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SVETLANA VELISEVICH

ALEXANDER POPOV

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Evaluation of cone and seed quality of Siberian stone pine (*Pinus sibirica* Du Tour) for plus-tree selection

Svetlana N. VELISEVICH[1], **Alexander V. POPOV**[2,*]
[1]Institute of Monitoring of Climatic and Ecological Systems SB RAS, Tomsk, Russia
[2]National Research Tomsk State University, Tomsk, Russia

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Abstract: Siberian stone pine (*Pinus sibirica* Du Tour) is a cone-bearing species with outstanding nutritious seeds, health-supporting properties, and nut oil. Currently, the main volume of pine nuts is harvested in natural forests, which causes damage to ecosystems. The best solution to this problem would be harvesting nuts on special pine nut plantations established on the basis of high-seeding clones. However, it is hampered by the low efficiency of the existing methods for assessing plus-tree productivity. This work focused on cone analysis in two sites used for plus-tree selection: a 42-year-old sparse forest plantation and a 186–224-year-old closed natural forest. The quality of cones and seeds was assessed for 20 traits characterizing the sequence of crop maturation, from the differentiation of the cone to seed ripening. The differences between the sites for most quality traits, except the number of developed and filled seeds, were insignificant. This means that both sites were equally promising for seed quality selection. An index of kernel weight per 1 m^2 of crown area was proposed to be used for the initial selection of plus-trees that combine a narrow crown with a large mass of kernels. Such trees can produce a greater yield from a smaller plantation area. In closed stands, the cone number per tree was strongly correlated with crown size; thus, this index can only be used for sparse forests or plantations.

Key words: Siberian stone pine, cone structure, seed quality, variation, stand density

1. Introduction

Siberian stone pine is the only nut-bearing species in Russia, and its seeds are a popular food product in this country and abroad. It has been introduced as a nut-producing species even outside its range (Zhao et al., 2014). The main volume of pine nuts is harvested in natural pine forests, but the taking away of nuts from them complicates the reforestation and the survival of the local fauna. Although establishing special nut-producing seed orchards is very difficult, plus-tree breeding stopped at the stage of phenotypic selection in native forests and creation on the basis of a few clone plantations of the first generation (Titov, 2007). The current methods for selecting plus-trees are ineffective. For example, in the still valid "Recommendations for the selection of Siberian stone pine plus-trees for seed productivity" (Iroshnikov and Titov, 2000), selection is recommended using the eye counting method, which can cause large errors. Zemlyanoy and Nekrasova (1980) suggested selecting the best trees based on the number of cones over the past 10–12 years, calculated from bark scars on seeding branches. However, this method has not found wide application due to the high cost and risk to life, since the branches must be manually climbed into the crown of 25 m mature trees. It was also found that on clone plantations of the first generation, the selection rank of the mother plus-trees, selected from natural forests, was not completely preserved (Il'ichev and Shuvaev, 2016; Zemljanoy et al., 2011). Plantations of the second generation, which have been successfully created for other nut-producing species (for example, Yuan et al., 2016; Afonso et al., 2020), are still not available for Siberian stone pine since there is no effective method for selecting plus-trees. This work is intended, at least in part, to fill this gap.

To get a better understanding of possible improvements in the function of seed orchards, researchers use cone analysis very widely to determine seed production (Bramlett, 1972; Matziris, 1998; Sivacioglu and Ayan, 2008; Yuan et al., 2016). The analysis of variability in different seed production traits allows the selection of traits that are the best predictors of seed efficiency. For example, in *Pinus tabuliformis* (Li et al., 2011; Yuan et al., 2014, 2016), *Pinus sylvestris* (Burczyk and Chalupka, 1997; Bilir et al., 2008; Sivacioglu et al., 2009; Sevik and Topacoglu,

* Correspondence: tomskceltic@gmail.com

2015), *Pinus pinea* (Calama and Montero, 2007; Ganatsas et al., 2008), *Pinus strobus* (Noland et al., 2006), *Pinus albicaulis* (Owens et al., 2008), and *Pinus koraiensis* (Kang and Lindgren, 1999), traits with a high level of variability have a high genotypic component. However, previous studies were based on the analysis of a small number of seed production-related traits characterizing the outcome of the crop formation, paying little attention to other contributing traits. Our work is aimed at a detailed analysis of the quality of seed production, taking into account seed losses.

For the variability in reproductive traits in the Siberian stone pine, information has only been provided for the number of cones per crown and traits that directly characterize the morphogenesis and growth of reproductive structures: cone size, seed number, and seed weight (Kuznetsova, 2003; Titov, 2010; Zemlyanoy et al., 2010, 2011). Depending on the interaction of the mother tree with its pollinators, traits of a more complex nature, such as the proportion of underdeveloped and empty seeds, seeds with underdeveloped endosperm, and the combination called "seed losses", have only been analyzed in two publications (Goroshkevich and Khutornoy, 1996; Goroshkevich, 2008).

In this study, we examined variation in and correlations among 20 traits related to the quantity and quality of cones and seeds of *Pinus sibirica* in two stands: a 42-year-old sparse forest plantation and a 186–224-year-old closed natural forest. We compared correlations between traits and their contributions to seed production, and proposed «index of kernel weight per 1 m^2 of crown area» as a final selection trait. The goal of this study was to provide new information for evaluating seed production in Siberian stone pine seed orchards by increasing our understanding of the variation among seed production-related traits.

2. Materials and methods

2.1. Study site

The study sites are located on the West Siberian Plain, Tomsk region, north of the Ob-Tom interfluve (Figure 1). According to the Köppen-Geiger climate classification scheme, this region is on the boundary of subarctic or boreal and warm summer continental or hemiboreal climates. Based on the Tomsk weather station record, the mean annual temperature was +1.2 °C, and the annual precipitation was 560 mm. It is south of the southern taiga subzone and south of the Siberian stone pine range. The soil is deep, loamy, fertile, and well moistened (Dyukarev and Pologova, 2011).

The first site (56°30'N and 84°38'E, sea level elevation 100 m) was a forest near a settlement that is reserved and managed as a source of seed for locals. It was designated as the "forest site" (FS). Area of this forest more than 6 ha, tree density was 80 trees per hectare, and canopy cover density was approximately 60% according to our calculations. In addition to the Siberian stone pine, *Abies sibirica* (Ledeb.)

