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Article

Performance of Scots Pine Seedlings from Seeds Graded by Colour

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Abstract: *Research Highlights:* One of the ways to improve the quality of a seedlot used in the forest nursery is the grading of seed by colour. *Background and Objectives:* The study is intended for forest's engineers and owners because it offers an alternative solution for forest seeds improvement before sowing. The success of forest establishment program mainly depends on the quality of Forest Reproductive Material. At this time usual practices during the seed processing is seed grading on size. This causes a lot of controversy about the possible reduction of genetic diversity through directional selection. *Materials and Methods:* Aiming to study the effect of seed coat colour on seedling performance, a one-year old container seedlings of *Pinus sylvestris* L. were planted at the post-fire site. Seedlings were produced from three fractions, previously graded in the visible wavelength range on a standard optical separator, plus control obtained without separation by colour. *Results:* Seedlings from different seed fractions performed differently in the first growing season after planting on the field. Seedlings from light seed fraction grow better in height, but those from dark seed fraction resulted with the highest survival rate. Light-dark seeds, which constitute the largest group in the initial sample by absolute weight, resulted with seedlings of the lowest growth rates and survival. The good results showed by seedlings from the control, for both growth rates and survival, indicate the weak effect of seed colour grading on seedlings field performance, but also the need for the more comprehensive studies in the future.

Keywords: reforestation; *Pinus sylvestris* L.; seed grading; seed coat colour; seedlings growth

1. Introduction

The success of forest establishment program, including afforestation, reforestation, and forest restoration [1,2] mainly depends on the quality of Forest Reproductive Material (FRM), i.e., seedlings [3,4] and seeds [5]. FRM is often subjected to transfer and trade [6,7], emphasizing the genetic component of FRM quality regarding the seed source [8] and genetic diversity of seed lot used for production of seedlings in nursery or for direct seeding on the field [9]. One of the usual practices during the seed processing, mainly aiming to provide the seedlot uniform on size for mechanized sowing, is seed grading on size [10–13] which raise the concern about reduction of genetic diversity by directional selection as reviewed by Ivetić et al. 2016 [14]. As an alternative or as an addition to grading seeds on size, grading of seed on colour can be used in seed processing, aiming in quality improvement for nursery sowing and direct seedling on the field, both ground-based [5] and aerial seeding [15].

The seed coat colour is under genetic control, and can be used as a phenotypic marker [16–20]. Some results show that darker seeds are more resistant to infection by damping-off fungi [17]. There are reports about no significant economic benefit [21] of grading of *Pinus sylvestris* L. seed on colour and the effect can be decreased by pale full seeds mimicking the empty seeds [22], but more studies reports evidence of possible use of the seed coat colour and spectrometric effect [23–28] as an indicator of seed germination and following seedlings growth. Yet, the results for seed germination are not consistent. For example, dark seed performed better in terms of mass, viability and germination compared to light seed [29,30], but opposite is reported for germination rate [21,31]. However, evidence shows that seedlings from the light seed overgrown those from dark seed [12,32].

This applicability of seed grading on colour resulted with design of different devices [33,34] for seed grading on a photonics [35], electronics [36] and mechanics [37] principles.

Aiming to improve the knowledge on applicability and effectiveness of seed grading based on a qualitative attribute, we tested the effect of the seed coat colour on height growth of one-year old container-grown seedlings colour, after the first growing season following the outplanting on a wild-fire disturbed site.

