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The influence of organic plant material on seed germination and development of Scots pine *Pinus sylvestris* L. seedlings

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Abstract. In this article we analysed the influence of plant-based organic admixtures on the germination process of seeds and the early development of Scots pine Pinus sylvestris L. seedlings. The intensity of dumpin-off diseases within the culture was recorded after applying each of the admixtures. Organic material of nettle Urtica dioica L., softwood litter, hardwood litter and peat were applied to the nursery substrate in two ways, either as an admixture in crushed form or in granulated form. None of the introduced admixtures influenced the germination of seeds or the survival rate of pine seedlings positively. The best results were obtained with a substrate without admixtures used as a control, which is the most common nursery substrate. The worst seed germination rate was observed on the substrate enriched with the organic material from nettles. In pots with granulated organic material from hardwood litter, significantly more seedlings showed signs of post-emergence dermatitis. In all other cases, there was no clear difference between the crushed or granulated admixture in either germination or survival of seedlings. We furthermore demonstrated that the process of granulating plant material leads to an approximately 10-fold increase in the bulk density of the granulated substance, which translates directly into volume reduction.

Keywords: nursery grounds, soil enrichment, natural fertilizers, seed germination, seed damping-off

1. Introduction

Under the conditions of forest nursery, germination of seeds and development of seedling root systems depend on the substrate that has the capability to store water and mineral nutrients supplied by fertilizer treatments. The substrates are usually mixed with mycorrhizal fungi, whose presence improves the growth of seedlings in container cultivation, and also facilitates their adaptation to the growth conditions (Szabla 2009; Buraczyk et al. 2012).

Forest litter mainly consists of shed vegetative parts and other non-living forest materials including animal droppings. It plays an important role in forest stand productivity as litter production and decomposition are functions of the site (Molenda, Żabko-Popowicz 1980). Over time, the soil is enriched by organic compounds released due to complex decomposition processes going on in the forest floor. Litter organic matter is a crucial component in nutrient transfer through the biogeochemical cycle in forest ecosystems (Dziadowiec 1990). Microorganisms decompose organic matter, a fraction of which is transformed into humus that integrates into the soil as a protective layer (Sayer 2006).

Frequent and repetitive agro-technical operations in forest nurseries can lead to humus losses, followed by disturbances in seedling growth. Additionally, in long-term forest nurseries, there was observed weakened activity of ectomycorrhizal fungi (Aleksandrowicz-Trzcińska 2004). Hilszczańska (2000) stated that substrates mixed with forest litter were more and more applied in the Polish forest nurseries to improve the survival rates of seedlings after planting. As it is very difficult to accumulate forest litter with no harm to the environment, Klimek et al. (2011) propose biomass collection during large-scale deforestation activities, for example, carried out during road developments. The study conducted by the authors of this study, in the Dobrzejowice Forest District, proved that the use of compost based on forest humus supported Scots pine seedling development. Above-ground parts of the seedlings

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treated with humus were almost half higher than those treated only with mineral fertilizer. In addition, there was observed a considerable increase in the number of saprophages in the soil (Klimek et al. 2011).

In forest nursery production, young seedlings are exposed to many hazards, resulting from, among others, abiotic factors, the presence of pathogens in the environment or mistakes in the conduct of treatments. Intensively exploited soil substrate often becomes a source of infection, as it contains numerous strains of pathogenic fungi. A special threat to the young generation of conifers grown in forest nurseries is posed by a widespread fungal disease called 'damping-off' that causes seedlings to topple over and die. Mańka (2005) divides damping-off diseases into two types: pre-emergence (infection symptoms within germinating seeds) and post-emergence (infection symptoms in several-week-old seedlings – narrowed root neck, instability, collapse and death).

Damping-off diseases are predominantly caused by: *Rhizoctonia solani* (J.G. Kühn), *Fusarium* spp. (Link), *Alternaria* spp. (Nees) and *Cylindrocarpon* spp. (Wol-lenw), and Oomycetes, such as: *Pythium* sp. (Pringsheim) and *Phytophtora* sp. (de Bary). The occurrence of a given pathogen in forest nursery depends first of all on climatic conditions (air temperature and humidity) prevailing at the time when plants are at the stage of seed germination (Mańka, Mańka 1993; Mańka 2005).

Damping-off diseases can be controlled by means of biological, chemical and mechanical plant protection products. Chemical control of pathogens proved to be the most effective. These include plant spraying and seed dressing with fungicide formulations (Hamera-Dzierżanowska 2014). Chemical methods are not desirable in the natural environment, so they have been applied only to a limited extent, and the use of other methods has been encouraged.