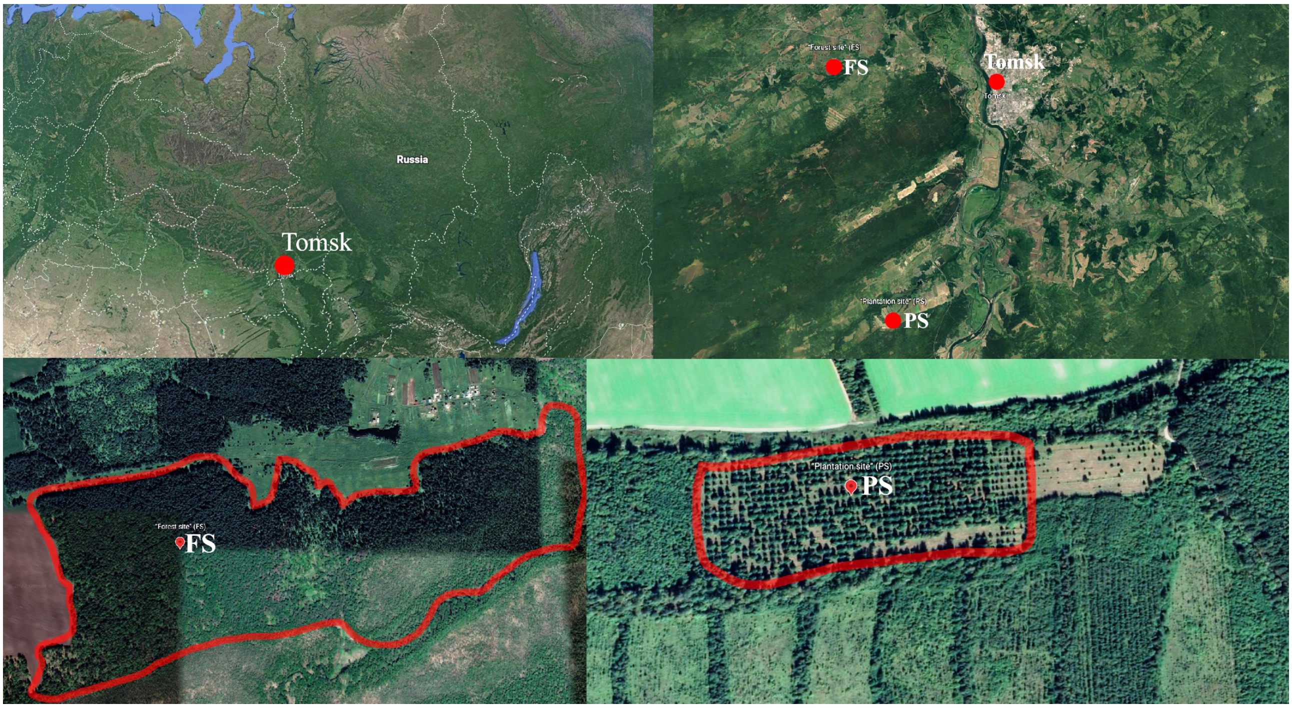

Figure 1. Location map of Tomsk region on the West Siberian Plain and two experimental sites —forest site (FS) and plantation site (PS).

and *Picea obovata* (Ledeb.) grow on the site as an admixture about 10%. Twenty-one trees were monitored for growth and sexual reproduction for 30 years (Goroshkevich et al., 2021), so these same trees were analyzed in this study. These trees were 186–224 years old, with a height of 24.5 m, diameter at breast height of 66 cm. The trees were unevenly distributed over the area; therefore, there were dominant and codominant trees among them, whose crowns formed the general level of the canopy and received full light from above but comparatively little from the sides.

The second site (56°14'N and 84°45'E, sea level elevation 134 m) was the "plantation site" (PS). The PS was created from local seeds, which were planted in 1977 for timber forest plantations with 0.75 × 3 m spacing. In 1987, when the trees reached 1–1.5 m in height, they were replanted at 8 × 8 m spacing on 6 ha PS (Bekh and Vorobjev, 1998). By 2019, when cones were harvested for analysis, the trees age had reached 42 years, a height of 10.5 m, diameter at breast height of 36 cm. The total tree mortality on the plantation trees was 7%. We analyzed 32 trees that have been monitored for growth and crown development since 2005 (Popov and Velisevich, 2021).

2.2. Data collection

Siberian stone pine is a species that exhibits masting behavior, i.e. high interannual variability in seed production (Pearse et al., 2016). Information on a masting cycle for Siberian stone pine was reported in a recent paper (Goroshkevich et al., 2021). For a detailed analysis of the harvest quality, the cones that ripened in 2019 were taken, since it was a year of unusually good seed production when the reproductive potential of trees was at a maximum. To count the number of cones per tree, all cones were shaken to the ground. Ten cones per tree were collected randomly for cone analysis.

2.3. Cone analysis

The size of the cone was estimated by cone length (CL) and cone diameter (CD). A female cone consists of a central axis supporting scales that bear naked seeds. Each female cone contains about a hundred scales, but not every scale has two basal ovules. Potentially ovuliferous fertile scales (FSc) that bore seeds (developed or underdeveloped) are located in the medial zone of the cone. Many of the small scales at the base and tip of each cone did not develop and remained as a cluster of very small sterile (ineffective) scales (Owens, 2006). Such sterile scales are also found in small numbers in the medial part, where fertile scales should be located. Therefore, the number of three categories of sterile scales was counted separately: base sterile scales (BSSc), top sterile scales (TSSc), and medial sterile scales (MSSc). The total number of scales (TNSc) was calculated as the sum of the four categories of scales: FSc, BSSc, TSSc, and MSSc (Figure 2).

Each ovuliferous scale (OSc) normally has two ovules and, accordingly, two seeds. However, not every potential