2. Materials and Methods

2.1. Seed Coat Colour Grading

The seedlings were produced from *Pinus sylvestris* L. seeds graded on the seed coat colour by a standard optical separator (LLC Smart Grade, Russia). The seed for this study was collected in a natural stand in Pavlovsky district of Voronezh region, Russian Federation (N 50.462169; E 40.096446; 83 m a.s.l.). According to the standard seed-processing protocol [38] seeds were de-winged in a drum-type wet de-winger (Dewinger 800—BCC AB, Sweden), and then dried in a chamber (DL1200—BCC AB, Sweden) on a moisture level of 7.5%. Empty seeds were eliminated by gravity method [39] using Gravity separator (Mini-Series—BCC AB, Sweden). From the resulting batch of seeds with a total mass of 1600 g, first the control fraction of 88 g was taken, and the rest of the seeds were separated by optical separator of conventional design (VIS-spectroscopy) in three seed-colour classes: 1—Light, 2—Light dark, and 3—Dark, with a mass of 376 g, 1002 g, and 134 g, respectively (Table 1 and Figure 1). Then, applying the visual (organoleptic) method [40], each of these three classes were described to have yellowish-white (Lutescens), ochre (Ochraceus), and dark-brown (Brunneus) seed coat colours [41], and finally, each class was tested by digital colour systems of Munsell and CMYKOG. Zone of possible separation of Scots pine seeds (Table 1) of the specified origin lay in the optical VIS of reflected wavelengths corresponding to the red colour [25]. After separation, seeds of all three fraction plus control were sown using an automatic precision Seeder (BCC AB, Sweden) in containers type Hiko V-120 SideSlit (BCC AB, Sweden). Seedlings were nursed from May to October of year 2017, in the Voronezh Forest Selection and Seed Center (VFSSC; currently—the Novousmansky branch of the Voronezh forest fire centre, Russia, the coordinates of the nodal point N 51.566944, E 39.243056). On 24 October 2017, the produced one-year old seedlings were removed from the container cells and outplanted on the experimental site.

Table 1. Colour classes and sample optical features of *Pinus sylvestris* L. seed used in the study.

Seed Colour Class (VIS-spectroscopy) [25]			**Organoleptic Method** [40]	**Munsell (CMYKOG) Colour System**	**Seed Mass in g (total of 1600 g)**
Name	**VIS Wavelenth Range, nm**	**Degree of Reflection, %**			
1—Light	650–715	70–85	Lutescens	4.9 Y 7.5/4.2 (C0, M0, Y35, K26, Or10, G0)	376
2—Light-dark	650–715	50–65	Ochraceus	9.8YR 6.0/4.1 (C0, M0, Y14, K40, Or36, G6)	1002
3—Dark	650–715	35–45	Brunneus	7.3YR 2.6/1.7 (C63, M70, Y85, K54,	134

				Or0, G0)	
Control	bulk	bulk	bulk	bulk	88

Figure 1. Characteristic type of *Pinus sylvestris* L. seeds in light (center), light dark (on the left), and dark (on the right) colour fractions used in the study. The photo was taken with a digital USB-microscope on a coordinate grid, between adjacent grid lines 0.1 mm.

2.2. Experimental Site, Experimental Design, and Planting Method

The seedlings produced from a different colour-seed fractions of Scots pine seed were established in 24 October 2017 on the territory of the 27th district of the left-Bank forestry of the Educational and Experimental Forestry Center (EEFC) of the Voronezh State University of Forestry and Technologies named after G.F. Morozov (VSUFT). Coordinates of nodal point N 51.827861, E 39.363806, 100.8 m a.s.l., at total area of 405 m^2 [12]. The planting site represents a pyrogenic disturbed area after the wildfire in 2010.

At a distance of 1.0 m with the help of a plow aggregated with a tractor in the sandy soil parallel furrows were cut to a depth of about 20 cm. then four adjacent furrows were selected, and 90 seedlings were planted in them. As weeds appeared, mechanized agrotechnical measures were carried out.

Seedlings were planted on a previously furrowed soil (without uprooting of the remaining stumps), in the bottom of the furrow under the Kolesov sword, on distance of 0.7 m (between seedlings in one row) × 1 m (between rows).

2.3. Meteorological Conditions during the Study

The average temperature for the growth season of 2018 was near normal values. The climatic summer was dry with precipitation values away bellow the normal for the region (Table 2).

Table 2. Meteorological data for the growing season 2018 in Voronezh Region (51.650, 39.250) adapted in http://pogoda-service.ru/climate_table.php.

