Cone weight was very variable and ranged from 5.6 to 11.8 g. Cones from seed orchard are characterized by greater dry weight than ESS cones (p = 0.0219) (Table 4), the mean weight was  $7.7 \pm 1.2$  and  $5.6 \pm 1.4$  g respectively. Cones originating from the Wegliniec ESS were characterized by the smallest mean dimensions and the lowest weight. Cones from the Gubin seed orchard are the largest in terms of length and width and weight (Table 2).

Table 4. Analysis of variance of characteristics of cones and seeds of Scots pine: significance of differences (P) between economical seed stands and seed orchards

Trait	Economical seed stands vs. seed orchards		
	P		
Fresh mass of cones	0.029		
Dry mass of cones	0.022		
Full seed number	0.142		
Number of empty seeds in cone	0.517		
Number of full seeds in cone	0.192		
Percent of full seeds in cone	0.252		
Percent of empty seeds in cone	0.252		
1000 - seed weight	0.037		

Cones differed in the total number of seeds per cone. The mean amount of seeds per cone was the following:  $27.82 \pm 6.7$  for ESS and  $35.02 \pm 2.1$  for seed orchard. The lowest number of seeds in the Bolewice ESS was  $18.7 \pm$ 5.2, with 31 % of empty seeds and the Gubin seed orchard, 27.3  $\pm$ 6.8, and 12 %, respectively. The highest number of seeds per cone were found in the Goleniów  $ESS - 36.9 \pm 6.1$  (15.4 % empty seeds) and Międzyrzecz  $SO - 36.3 \pm 6.4$  (11.8 %). No differences in the total number of seeds and filled and empty seeds were found when differences between seed orchards and stands were analysed (Table 5). The percentage contribution of empty seeds for all of the analysed sites of origin was not statistically significant.

Table 5. Results of seed stands and seed orchards seeds of Scots pine measurements. Different letters denote significant differences between features at p < 0.05 (Tukey test).

	Length [mm]		Width [mm]		Area [mm²]	
Object	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Bolewice ESS	4.75 cd	0.51	2.82 c	0.24	9.42 b	1.36
Międzyrzecz SO	5.14 a	0.30	3.14ab	0.29	11.30 a	1.35
Goleniów ESS	5.18 a	0.30	3.12 ab	0.30	11.51 ad	0.98
Nowogard SO	4.90 bc	0.39	3.02 a	0.27	10.35 c	1.33
Gubin ESS	4.62 d	0.44	2.83 c	0.28	9.44 b	1.64
Gubin SO	4.98 abc	0.37	3.23 bd	0.31	11.37 a	1.61
Węgliniec ESS	4.35 e	0.26	2.74 c	0.22	8.40 e	0.77
Obomiki Śl. SO	5.19 a	0.39	3.38 d	0.30	12.33 d	1.55
Syców ESS	5.04 ab	0.31	3.13 ab	0.23	11.24 a	1.10
Svców SO	5.19 a	0.39	3.02 a	0.31	11.20 ac	1.58

The mean seed length was  $4.78 \pm 0.33$  mm in ESS and  $5.07 \pm 0.13$  mm in seed orchards, and the mean width was  $2.92 \pm 0.18$  mm and  $3.15 \pm 0.15$  mm, respectively. Detailed seed dimensions for the analysed origins are presented in Table 5. Significant differences among seed length, seed area and seed width for all examined populations were demonstrated (Table 6).

Table 6. Analysis of variance of Scots pine seed dimensions: significance (P) of impact of Objects and Type of seed source

		Р			
Source of variation		Seed length	Seed width	Area of projection	
Objects	9	<0.001	<0.001	<0.001	
Type of seed source: stands vs. seed orchards		<0.001	<0.001	<0.001	

Subsequent stage of the study consisted in determination of seed weight (Table 2). The lowest weight of 1000 seeds  $(5.2 \pm 0.01 \text{ g})$  was found for the Bolewice ESS, whereas the highest  $(9.1 \pm 0.02 \text{ g})$  for the Oborniki \$1. SO. Seed orchards and stands differed significantly in the weight of 1000 seeds (p = 0.030), yet the differences in weight of all seeds obtained from one cone was not statistically significant (Table 4).

#### **Discussion and Conclusions**

Scots pine cones are characterized by high variability in terms of size. Their length ranges from 1.9 to 7.0 cm and width from 1.2 to 3.2 cm (Białobok et al. 1993). Dimensions of investigated cones are within the limits characterizing this species in Europe. It is stated that cone size is genetically controlled (Sivaciošlu and Ayan ż2008). It also depends on numerous factors such as climate, soil fertility, type and age of the stand and location of the cone in a tree (Aniszewska 2006, Bilir et al. 2008). Mean cone weight in Poland within the period 1996– 2003 is 6.30 g (Bodył and Załęski 2005). In our study size and weight of cones from seed orchards are above the national average, which may confirm the efficiency of intensive management of the environment (growth conditions) of a seed orchard.