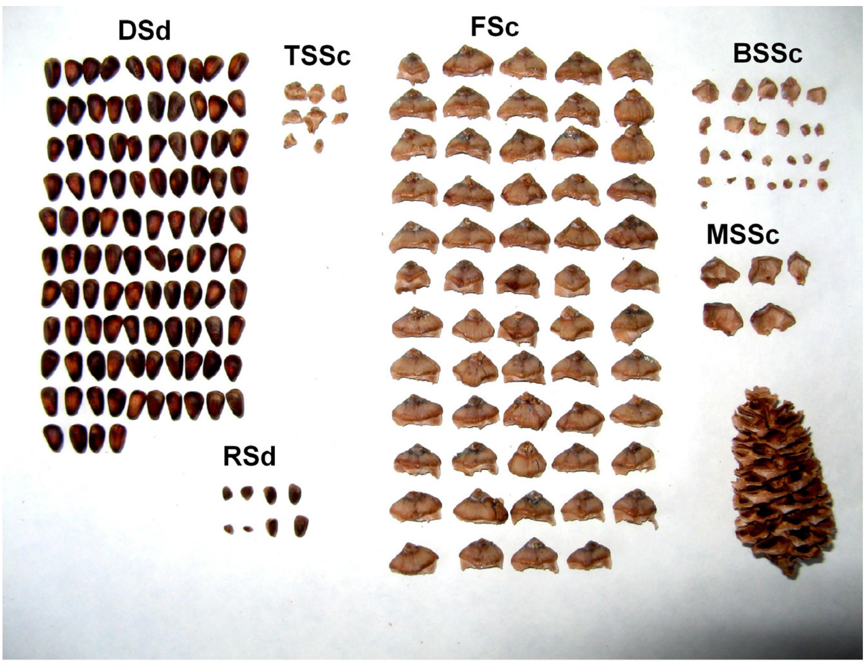

Figure 2. Seed cone structure analysis diagram on the scales and seeds of different ranks: fertile scales (FSc), base sterile scales (BSSc), top sterile scales (TSSc), medial sterile scales (MSSc), developed seeds (DSd), rudimentary seeds (RSd).

OSc in the medial zone of the mature cone has two seeds since some FSc have one seed instead of two. This is due to the death of a part of the seeds during the 15–16 months of bud development; therefore, the final seed number in the mature cone differs from the initial ovule number. The number of OSc was calculated as twice the number of scales in the medial zone ([FSc + NSSc] × 2). The total seed number (TSd) was calculated as the sum of the two types of seeds: developed seeds (DSd) and rudimentary seeds (RSd). RSd were very small seeds that aborted soon after pollination and contained only a small aborted megagametophyte (Owens, 2006).

On the radiographs (Figure 3), obtained by the method of radiographic studies (Shcherbakova, 1965), three categories of seeds were counted. Filled seeds (FilSd) were counted as the number of seeds that contained a well-developed megagametophyte and an embryo that filled about 90% of the length of the megagametophyte (Owens, 2006). Defective seeds (DefSd) were counted as seeds with a damaged megagametophyte (Owens, 2006). Empty seeds (EmpSd) were counted as the number of seeds that did not contain all tissues essential for germination. Seed efficiency (SdEf) was calculated as the FilSd percentage of the number of OSc (FilSd × 100 / OSc).

To determine the weight of one seed (SdW), 100 filled seeds from each tree were weighed, and then the resulting value was divided by 100. To determine the weight of filled seeds per cone (SdW/Cone), the weight of one seed was multiplied by the total seed number. The kernel weight (KW) of one seed and kernel weight per cone (KW/Cone) were calculated in the same way.

The index of kernel weight per 1 m^2 of crown area (KW/Crown) was calculated as kernel weight per cone multiplied by the total cone number and divided by the crown cover.

The list of abbreviations for the analyzed traits is given in Table 1.

2.4. Statistical analysis

The data set was composed of 21 trees and 210 cones for FS and 32 trees and 320 cones for PS. The results of the seed production traits were first submitted to the Shapiro–Wilk test to verify the normality of the data and then subjected to analysis of variance (ANOVA; Statistica 6.0). Pairwise comparisons were established using Newman-Keuls posthoc test or the chi-square test. All probabilities were appreciated at 5%. The variability of analyzed traits was expressed as CV-the coefficient of variation (standard deviation/mean) × 100). A Spearman rank correlation analysis was used to establish the relationship between traits.

3. Results

3.1. Tree productivity

Crown areas in FS varied widely (Table 2) due to larger growing gaps within the stand. In PS, variation in this trait was much less due to the gap condition is similar for crown formation. In FS trees, the crown area strongly affected the cone number; a significant correlation was found between these traits ($r = 0.624$, $p \leq 0.05$). In PS trees, the relationship between crown area and cone number was very weak ($r = 0.162$), demonstrating the independence of the cone number from crown growth. Variation in the number of cones per tree was much higher in FS. In PS, the factor of spatial heterogeneity was absent; the variability in cone production was lower than that of FS.

3.2. Cone structure

The variability of the CL, expressed as CV, was medium (11.4 for FS, and 14.1 for PS). The variability of the CD (9.9 for FS, and 7.8 for PS) and TNSc (8.1 for FS, and 9.4 for PS) were low, less than 10%. The MSSc, which characterizes the potential seed losses due to insufficient pollination, had a large variability (51.1 for FS, and 70.5 for PS).

There were no significant differences between sites in the mean values of CL, CD (Figure 4) and TNSc (Figure 5a), although in PS, cones were more variable in size. At both sites, more than half of all scales were fertile (Figure 5b) and at both sites, there were 3 trees that were promising for breeding because they had more than 60% of FSc. Percentage of BSSc and TSSc were also the same for both sites. Significant differences were established only in the proportion of MSSc (Figure 5c).

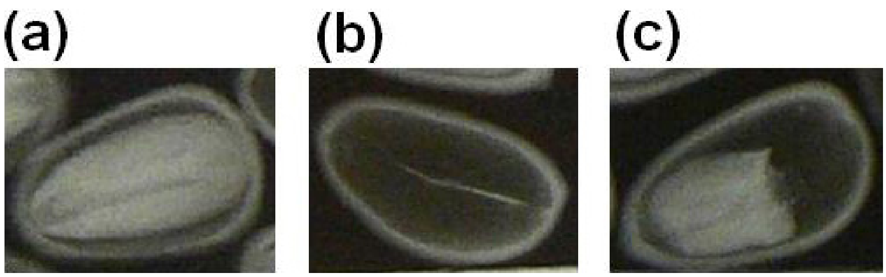

Figure 3. The fragment of seeds radiographs: **(a)** filled seed have a light colored megagametophyte; **(b)** empty seed are dark in color and does not contain all the tissues essential for germination; **(c)** defective seed have a damaged megagametophyte.

Table 1. The list of abbreviations for the analyzed traits.