Month	Average Temperature, °C (Mean±SE)	Temperature Normal Ratio, °C	Rainfall, mm	Rainfall Normal Ratio, mm
May 2018	15.18 ± 0.28	14.8	22	44
June 2018	18.22 ± 0.23	18.5	42	67
July 2018	20.45 ± 0.16	20.5	26	72
August 2018	19.69 ± 0.31	19.2	17	55

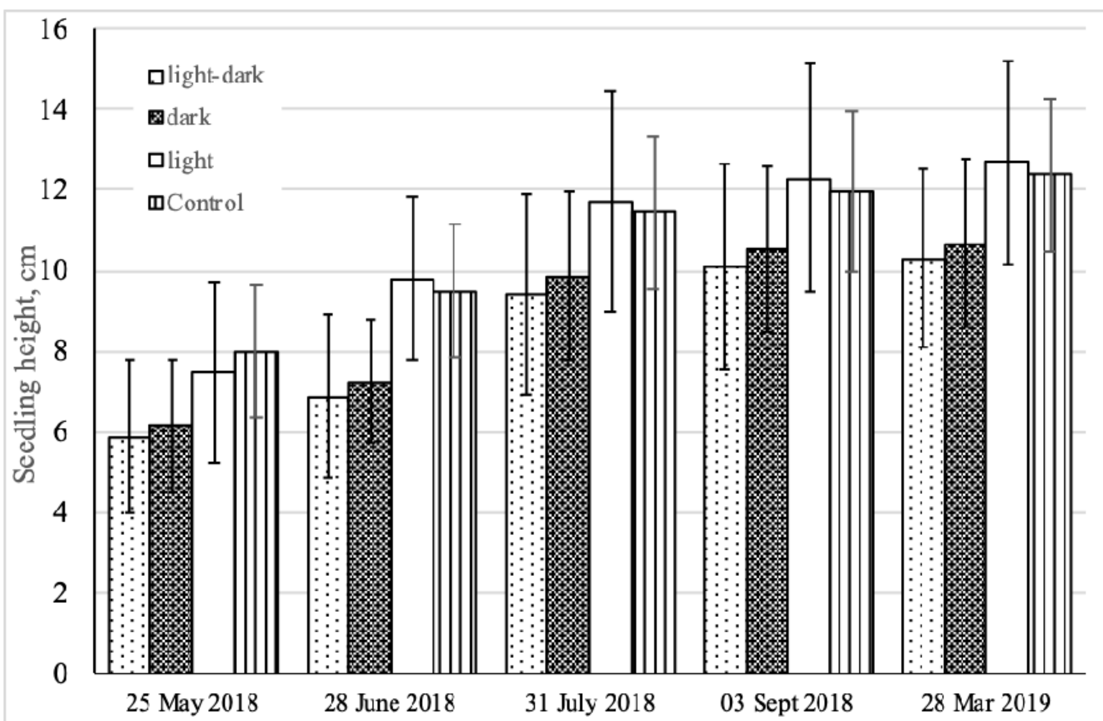
September 2018	13.33 ± 0.33	13.3	28	53

2.3. Data Collecting and Processing

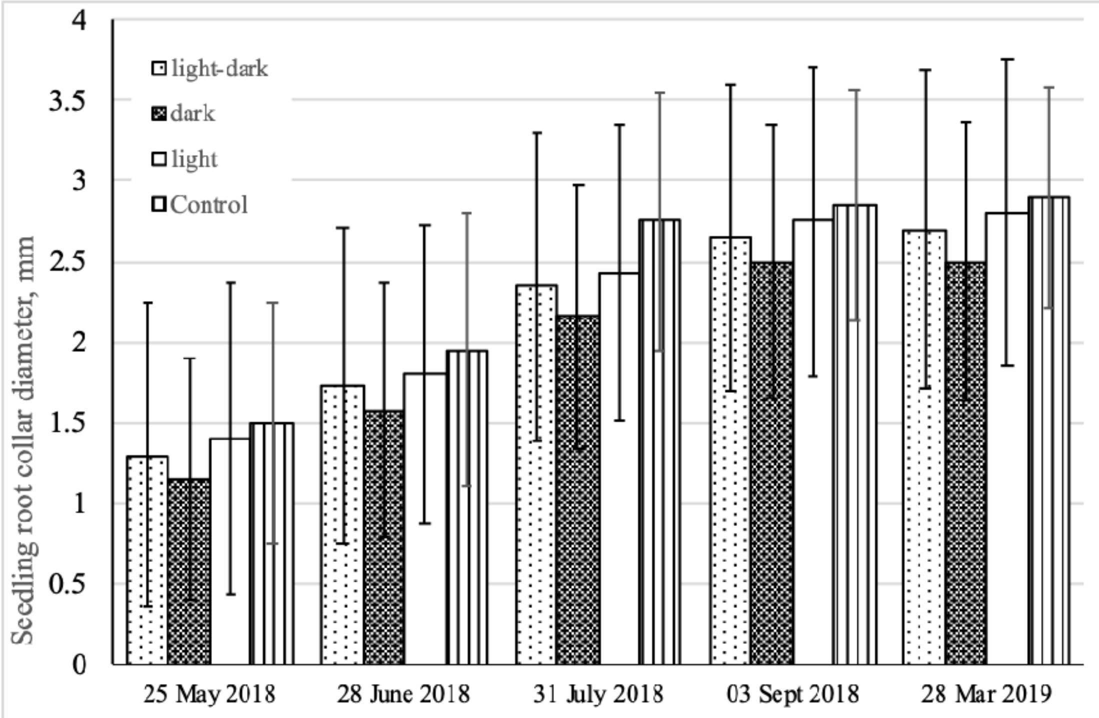
After outplanting at the field site, seedlings were monthly (from May to September, 2018, figure 2), measured for height (a distance between the root collar and the apical bud, cm), Root Collar Diameter (RCD, mm) a Despite the availability of alternative automated methods [42], height measured using the ruler, and RCD measured using the digital Vernier caliper.nd survival (%). Despite the availability of alternative automated methods [42], height measuring using the ruler, and RCD—using the digital Vernier calliper. The final measurement of survived seedlings (n = 271) for height and RCD was done on 28 March 2019—Table 3 and Figure 2.

Table 3. The growth of *Pinus sylvestris* L. seedlings produced from different seed colour classes in the first growing season after the outplanting at experimental site. Seedlings were measured at the end of the growing season (28 March 2019). Mean values followed by the different letter are statistically different used Tukey's HSD test (α = 0.01)

Characteristics	Seed Colour Classes	Root Collar Diameter, mm	Shapiro–Wilk's W	Height, cm	Shapiro–Wilk's W
The average values (Mean±SE). Mean values followed by the different letter are statistically different ($p < 0.05$).	**Control**	2.9 ± 0.08	W = 0.95085, p = 0.00598	$12.4^a \pm 0.22$	W = 0.98059, p = 0.31342
	1—Light	2.8 ± 0.12	W = 0.92449, p = 0.00051	$12.7^a \pm 0.31$	W = 0.95540, p = 0.01611
	2—Light-dark	2.7 ± 0.13	W = 0.89588, p = 0.00012	$10.3^b \pm 0.29$	W = 0.95251, p = 0.02376
	3—Dark	2.5 ± 0.1	W = 0.96631, p = 0.05341	$10.7^b \pm 0.25$	W = 0.95077, p = 0.01739
Survival, %	**Control**	78.2			
	1	79.3			
	2	72.3			
	3	83.5			



