The effect of seed size grading on seed use efficiency and height of one-year-old container-grown Scots pine (*Pinus sylvestris* L.) seedlings

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Abstract

The seed of *Pinus sylvestris* L. originating from natural forest in Voronezh region, Russian Federation, was graded in four classes based on seed diameter size, using the operational equipment of screens with round openings. The ungraded seedlot was used as control. Seedlings were produced in Voronezh containerized forest nursery, from March to September 2015, following the standard procedure for *Pinus sylvestris*. Seed from each size class, plus control, is sown in containers with 120 cm^3 cells, filled with peat-substrate, grown in greenhouse for eight weeks, followed by hardening in growing area until the end of the growing season. The results shows a week positive effect of seed grading on height of one-year old container-grown seedlings, but significant improvement of seed use efficiency by reducing the number of empty cells. Based on results of this study, we can recommend grading of *Pinus sylvestris* seed on two size classes.

Keywords

Seed grading; Seed size; Seedling quality; Container-grown seedlings

Contents

1 Introduction

Improvement of forest restoration, reforestation, and afforestation success is an imperative facing the climate change and increased frequency and intensity of natural disturbances, including strong winds, heat- and drought-induced physiological stress, forest fires, pests and diseases outbreaks. The quality of forest reproductive material (FRM) has a key role in achieving this success. This role is recognized in Strategy of Forest Complex Development until 2030 (Anonymous 2018), launched recently by the Russian Federation (September 20, 2018), which emphasize the significance of FRM production and quality.

Pinus sylvestris L. is the most spread pine species in Europe, with high importance in forestry, for pulp industry and sawn timber products. In Russian Federation, Pinus sylvestris is one of the most important forest-forming tree species (after larch), with constant increase of area, from 113.5 million of hectares in 1988 to 122.2 million of hectares in 2010, accounting for 15.6%, of forested land area (FAO 2012).

While the provenancing and seed collection strategy are the most important management practices in maintaining the genetic diversity (Ivetić and Devetaković 2017), the first step in improving of tree seed physical quality is grading on size. The obvious practical benefit of seed grading on size, is achieving the size uniformity for applying the mechanical sowing. Further, grading on size provide more uniform seed germination, followed by the more uniform seedling density in the seedbed. There are evidences that seed size of *Pinus* species is positively correlated to the germination, seedlings survival, and growth (Burgar 1964, Dunlap and Barnett 1983, Belcher et al. 1984) mainly through providing uniform speed of germination (Barnett 2008); although the long-term effect is disputed (Mikola 1980; Sluder 1991; Castro 1999). There are also an opposite reports on week or lack of effect of seed size on seedling development of Pine species, calling into question the justification of seed grading (Dumroese and Wenny 1987; Sluder 1991; Bladé and Vallejo 2008). In addition, there is a concern that directional selection by seed grading can change the genetic constitution of the whole seedlot (Ivetić et al. 2016).

Despite controversy and conflicting reports on effect of seed size grading, this is still a standard procedure in tree seed centers', of high practical significance especially in container nurseries equipped with mechanical sowing devices. *Pinus sylvestris* seed grading on size (e.g. in Russian Federation - Novikov 2000, 2001, 2002, 2008, 2017; Sviridov et al. 2001; Gomzyakov and Novikov 2008; Gomzyakov et al. 2010; Drapalyuk and Novikov 2018; Novikov and Novikova 2018; and in Serbia – Ivetić 2013) is done by screens with openings of different size, usually separating three to four size classes, plus impurities which are larger than openings at the top, or smaller than openings at the lowest screen; followed by gravity separation for removing of empty and undeveloped seeds, and remaining impurities, and finally by pressure-vacuum separation for removing of damaged seeds. Although existing methods for seed grading are efficient at operational level, having on mind the need for enormous quantity of FRM worldwide (Haase and Davis 2017), there is a constant need for innovative approaches (e.g. Near Infrared Spectroscopy - Farhadi et al. 2015) and development and design of new devices based on biophotonic effect (Drapalyuk and Novikov 2018).

In this study, we have tried to answer the following questions, related to one-year old container-grown *Pinus sylvestris* seedlings: 1) Is there a relationship between seed size and seedlings height? 2) Is there a relationship between seedling/container position and seedlings height? 3) Does seed grading on size improve seed use efficiency?