Abbreviation	Trait
CL	Cone length
CD	Cone diameter
FSc	Fertile scales
BSSc	Base sterile scales
TSSc	Top sterile scales
MSSc	Medial sterile scales
OSc	Ovuliferous scales
TSd	Total seed number
DSd	Developed seeds
RSd	Rudimentary seeds
FilSd	Filled seeds
Def Sd	Defective seeds
EmpSd	Empty seeds
SdEf	Seed efficiency
SdW	Seed weight
SdW/Cone	Seed weight per cone
KW	Kernel weight
KW/Cone	Kernel weight per cone
KW/Crown	Kernel weight per crown

Correlation analysis between cone size and structure showed some differences between sites (Table 3). In PS, the relationship between CL and CD was stronger than that in FS. In PS, cones were more round in shape, since the diameter increased with increasing length. In FS, the CL was practically not related to the CD; the cones were more varied in shape, including those that were almost round and those that were elongated. At both sites, the CL was positively associated with TNSc and especially with FSc in the medial zone. An increase in the proportion of BSSc and TSSc at both sites leads to a reduction in the length of the cone. MSSc did not affect the size of the cones at either site.

3.3. Seed quality

In the FS, the TSd and DSd was significantly greater than that in PS (Figure 6). At both sites, there was a very high level of variability in the number of RSd —from 0 to 30 at FS and from 0 to 40 at PS. According to the percentage of DSd and RSd from the TSd, the sites did not differ significantly. Some trees had almost no RSd; most of them were limited, and only a few trees reached 40%–50%. For example, in PS, three trees had above 10% underdeveloped seeds, one tree above 20% and one above 45%. In FS, the increased proportion of underdeveloped seeds was almost the same: four trees had 20% underdeveloped seeds.

Comparative analysis of seed fullness showed no significant differences between sites in the number of FilSd, DefSd, and EmpSd (Figure 7), and there was large variability in these traits for both groups of trees. Trees without seeds in cones or with EmpSd were not found in our work. In FS and PS, 10% and 12% of trees, respectively, had more than 95% full seeds in their cones. But both sites had two trees each with cones that had more than 50% EmpSd. The proportion of DefSd was very small at both sites, compared to the proportion of EmpSd.

If the traits of seed quality were arranged sequentially in a row: TSd → DSd → FilSd as the final indicator of their development, then the level of their variability (expressed as CV) gradually increased, but was almost the same for FS (15.6 → 18.8 → 20.9, respectively) and PS (18.3 → 22.4 → 29.8, respectively). The variability became much greater when analyzing seeds that died at different stages of development in a row: RSd → DefSd → EmpSd (85 → 104 → 117 for FS, 99 → 106 → 69 for PS, respectively).

3.4. Seed efficiency

In FS cones, if each of the potential OSc in the medial part of the mature cone had 2 seeds, then with an average FSc of 48.6, the initial OSc would be 97.2 (Figure 8a). However, 6.4% of the scales in the fertile zone of the cone did not have seeds, i.e. were sterile. In addition, 14% of FSc, on average, had one seed instead of two, i.e. they were single-seeded, so the TSd was potentially 79.3%, on average. Some of the seeds (4.3%, on average) were underdeveloped, lacking an embryo and endosperm. Of the DSd, 12.5%

Table 2. Crown area and cone number in FS and PS.

Trait	Site	Mean	Standard deviation	Minimal-maximal value	CV, %
Crown area, m^2	FS	57	18.1	28–117	31.8
	PS	29	6.2	16–41	21.7
Cone number	FS	509.2	289.1	179–1327	56.8
	PS	63.7	43.6	12–260	39.2

FS: forest site, PS: plantation site. Sample size is 21 for FS, 32 for PS. CV: coefficients of variation.

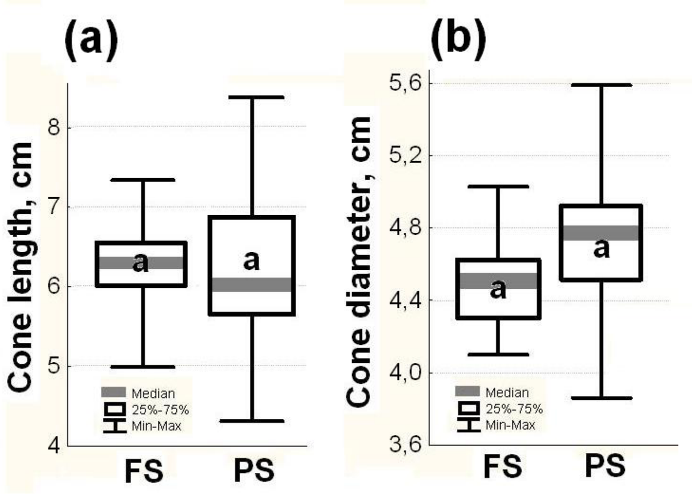

Figure 4. Cone length **(a)** and cone diameter **(b)**. Letter indices indicate significant differences (p ≤ 0.05) between means calculated by Newman–Keuls test. FS: forest site, and PS: plantation site. Sample size is 21 for FS, 32 for PS.

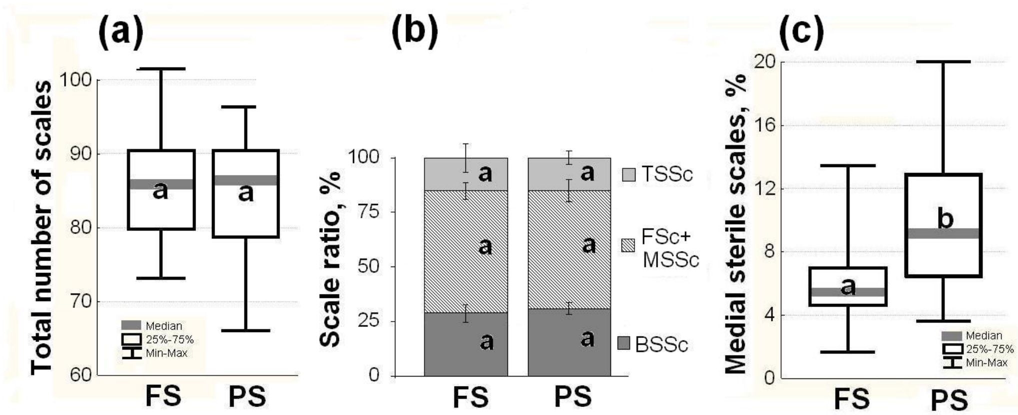

Figure 5. Cone structure on the scales of different ranks: Total number of scales **(a)**, scale ratio, % **(b)** and medial sterile scales **(c)** in FS (forest site) and PS (plantation site). Top sterile scales (TSSc), fertile scales (FSc), medial sterile scales (MSSc), and base sterile scales (BSSc). Letter indices indicate significant differences (p ≤ 0.05) between means calculated by Newman–Keuls test **(a, b)** and chi-square test **(c)**. Error bars **(b)** represent standard deviation. Sample size is 21 for FS, 32 for PS.

were completely empty, and 3.3% had an underdeveloped endosperm. The proportion of FilSd in the cone (seed efficiency) was 68.5% of the potential (Figure 8b). Thus, 31.5% of OSc did not form seeds of full value; these were considered seed losses (Figure 8c). Of the ineffective OSc, 54.9% did not produce seeds, 10.8% were underdeveloped, 7.2% were EmpSd of normal size, and 27.1% had seeds with an underdeveloped endosperm.