The analyzed economic seed stands and seed orchards, significantly affects the cone and seed morphological characteristics. The ESSs are at the cutting age and produce smaller cones with lower 1000-seed weight than seed orchards. Seed weight depends on numerous factors. The tree age has considerable importance, the older the tree stand, the weight of seeds is lower (Białobok et al. 1993). Seeds from an orchard were found to be heavier than seeds from stands (Hadders 1963). Our results show a similar effect, after excluding two provenances: Rychtal (no statistical difference) and Goleniów (lower seeds from the orchard). The heavier seeds produced by the seed orchard trees are attributed to the better environmental conditions found in the seed orchards.

The seed size is linked to the cone size: the larger are cones in terms of dimensions and weight, the larger are the seeds (Verheggen and Farmer 1983, Matziris 1998). Aniszewska (2008 after Daszkiewicz 1961) states that seeds extracted from cones larger than 5 cm were 20 % larger and heavier than from cones smaller than 4 cm.

Bodył and Załęski (2005) state that on permanent observation plots within the period 1996 – 2003, the mean amount of seeds for Scots pine per one cone was 16.59 seeds. In the presented study the mean amount of seeds was 27.82 for ESS and 35.02 for seed orchards. Rożkowski et al. (2007) provide lower mean numbers of seeds per cone, which were 19.16 in ESS and 28.7 in seed orchard.

In northern Sweden the number of seeds per cone in a seed orchard of Scots pine ranges from 9.3 to 21.8 (Yazdani et al. 1995). The age of seed orchard influences the number of seeds per cone: the older the orchard the greater the number of seeds per cone (Sivaciošlu and Ayan 2008). In our study, the difference in number of seeds per cone between seed orchard and seed stand was not statistically significant, yet ESSs are mature stands, whereas the analysed seed orchards are young. It can be expected, that the amount of seeds produced by these orchards will continue to increase.

The weight of analysed seeds did not differ from the standards of weight established in Poland. The weight of examined seeds ranged from 5.2 to 9.1 g. According to Antosiewicz (1970), the mean weight of 1000 seeds of Scots pine for Poland is 6.2 g, yet it may range from 4.5 to 8.5 g. The mean weight of 1000 seeds in Poland in the period 1996-2003 was 6.54 g (Bodył and Załęski 2005). Worthy of noting is the seed orchard in Gubin, the seeds of which are characterized by the greatest weight among the tested origins, significantly over the range provided by Antosiewicz. Detailed study on the variability of seed structure of Scots pine was conducted by Wrześniewski (1982), seeds with the weight of 6 to 8 mg swell and germinate at the highest rate and their sprouts have the highest growth rate. Seedlings obtained from heavier seeds attain greater size Mikkola (1980). Pine seeds originating from orchard seeds, in the 2 year of cultivation were characterized by greater dry weight of the shoot and needles and greater survivability than seedlings originating from stands (Wennstrom 2001).

Heavy seeds, contrary to medium and small seeds, are characterized by higher content of proteins, carbohydrates and lipids (Khan and Shankar 2001). Climatic conditions and site conditions affect the seed weight. In the north, were climatic conditions are harsher, and the temperature during the vegetation season is lower, the mean seed weight of the Scots pine is lower than in the central and southern parts of its distribution area (Reich et al. 1994).

Seeds from seed orchards are characterized by greater weight than seeds from seed stands (Wennstrom 2001, Bilir et al. 2008). Our study confirmed this trend. Seeds from orchards were characterized by greater length and width. The greater seed weight may be resulted from selection of trees for the establishment of seed orchards. Seed orchards are established in a wide spacing, which in Poland is  $6 \times 6$  m. Moreover, they are fertilized according to the needs which may influence the weight of the seeds.

Overall seed quality is related to a number of factors, the most important among which appears to be the proportion of empty seeds. They are often produced as

a result of self-pollination (Lindgren 1975). In our study the number of empty seeds was 24.54 % for ESSs and 16.85 % for seed orchards. Yazdani et al. (1995) provides that the percentage of empty seeds in seed orchards ranges from 12 to 39 %. In Swedish seed orchards the percentage of empty seeds is 12 and 27 % at the top and bottom of grafts, respectively (Prescher et al. 2005). The number of empty seeds declines with the age of orchard (Bilir et al. 2008); therefore, we suspect that in the following years the number of empty seeds in the studied seed orchards will decline.

Seeds originating from seed orchards are considered as genetically enhanced due to the tree selection process prior their establishment. Selection is carried out for the increase of growth, shoot straightness and wood quality (Stener 2015). Genetic gain is the measure of efficiency of the selection process. Theoretical genetic gain for Scots pine originating from the first-generation seed orchard (phenotypic tree selection for orchard) is estimated at 10 % in comparison to non-selected trees. Orchard contamination with foreign pollen decreases the genetic gain to 8 % (Stener 2015 after: Rosvall et al. 2001). Scots pine offspring from the first-generation seed orchards differ from unselected stands: in height by 9.2 %, in stem diameter by 5.4 % and 18.9 % in stem volume (Andersson et al. 2007).

Phenotypic selection is the oldest and easiest way to select the best trees. Thanks to this selection we can observe in seed orchards also a genetic gain expressed in biometric features of cones and seeds.

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