2 Material and Methods

2.1 Seed grading

Cones of *Pinus sylvestris* are collected from selected seed trees in natural forest, in September 2014. This seed source is located in the Pavlovsky district of the Voronezh region, Russian Federation (Latitude 50.462169; Longitude 40.096446; Altitude 83 m asl). Seed was extracted from cones and further processed (pre-cleaning, extraction, de-winging) using the standard procedures and equipment (BCC AB, Landskrona, Sweden) in Voronezh Forest Selection and Seed Center.

From the original seedlot, four random samples of 0.5 kg were extracted: one was left as the control and the remaining three were graded on size, i.e. seed diameter (Table 1) by Cleaner & Seed Sizer (Mini-Series - BCC AB), using the screens with round openings. In the course of work, some seeds stuck in sieves were observed, which leads to a decrease in the efficiency of using the device.

Table 1. Size classes and sample size of *Pinus sylvestris* seed used in the study

Seed size group	Seed size (mm)	Sample seed quantity
1 - Extra Small	ES ≤ 2.25	3 × 40 (120)
2 - Small	2.25< S ≤ 2.75	3 × 40 (120)
3 - Medium	2.75< M ≤ 3.25	3 × 40 (120)
4 - Large	L > 3.25	3 × 40 (120)
Control	bulk	4 × 40 (160)

2.2 Seedlings production

Seedlings for this study were produced in Voronezh containerized forest nursery, from March to September 2015, following the standard procedure (Landis et al. 1998) for *Pinus sylvestris*: mechanized peat-substrate filling and seed sowing in containers (Hiko V-120 SS – 120 cm^3), followed by growing in greenhouse for eight weeks, with automatic maintenance of humidity and temperature, and hardening in growing area with automatic irrigation until the end of the growing season. From each sees size class, a total of 120 (3 container trays X 40 cells) seeds were sown, plus 160 (4 container trays X 40 cells) seeds from the control group, resulting wit total of 640 sown cells.

In order to investigate relationship between seedling/container position and seedlings height, containers sown with seeds of different size classes (plus control) are kept together, and the same arrangement with position of each container relative to the sides of the world is maintained in the greenhouse and at the growing area (Figure 1).

Height of all established seedlings was measured at the end of growing season, from root collar diameter to the top of the seedling.

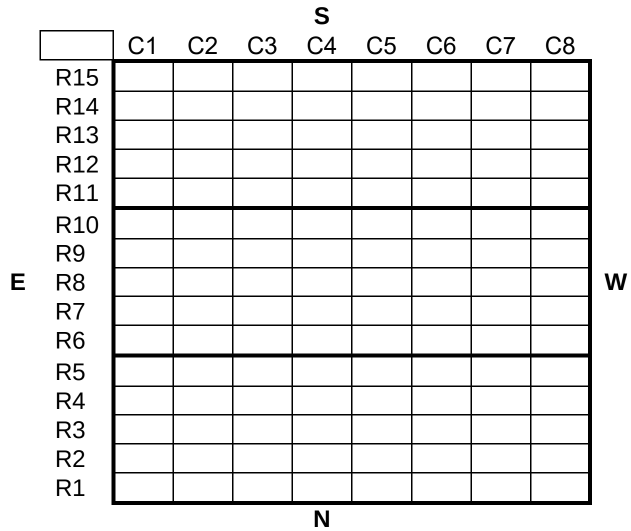


Figure 1. The arrangement with position of each container relative to other containers and to the sides of the world: S – South, N – North, E – East, W – West, C – column, R – row).

2.3 Statistical Analysis

The one-way ANOVA was used to test differences between mean values of seedlings height from different seed size classes. Descriptive statistics included number of samples, mean value, standard deviation, variance, minimum value, and maximum value. Mean values were separated using Tukey's HSD test for unequal number of samples, with a significance level of alpha = 0.05. In order to visualize height of seedlings, as well as number of empty cells, relative to their position, the colored matrices with height values are produced.