Similar calculations were also made for PS. For PS, with an average FSc of 45.8, the initial number of OSc would be 91.6 (Figure 8a). However, 11% of the scales in the cone fertile zone were sterile, and 10% of the fertile scales had one seed instead of two; thus, the percentage of TSd was 72.3%. Among the TSd, 4.4% were underdeveloped; of the DSd, 10.7% were EmpSd, and 4.6% had an underdeveloped endosperm. Therefore, FilSd in the cone (seed efficiency) was 69.5%. (Figure 8b). Thus, 30.5% of OSc did not form FilSd. Of the ineffective OSc, 54.8% did not produce seeds, 10.4% were underdeveloped, 10.4% developed into EmpSd of normal size, and 24.4% developed into seeds with a defective endosperm (Figure 8c).

Analysis of the seed losses ratio (Fig. 8c) and Spearman rank correlation between number of FilSd and seed loss traits (Table 4) showed that losses occurred due to the large

Table 3. Spearman rank correlation between traits of cone size and structure.

	CL	CD	TSc	BSSc	TSSc	FSc	MSSc
CL		0.175	**0.631**	**–0.493**	**–0.796**	**0.712**	0.027
CD	**0.580**		0.307	0.106	0.121	0.191	0.181
TNSc	**0.386**	0.231		–0.157	**–0.496**	**0.444**	–0.014
BSSc	**–0.574**	–0.200	–0.179		0.317	**–0.808**	0.255
TSSc	**–0.599**	–0.074	–0.201	**0.533**		**–0.777**	–0.141
FSc	**0.687**	0.155	0.232	**–0.838**	**–0.865**		–0.091
MSSc	0.107	–0.123	0.304	0.056	–0.107	0.038	

Coefficients in the upper right corner indicate forest site, in the lower left - plantation site. Cone length (CL), cone diameter (CD), total number of scales (TSc), base sterile scales (BSSc), top sterile scales (TSSc), fertile scales (FSc), and medial sterile scales (MSSc). Correlations significant at $p \leq 0.05$ marked bold. Sample size is 21 for FS, 32 for PS.

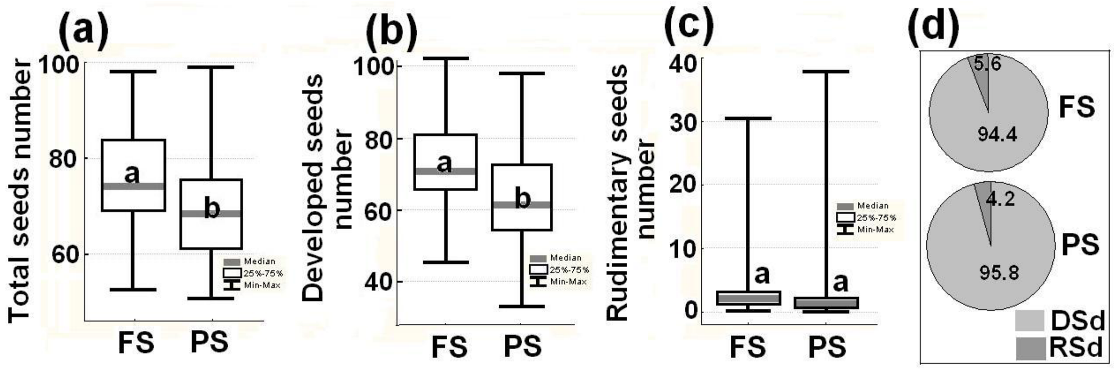

Figure 6. Seeds of different developmental class in forest site (FS) and plantation site (PS): **(a)** total seed number, **(b)** developed seed number (DSd), **(c)** rudimentary seed number (RSd), **(d)** proportion of DSd and RSd (%); the numbers in the sectors of the circle indicate the percentage of DSd and RSd. Letter indices in boxes indicate significant differences ($p \leq 0.05$) between means calculated by chi-square test. Sample size is 21 for FS, 32 for PS.

number of single-seeded fertile scales at both sites. They were followed by losses due to DefSd as only found in FS. In FS, good cones with minimal seed losses (< 20%) were found in two trees, and bad cones with large seed losses (> 50%) were found in only one tree. In PS, minimum seed loss was observed in 6 trees, and maximum seed loss was also found in 6 trees.

3.5. Seed weight

One seed in PS weighed 293 mg, on average. This was significantly more than FS, where the average SdW was only 260 mg (Figure 9). The weight of FilSd per cone was slightly more in FS (16.5 g) than in PS (15.8 g), since there were slightly more seeds in the cone. In FS and PS, 17 and 16% of trees, respectively, had a total mass of seeds in a cone of more than 20 g. A similar relationship was shown for the kernels. The average KW of one seed in PS (137 mg) was not significantly higher than that in FS (126 mg). When recalculated per cone, the KW/Cone in PS was 7.7 g; in FS, it was insignificantly higher at 8.0 g. In FS, 13% of trees had a KW/Cone more than 10 g, and in PS, 19% were higher than 10 g.

The correlation analysis between traits related to seed weight and cone structure showed the similarity between the sites (Table 5). At both sites, SdW and KW were more independent of cone structure, but the correlations were significant for counts that were made per cone. At both sites, SdW/Cone and KW/Cone were more strongly correlated with CD and weaker with CL. FSc, TSd, and OSc were positively and significantly correlated with SdW/Cone and KW/Cone. The traits characterizing seed losses (single-seeded fertile scales and DefSd) had no effect on the SdW and KW, both for one seed and when calculated per cone. However, with the third trait of seed losses — EmpSd— the correlations were positive and significant.