A



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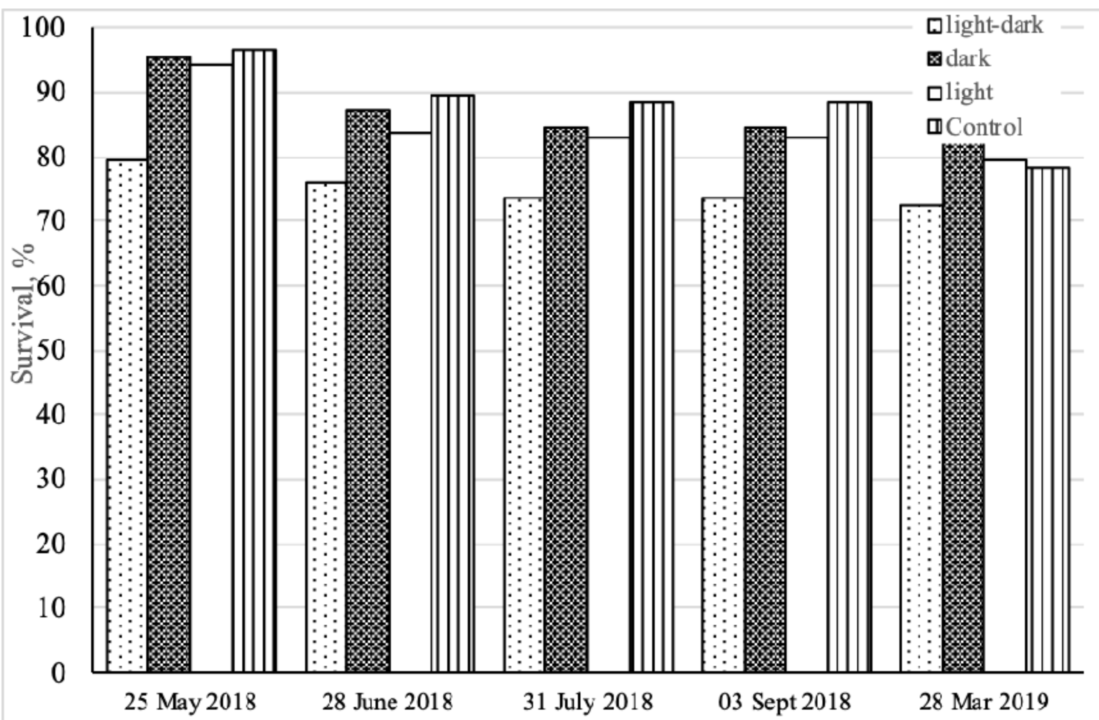

C

Figure 2. Dynamics in *Pinus sylvestris* L. seedlings height (**A**), RCD (**B**), and survival (**C**), as measured in the first juvenile stage.

The data collected by measurement of height and RCD on the final measurement were processed by use of the StatSoft Statistica Software (Version 7.0, Moscow, RU). After calculation of descriptive statistics (Mean ± SE), data were tested for the normality by the use of Shapiro–Wilk's test. The null hypothesis that the data are normally distributed was not rejected for the height given that $p > \alpha$ ($\alpha = 0.01$), but it was rejected for RCD given that $p < \alpha$ (except seedlings from dark seeds with $p = 0.05341$). Because of that, the One-Way ANOVA was performed only for the seedling's height (Table 4). Finally, the mean values were separated using Tukey's HSD test for unequal number of samples ($\alpha = 0.01$).

Table 4. Analysis of Variance (One-Way ANOVA) of one-year old container *Pinus sylvestris* L. seedlings height from different seed-colour classes.

Title	SS	*df*	MS	*F*	*p*
Height, cm	313.01	3	104.34	20.602	0.00000

3. Results

Seedlings produced from the light fraction showed the significantly ($p = 0.00000$) higher value for height (12.7 ± 0.3 cm), compared to seedlings produced from dark (10.7 ± 0.3 cm) and light-dark (10.3 ± 0.3 cm) fractions, but they were not significantly taller than seedlings produced from the control (12.4 ± 0.2 cm) (Table 3). In the first quarter of the growing season, seedlings from control was the tallest, but from the middle of the growing season, seedlings produced from the light fraction were tallest and remain the tallest until the end of research (Figure 2A).

The ranking of seedlings from different seed colour fractions was opposite for RCD. Seedlings from the control was the thickest (2.9 ± 0.08 mm), followed by these from light fraction (2.8 ± 0.12 mm), from light dark fraction (2.7 ± 0.13 mm), and finally by those produced from the dark fraction (2.5 ± 0.1 mm). In the first quarter of the growing season, seedlings from the control had the largest RCD and retained it until the end of research (Figure 2B).

Seedlings produced from the dark fraction were the highest survival rate (83.5%), compared to seedlings produced from light (79.3%) and light-dark (72.3%) fractions. At the same time, seedlings from the control had a survival rate of 78.2% (Table 3). In the first quarter of the growing season, seedlings from the control had the highest survival rate, but by the end of research, they exceeded the survival of the dark and light fractions, respectively (Figure 2C).