3 Results

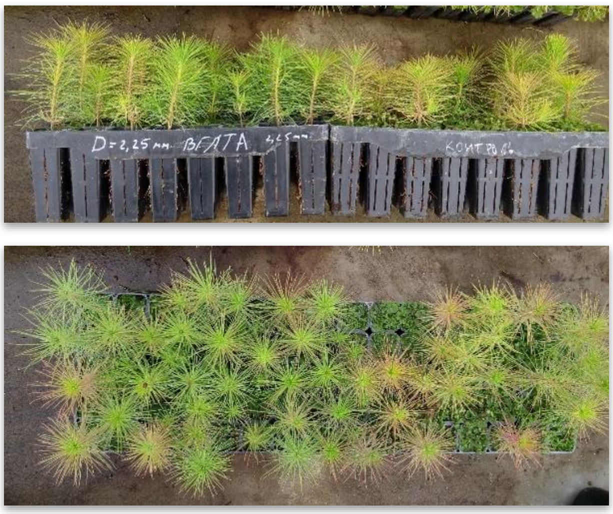
The seed size have significant effect on one-year old *Pinus sylvestris* seedlings height (Table 2, Table 3, and Figure 2). However, this effect is not necessarily positive. The highest mean value of height (116,3 mm) is recorded for seedlings from large seed size class (>3.25 mm), but the lowest mean value of height (98,8 mm) is recorded for seedlings from small seed size class (2.25- 2.75 mm) – much lower compared to (108,6 mm) seedlings from extra small class (<2.25 mm). The mean value of seedlings height from control group is above the average for all measured seedlings.

Table 2. Descriptive statistics (N=470) of one-year old container *Pinus sylvestris* seedlings height from different seed-size classes: N – number of seedlings, SD – standard deviation, Var – variance, min – minimum value, max – maximum value). Mean values followed by the different letter are statistically different (p<0.05).

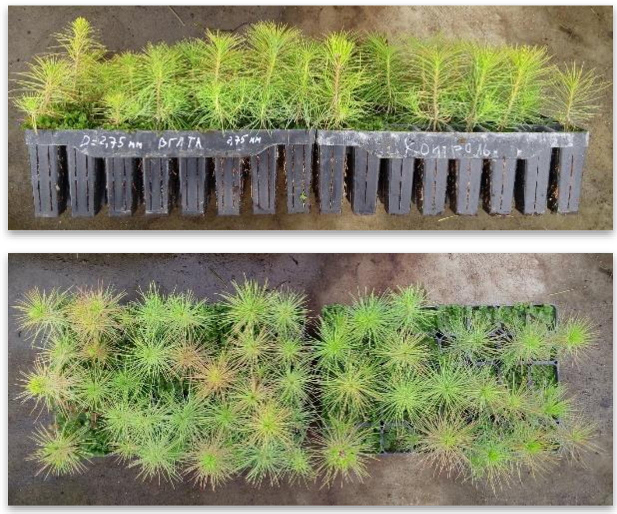
Size Class	HT (mm)	N	SD	Var	Min	Max
Control	$113{,}2^{a}$	74	17,71	313,6	40	140
ES	$108{,}6^{a}$	94	23,12	534,4	33	151
S	$98{,}8^{b}$	90	23,19	538,0	20	142
M	$111{,}7^{a}$	106	24,31	590,9	36	160
L	$116{,}3^{a}$	106	22,49	506,0	60	183
All Groups	109,9	470	23,20	538,1	20	183

Table 3. Analysis of Variance (One-Way ANOVA) of one-year old container *Pinus sylvestris* seedlings height from different seed-size classes.

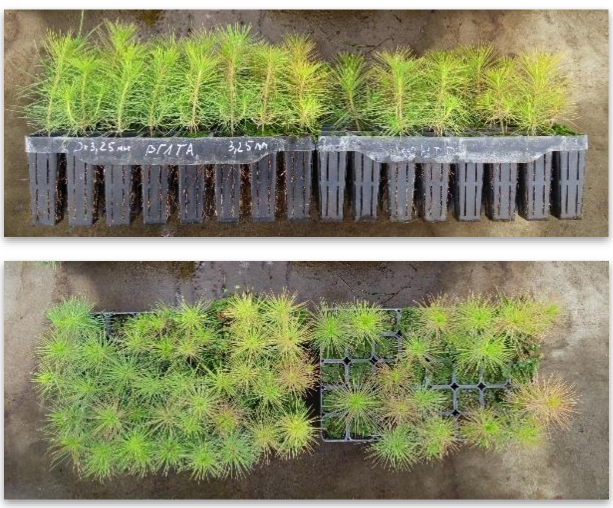
	SS	df	MS	SS	df	MS	F	p
HT (mm)	16734,89	4	4183,722	235645,2	465	506,7639	8,255762	0,000002