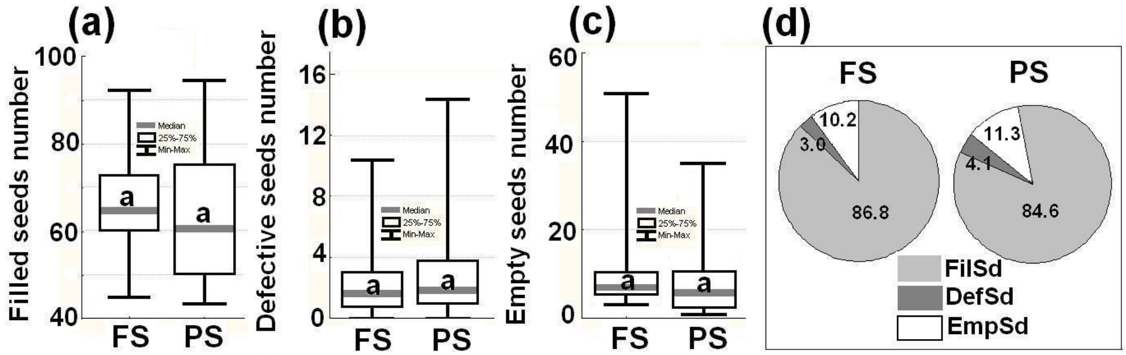

Figure 7. Fullness of seeds in forest site (FS) and plantation site (PS): **(a)** filled seed number (FilSd), **(b)** defective seed number (DefSd), **(c)** empty seed number (EmpSd), **(d)** proportion of FilSd, DefSd and EmpSd (%). The number in the circle sector indicates the percentage of seeds. Letter indices in boxes indicate significant differences between means calculated by chi-square test. Sample size is 21 for FS, 32 for PS.

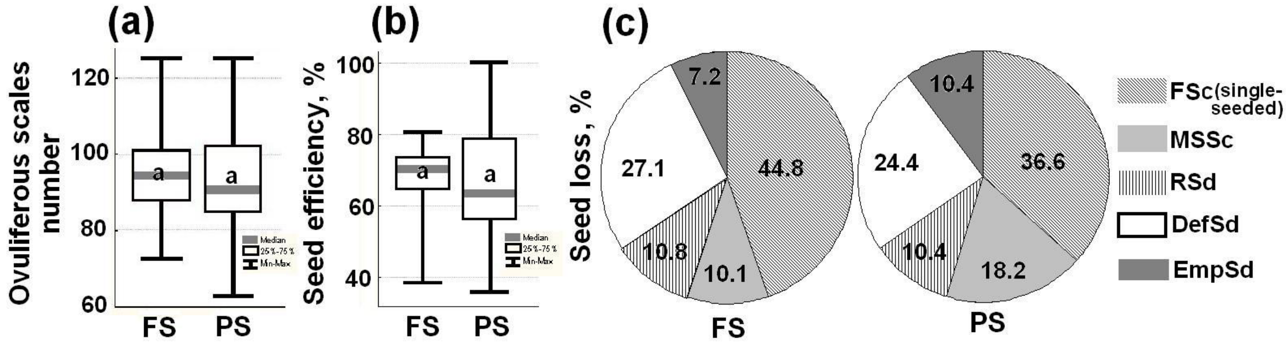

Figure 8. Seed efficiency and losses in forest site (FS) and plantation site (PS): Ovuliferous scales number (OSc) **(a)**, seed efficiency (SdEf) **(b)**, seed losses **(c)**. The number in the circle sector indicates the percentage of loosed scales and seeds. Fertile scales (FSc), medial sterile scales (MSSc), rudimentary seeds (RSd), defective seeds (DefSd), empty seeds (EmpSd). Letter indices in boxes indicate significant differences between means calculated by chi-square test. Sample size is 41 for FS, 49 for PS.

3.6. Index of kernel weight per crown cover

In FS, the number of cones was closely correlated with the crown area (r = 0.624); therefore, kernel weight per crown (KW/Crown) was closely correlated with the crown area (r = 0.495, significant at $p \leq 0.05$). Due to such a close connection in dense forests, it was difficult to find trees that actually had both a narrow crown and a large mass of kernels. In PS, the number of cones was very weakly correlated with the crown area (r = 0.162); therefore, KW/Crown was also weakly correlated with the crown area (r = 0.230), making the search for high-yielding trees with a narrow crown more effective in a sparse plantation. The average PS value of the KW/Crown index was 18.9 g/m^2, with an average crown area of 29 m^2. Six trees with a KW/Crown of 34.8 g/m^2 and an average crown area of 22 m^2 were selected. These trees are promising for the establishment of a special nut plantation, of which the breeding effect can be almost doubled.

4. Discussion

4.1. Cone structure and seed quality

In Siberian stone pine, the development cycle of the female cone is extended for three years (Nekrasova, 1972); therefore, the structure of mature cones develops over a three-year period under the influence of various factors. All seed-production traits were divided into three qualitatively different groups. The first group was associated with the influence of the mother plant on the traits of initiation, differentiation, and growth of cones and seeds (Goroshkevich and Khutornoy, 1996). The second group of factors was determined by the conditions of pollination and the quantity and quality of pollen (Nekrasova, 1983; Owens and Fernando, 2007). The third group was formed by the interaction of pollen and ovule, male and female gametes, and the compatibility of their genotypes in the processes of fertilization and embryogenesis (Kosiński 1987; Nikkanen et al., 2000; Owens, 2006; Owens et al.,

Table 4. Spearman rank correlation between filled seeds number and seed losses traits.

Seed losses traits	FS	PS
FSc (single-seeded)	**–0.509**	**–0.444**
MSSc	–0.171	–0.270
RSd	–0.145	–0.107
DefSd	**–0.421**	–0.296
EmpSd	–0.046	–0.266

Forest site (FS) and plantation site (PS). Fertile scales (FSc), medial sterile scales (MSSc), rudimentary seeds (RSd), defective seeds (DefSd), empty seeds (EmpSd). Bold marked correlations are significant at p ≤ 0.05. Sample size is 21 for FS, 32 for PS.

2008). The traits used in this work were formed under the influence of different ratios of these three groups of factors.

The first two groups of factors determined the structure of the cones. It was characterized by the ratio of scales of different ranks. There were no significant differences between sites in the number of fertile scales, base sterile, and top sterile scales, but trees in PS had an increased number of MSSc, indicating possible deviations in the process of pollination (Nikkanen et al., 2000; Owens et al., 2008). Trees in PS were relatively young, and not all of them had formed a sufficient male zone in the crown, while the nearest mature forest was located 10 km from PS. Therefore, it is very important to consider the location of mature Siberian stone pine forests in crop management practices that reduce self-fertilization.