4. Discussion

The seed coat is the seed's primary defense against adverse environmental conditions [43]. *Pinus sylvestris* L. is a species known for a large natural variation in seed color, ranging from pale yellow to black [44]. Scots pine seed colour varies from tree to tree and becomes darker for each subsequent harvest date [45]. The same study shows that seed colour is not a reliable measure of seed maturity. It seems that seed colour depends on tree age. Although the seed coat colour is under genetic control, and can be used as a phenotypic marker [16–20], Mukassabi et al. [29] noticed that younger trees produced more dark winged seeds than older trees. The uncertainty about level of genetic control leave the open question of effect of seed grading on colour on genetic structure of seedlot.

Aiming to assess the effect of seed coat color to seedlings quality, we have measured seedlings height and RCD as the most widely used attributes measured in seedling quality assessment [13,46]. Stem diameter is a seedling attribute that forecasts both survival and growth, and is considered the single most useful morphological attribute to measure [46,47], and was reported as better (compared to shoot height) measure of seedling quality[48,49]. Yet, seedlings height is easier to measure, and it is widely used in operational forestry. While RCD is positively and significantly correlated with both survival and growth after seedlings planting at the field, seedlings height can have both, positive and negative effect on seedlings field survival and growth, as reviewed by Ivetić and Devetaković [50]. Their combination as the HD (height/RCD) ratio was the most reliable plant attribute to forecast Austrian pine seedlings field performance [4] and there is evidence that initial seedling height and RCD are equally related to the field performance [3]. Our results show a consistent effect of seed colour on seedlings field growth of height and RCD. Seedlings from the light fraction and control reached the highest mean values of height and RCD, but the difference between mean values of RCD from other two fractions (light dark and dark) was not significant, indicating the weak effect of seed grading on colour. Although there are contradictory results reported for some attributes of seed quality, including germination (see Introduction section) our results are consistent with previous reports that seedlings from the light seed overgrown those from dark seed [12,32]. Considering uncertainty on nature of control of seeds coat colour (genetic and/or age), grading of seed on colour should be defined differently for different seedlots (i.e., provenances and seed tree age).

Results for survival were not consistent during the first growing season on the post-fire site, ending with the higher survival percent of seedlings from dark fraction. The reasons for these results are unclear and require further investigation.

Despite the relatively weak effect of seed colour on growth and survival of one-year old *Pinus sylvestris* L. seedlings outplanted at the post-fire field, there are indications that this method of seed grading can be improved [51,52]. Up to date, seed grading on colour was studied by organoleptic method [21,40] and by use of machine vision in Munsell and CMYKOG systems [53,54]. Currently, the colour classification of seeds is usually based on the assessment of the predominant pigment colour. The accuracy of this approach depends on the individual characteristics of the researcher's visual perception [55], which can significantly reduce its effectiveness. Machine vision eliminates this deficiency and in combination with pneumatic injector, separation is widely used in agriculture and food industry when sorting biological objects by colour. Technological schemes of a compact, simple, fast separator that is able to conduct a high-quality analysis of the spectrometric characteristics of seeds by implementing of the microprocessor analysis algorithms have already been created and patented by the authors (RU Patent 2,675,056 [33,48], RU Patent 2,682,854 [34], and RU Patent 2,687,509 [56]). For the better understanding of interactions between seedling growth and spectrometric and morphometric parameters of the seed, additional studies are planned in the future. One direction of further research will be to follow the path of each seed fraction from seed processing to the seedlings field growth and performance, by integrating all parameters of FRM into the *FRMLibrary* database, a relational model of which is already under development [52].

5. Conclusions

The results of this study show that:

- *Pinus sylvestris* L. seedlings produced from light seed fraction grow better in height, compared to other colour fractions, with an average survival rate;
- Light-dark seeds, which constitute the largest group in the initial sample by absolute weight, results with *Pinus sylvestris* L. seedlings of the lowest growth rates and survival;
- Dark seed fraction, which had the lowest partition in the initial sample, resulted with seedlings of the highest survival rate.
- The good results showed by seedlings from the control, for both growth rates and survival, indicate the weak effect of seed colour grading on seedlings field performance.
- For the present dataset, the conclusion points at a potentially strengthened hypothesis for a future study rather being conclusive in itself.

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