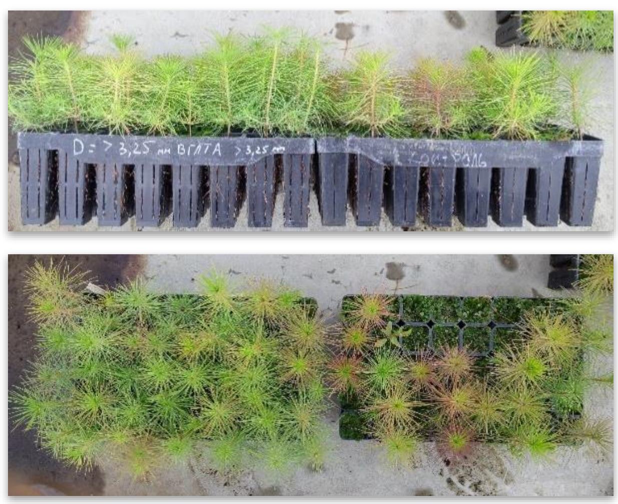
ES ≤ 2.25



2.25< S ≤ 2.75



2.75< M ≤ 3.25



L > 3.25

Figure 2. Containers with one-year old *Pinus sylvestris* seedlings from different seed size class (left container) and control (right container at each photo): upper left – extra small (≤ 2.25 mm), upper right – small (2.25- 2.75 mm), down left – medium (2.75- 3.25 mm), and down right – large (> 3.25 mm) seed class.

There is no obvious pattern in seedling height distribution in containers with seedlings from different size classes (Figure 3). However, it is obvious that control shows significantly more empty cells (53.75 %), compared to extra small, small, medium, and large class (21.67%, 25%, 11.67% and 11.67%, respectively).