The second and third groups of factors, which together affect the success of the interaction between pollen and ovule, fertilization, and embryogenesis, determine the quantity and quality of seeds, considering their losses at successive stages of development. A study on the role of physiological and genetic factors in seed losses in *Larix decidua* (Kosiński, 1987) showed that 34% of unsound seeds were formed due to lack of pollination and poor pollen quality; 30% was due to embryo degeneration as a result of self-pollination, and only 9% was due to the disruption of megasporogenesis. Using *Pinus monticola* as an example, Owens and Fernando (2007) demonstrated that the main losses occurred during the interaction of the pollen grain and the ovule. About 25% of ovules aborted because of incompatibility at fertilization. That is, the process of pollination and fertilization is the most dramatic moment in the formation of a crop. In this study, the number of RSd formed due to the early incompatibility mechanism did not differ statistically between sites (Owens, 2006). Perhaps there were no differences due to the very large variability of this trait, but in FS, the TSd was significantly higher than that in PS due to the higher number of DSd (Figure 7), indicating problems with cone pollination in PS. The seed fullness (Figure 7) and seed losses (Figure 8) were almost the same in FS and PS. The number of ineffective ovuliferous scales also differed slightly (31.5% in FS and 30.5% in PS). At both sites, the most significant yield losses were due to single-seeded fertile scales (45% in FS and 37% in PS), followed by seeds with a defective endosperm (27% and 24%, respectively). This was confirmed by the significant Spearmen rank correlation between the FlSd and seed loss traits (Table 4). The remaining loss categories were small at both sites. It is generally consistent with the data presented by Ganatsas et al. (2008) for *Pinus pinea* cones, which had few empty seeds, and the main losses were due to not fully developed seeds. In this study, there were no significant differences between sites in the number of OSc and SdEf, suggesting

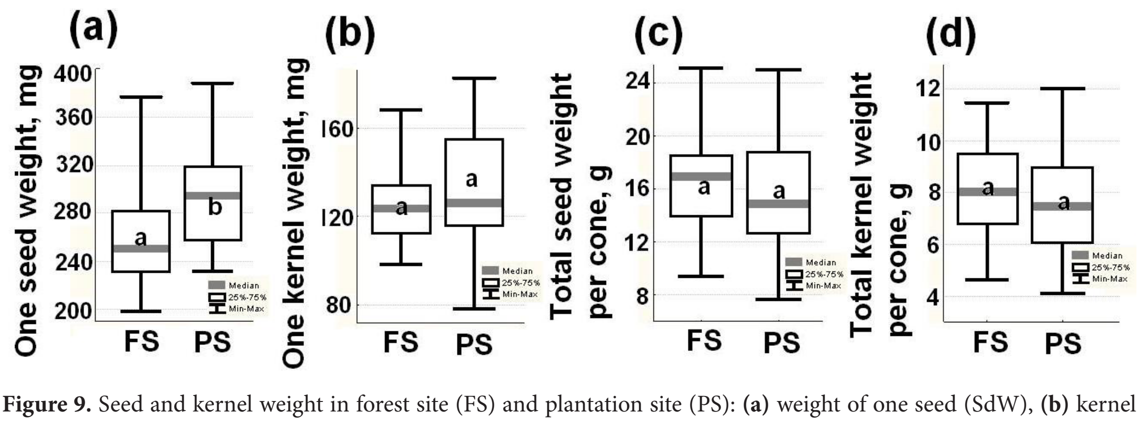

Figure 9. Seed and kernel weight in forest site (FS) and plantation site (PS): **(a)** weight of one seed (SdW), **(b)** kernel weight of one seed (KW), **(c)** weight of FilSd per cone (SdW/Cone), **(d)** kernels weight per cone (KW/Cone). Letter indices in boxes indicate significant differences between means calculated by chi-square test. Sample size is 21 for FS, 32 for PS.

Table 5. Spearman rank correlation between seed weight traits and cone structure traits.

	SdW	KW	SdW/Cone	KW/Cone
CL	−0.110 0.327	−0.065 0.272	0.374 **0.577**	0.352 **0.542**
CD	**0.694** **0.695**	**0.657** **0.650**	**0.754** **0.540**	**0.676** **0.530**
FSc	−0.114 −0.082	−0.112 −0.076	**0.487** **0.525**	**0.460** **0.502**
FSc (single-seeded)	−0.116 −0.095	0.272 0.079	0.082 0.039	0.275 0.089
OSc	−0.194 −0.083	−0.090 −0.088	**0.481** **0.422**	**0.474** **0.413**
TSd	−0.134 0.019	−0.170 −0.057	**0.459** **0.544**	**0.421** **0.524**
FilSd	0.409 0.146	**0.580** **0.560**	**0.451** 0.082	**0.563** **0.584**
DefSd	0.091 0.181	0.253 0.310	0.062 0.294	0.242 0.292
EmpSd	**0.536** 0.193	**0.471** **0.531**	**0.624** 0.161	**0.465** **0.566**

The first rows of values at each column represent the FS (forest site) and second – PS (plantation site). Cone length (CL), cone diameter (CD), fertile scales number (FSc), ovuliferous scales number (OSc), fertile scales number (FSc), total seed number (TSd), filled seed number (FilSd), defective seed number (DefSd), empty seed number (EmpSd), weight of one seed (SdW), kernel weight of one seed (KW), weight of filled seeds per cone (SdW/Cone), kernel weight per cone (KW/Cone). Correlations significant at $p \leq 0.05$ marked bold. Sample size is 21 for FS, 32 for PS.

that the breeding potential for seed quality is the same for both sites.

The SdW and KW in the cone is the most important trait for breeding. In PS, the average SdW was 30 mg more than that of FS, and KW was 9 mg more. However, the SdW/Cone and KW/Cone in FS was greater than that in PS since there were more seeds in the cone in FS. Approximately the same percentages of trees in FS and in PS had a SdW/Cone of more than 20 g; therefore, the possibilities for selecting the best trees for this trait at both sites were the same.