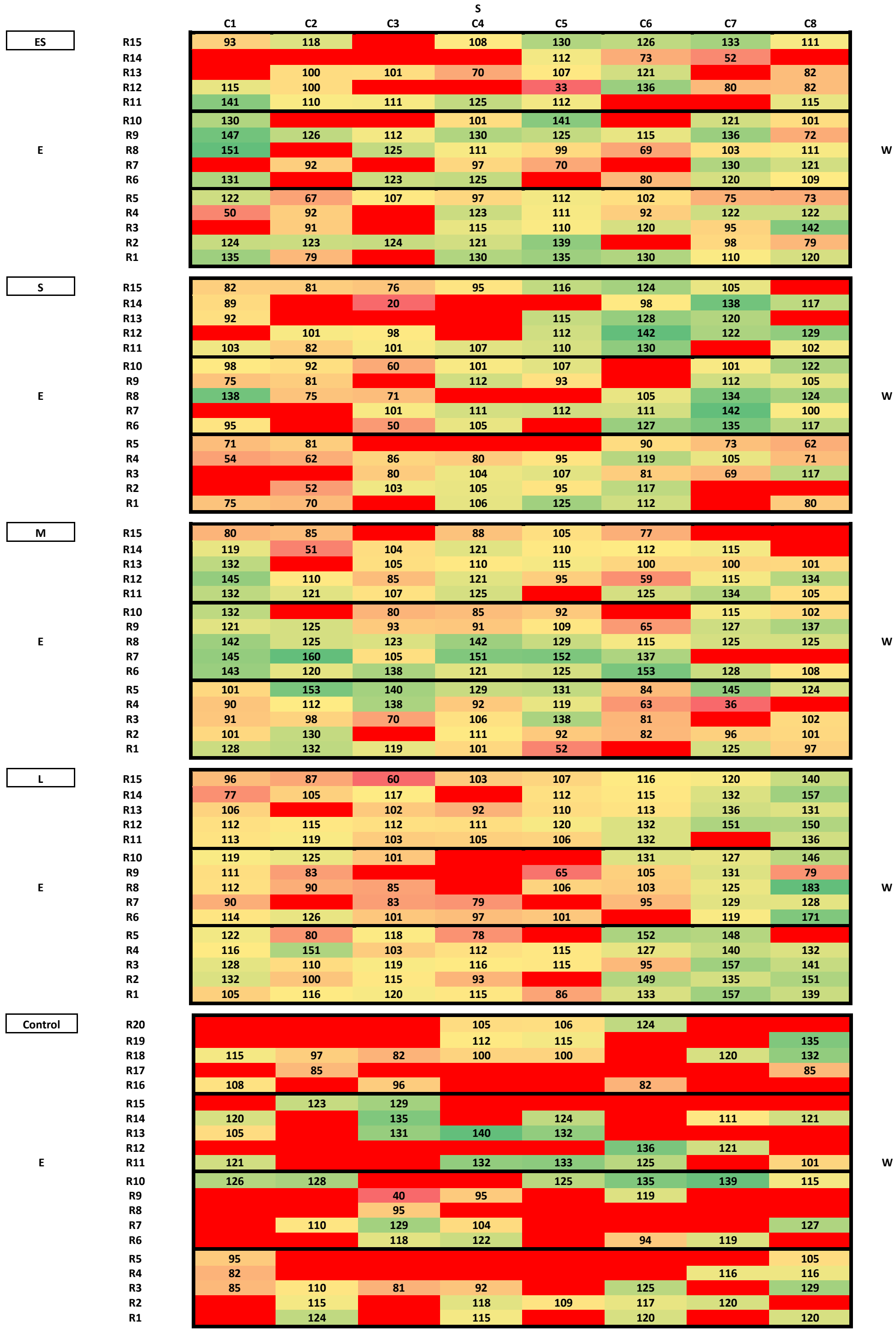


Figure 3. The matrix of one-year old *Pinus sylvestris* seedlings height (shades of red – lower than average, shades of yellow – around average, shades of green – above average) and empty cells (red color) distribution from different size classes.

4 Discussion

4.1 Is there a relationship between seed size and seedlings height?

There are evidences that seed size and mass have a positive effect on *Pinus sylvestris* seedling height (Mikola 1980; Reich 1994; Winsa and Bergsten 1994). Our results shows a similar effect, although seedlings from extra small seed size class are significantly taller than seedlings from small class. However, due to small differences in height of seedlings from different size classes, we cannot recommend grading of seed in more than two size classes. The boundary between these two classes should depend on seedlot provenance, since seed mass of *Pinus sylvestris* differs with latitude of stand origin (Reich et al. 1994). This week effect of seed size on seedlings height can be explained, to some extent, by favorable and uniform growing conditions in the nursery, as previously have been reported that seed mass effect on *Pinus sylvestris* seedling height is stronger under harsh (i.e. direct seeding in the field) compared to optimal (i.e. sowing in nursery) conditions (Wennström et al. 2002). The mean value of seedlings height from the control group is above average, overtopping all seed size classes except seedlings from the large class. So, from aspect of seedlings height, and similar to findings of Dumroese and Wenny (1987) for *Pinus monticola* Dougl. ex D. Don and Bladé and Vallejo (2008) for *Pinus halepensis* Mill., small improvement in seedling height obtained from seed grading do not seems to justify the increased operational costs.

4.2 Is there a relationship between seedling/container position and seedlings height?

There is no obvious pattern of *Pinus sylvestris* seedlings height or of empty cells related to their position. This can be explained by uniformity of growing conditions achieved in both nursery greenhouse and growing area. Here it should be emphasized that this was a small scale experiment (13 container trays) which enable the easy achievement of optimal and uniform growing conditions. On large scale production, it can be expected that seedlings and container cell position are affecting both, seedlings height and number of empty cells at the end of the first growing season.

4.3 Does seed grading on size improve seed use efficiency?

From operational aspect, seed grading seems to be fully justified. More than half cells sown with seed from control (ungraded) seedlot ended empty after the first growing season. In the same time, the percent of empty cells sown with graded seed ranged from 11.67% (medium and large size class) to 25% (small size class). The reasons from this can be numerous, including: cleaning by removal of empty and damaged seeds and other impurities; efficiency of sowing machine, which depends to some extent on seed size uniformity; and improved seed germination parameters (rate, speed, uniformity). Seed grading improved the overall seed quality, which can result in higher survival and growth rates, as it is previously shown for *Pinus sylvestris* (Winsa and Bergsten 1994). In our study, seed grading on size did improved the *Pinus sylvestris* seed use efficiency, i.e. the seedling-to-seed ratio (Belcher et al. 1984).

5 Conclusions

The grading on size of *Pinus sylvestris* seed had a week positive effect on one-year old container-grown seedlings. However, the grading on seed size significantly improved the seed use efficiency by reducing the number of empty cells after the first growing season. Based on results of this study, we can recommend grading of *Pinus sylvestris* seed on two size classes.

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