An analysis of the correlations between traits characterizing the mass of seeds and traits characterizing the structure of cones (Table 5) showed that SdW and KW were more independent of cone structure, but when for counts measured per cone, the correlation was significant. This was due to the close relationship between various traits of the cones and seeds, as shown by the example of various pine species (Ying et al., 1985; Reynolds and El-Kassaby, 1990; El-Kassaby and Cook, 1994; Matziris, 1998; Sivacioglu and Ayan, 2008; Yuan et al., 2016). In previous studies, it was demonstrated that the larger the cone, the larger the seed mass (Wittwer et al., 1997; Calama and Montero, 2007; Boutheina et al., 2013). In this study, SdW at both sites was significantly correlated with CD but weakly related to CL. Presumably, if there are few DSd in a cone, for example, if most FSc have not two developed seeds, except for one, then the weight of one full seed will increase, since one seed will have more opportunities for nutrition. Analysis of Table 5 did not confirm this presumption since, at both sites, the number of single-seeded FSc was weakly related to the weight of seeds. Similar presumptions were made about EmpSd. If the seed dies in the early stages of development and is empty, then the adjacent successful seed should receive more nutrition and increase in weight, which was confirmed in this study. The dependence of EmpSd on the SdW was significant. Unfilled and DefSd that died at later stages of development had little effect on the redistribution of nutrition in the cone. This is probably why the relationship between the

DefSd and SdW was weak. In general, both sites were characterized by a similar relationship between SdW and cone structure traits.

4.2. Variability of cone and seed traits

Many researchers consider the phenotypic coefficient of variation (CV) in seed production traits as an important tool for tree improvement programs. CV explain degree of difference in the selection materials and the range of variability and stability of traits in tree populations (Wittwer et al., 1997; Goroshkevich, 2008; Sivacioglu and Ayan, 2010; Rawat and Bakshi, 2011; Yuan et al., 2016; Kaviriri et al., 2021). However, the level of variability of each trait is determined by its nature (Goroshkevich, 2008).

Our results show that in young PS trees, the level of variability in all traits of cones and seeds was slightly higher than that in FS, although these differences were not fundamental. Cone size was a relatively stable trait. In this study, a small CV, less than 10%, for CL and CD was observed within the range presented by other authors (Sivacioglu and Ayan, 2010; Kaviriri et al., 2021). Of the traits characterizing the structure of the cone, the most stable was the initial TNSc. Its coefficients of variation in this study (8–10%) were also consistent with the data presented by Sivacioglu and Ayan (2010) for *Pinus nigra* and Kaviriri et al. (2021) for *Pinus koraiensis* but less than those presented by Yuan et al. (2016) for *Pinus tabuliformis* (CV 21%).

If the other traits of the cone structure were arranged sequentially in a row nearing the final indicator, SdEf, then the level of their variability would naturally and significantly increase. Relatively simple traits, such as FSc and OSc, in this study had an average level of variability (CV 15%–20%). The variability became greater when analyzing TSd per cone and FilSd per cone (CV 20%–30%). They were similar to those obtained in other pine species (Sivacioglu and Ayan, 2008, 2010; Yuan et al., 2016; Kaviriri et al., 2021). The highest level of variability in the resulting trait was SdEf (CV 28% for FS and 32% for PS), which is also highly variable in other pine species (Sivacioglu and Ayan, 2008, 2010; Yuan et al., 2016).

4.3. Seed producing index

The quality of the cones and seeds were analyzed in detail, and at both sites, trees could be selected with very large cones or with very large seeds. Presumably, such genotypes can be used as additional selection objectives for specific uses in small breeding projects aimed at the production of souvenir products, for example, very large cones or seeds. However, the traditional product, which is widely demanded in the world market, is vacuum packed seed kernels or nuts. Considering that the land under the plantation where seeds are harvested is also a valuable resource, it is advisable to calculate the yield based on the area occupied by the crown of the tree. Consequently, for the selection of Siberian stone pine, it is relevant to search for trees combining a narrow crown with a large total mass of kernels.

The previously discussed traits had different levels of variability. The more variable a trait is, the more complex its genotypic component; this is considered a foundation for elite clone selection (Yuan et al., 2016; Kaviriri et al., 2021). Direct signs of cone morphometry (cone size and number of scales) are more stable and therefore less predictable for plus-tree selection (Sivacioglu and Ayan, 2008; Yuan et al., 2016; Loewe-Muñoz et al., 2019; Kaviriri et al., 2021). The calculated quality traits, for example, the number of sound seeds per cone and seed efficiency, have a higher level of variability; therefore, selection is carried out according to these traits (Wittwer et al., 1997). However, most researchers agree that the most variable trait is the number of cones per tree (El-Kassaby and Cook, 1994; Li et al., 1996; Burczyk and Chalupka, 1997; Kang and Lindgren, 1999; Sivacioglu et al., 2009; Yuan et al., 2014, 2016), as this trait is under strong genetic control and therefore is used as a reliable selection criterion (Kaviriri et al., 2021). Different seed producing indices can be calculated based on the number of cones in the crown. For example, Yuan et al. (2016) suggested using relative female strobili, and Carrasquinho et al. (2010) considered the weight of the harvested cones per square meter of crown area as the best criterion for selection.

In this study, we intended to evaluate trees not only by crop weight but also by considering crown area; therefore, the KW per 1 m^2 of crown cover (KW/Crown) was used as a seed producing index. The high correlation between the number of cones and the crown area in dense FS made it difficult to find trees that combined a narrow crown with a large mass of kernels. In contrast, in PS, the number of cones and the total KW were very weakly related to the crown area; thus, at this site, the search for high-yielding trees with a narrow crown gave the best results.

5. Conclusion

Analysis of 20 traits characterizing the quality of cones and seeds showed insignificant differences between two sites, namely a young sparse plantation and a mature closed natural forest, that were used for plus-tree selection. The absence of significant differences in seed losses and SdEf suggests that the breeding potential for seed quality was the same for both sites. Since the quality of cones and seeds was about the same in both sites, trees with very large cones or very large seeds can be used as additional selection objectives for specific uses in small breeding projects aimed at the production of souvenir products, for example, very large cones or seeds. However, the traditional product, which is widely demanded in

the world market, is vacuum packed seed kernels or nuts. Considering that the land under the plantation where seeds are harvested is also a valuable resource, it is advisable to calculate the yield based on the area occupied by the crown of the tree. Consequently, for the selection of Siberian stone pine, it is relevant to search for trees combining a narrow crown with a large total mass of kernels. Therefore, as a final selection trait, the index of kernel weight per 1 m^2 of crown area was proposed. Based on this index, it was possible to implement initial breeding of trees that combine abundant cone-bearing with a small narrow crown, thus providing a greater yield per unit plantation area. Since in closed stands, the number of cones per tree was strongly correlated with crown size, this index should only be used for sparse forests or plantations. The results of this study can be included in the *Pinus sibirica* improvement program for pine nut production.

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Conflict of interest
The authors declare that they have no conflict of interest.

Contribution of authors
S. Velisevich: study conception and design, drafting of manuscript, acquisition of data and interpretation of data analysis. A. Popov: acquisition of data and interpretation of data analysis.

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