Biological methods include application of biocontrol agents, such as natural antagonists of fungi causing damping-off diseases. Mańka and Mroczkiewicz (199) proved the effectiveness of *Mycelium radicis atrovirens* (Melin) in damping-off control in Scots pine seedlings. Grosch et al. (2006) described three *Trichoderma* sp. (Pers.) strains as potentially effective biological agents against pathogenic fungus *R. solani*. Biological methods are perceived as the least invasive and harmful to the natural environment, nevertheless, their effectiveness has been still in question.

Among mechanical control methods, the most common is the expensive and energy-consuming method of soil steaming with the use of a pipe and boiler system (Rutkowski, 1995). This treatment has been repeatedly modified to improve its effectiveness, for example, by steaming loosened soil covered with plastic film, but the treatment was effective only down to the depth of 25 cm (Górski 2006). Studies have been carried out on the control of damping-off diseases with the use of ultraviolet radiation (Słowiński 2011). Słowiński and Stępniewska (2010) examined the microwave radiation effects on the growth of Scots pine seedlings and the occurrence of *R. solani*.

Common nettle *Urtica dioica* L. has been by and large used as a biological plant protection product against insect pests (aphids, spider mites) as well as fungal diseases (grey mould, powdery mildew). Common nettle has been proven to be effective in control of pathogen *R. solani* (Hadizadeh et al. 2009). Also, this plant is a soil fertility indicator, as it grows on nitrogen-rich soils, and thus, nettle constitutes a rich source of nutrients (Asgarpanah, Mohajerani 2012) that can potentially enrich the nursery substrate.

2. Study aim and scope

The aim of the present study was to explore the possibility of using four types of plant material, both crushed and granulated, as a natural means to enrich the nursery substrate with nutrients and mycorrhizal fungi, with the assumption that such treatment would have positive effects on the process and rate of seed germination and early development of Scots pine seedlings.

Demonstration of the effectiveness of the tested organic matter in the broadly understood fertilization of the nursery substrate could reduce the use of mineral fertilizers, whose production, taking into account the scale of national forest nursery, constitutes a significant burden on the natural environment.

3. Methods

Four types of organic material were tested:

- common nettle shoots and leaves,
- coniferous litterfall – because of the presence of mycorrhizal fungi (Sayer, 2006), beneficial for plant growth,
- deciduous litterfall – because of the presence of mycorrhizal fungi (Sayer, 2006), beneficial for plant growth,
- natural peat.

Nettle shoots were collected in the immediate vicinity of the Warta river bed in Myszków on 9 June, 2016. So as to avoid the high contents of cellulose fibres, merely plant tops (non-woody shoots with leaves) were collected. The presence of fibres would obstruct crushing and then granulating plant material.

Coniferous (spruce) and deciduous (mainly beech) litter-fall was collected on 16 June, 2016, in tree stands situated in close proximity of the Wolski Forest in Kraków. The material did not contain humus or large plant elements, such as cones or branches, as this would hinder biomass fragmentation.

All the above 3 types of plant material were harvested at several randomly selected points to eliminate the error resulting from local conditions.

The fourth type of organic material under the study, that is, natural peat, was obtained from the Nędza forest nursery (the Rudy Raciborskie Forest District) in July 2016.

All 4 types of admixture materials were placed in envelopes and dried at a temperature not exceeding 38ºC, with the use of laboratory moisture analyser, until their humidity was 18%. No higher temperatures were applied intentionally, so as to avoid damage to the plant material or mycorrhizal fungi. The temperature applied did not inhibit the activity of pathogenic organisms present in the plant material. For example, Nagrodzka et al. (2016) showed that similar temperatures had no negative effects on the occurrence and development of *R. solani*. After drying, all 4 types of plant materials were crushed in a mill for plant biomass.

The samples of each admixture under the study were placed in three 1-litre cylinders and their volume density was measured on a pre-calibrated balance. The arithmetic mean was calculated from the measurement results obtained.

In order to obtain granulates, a portion of each type of crushed plant material was granulated using a matrix with a unified final product diameter. A unit with flat matrix was used, that is, a pelletizing line from Kovo Novak MGL 200, with a maximum capacity of 150 kg/h. Afterwards, bulk density of granulated plant material was determined.

The next stage of work was washing and then sterilizing 180 pots (500 ml) with contaminated ethyl alcohol. Then each pot was filled with nursery substrate without mycorrhizae, that is, a mixture of natural peat (90%) and perlite (10%) (both obtained from the forest nursery in Nędza). Next, 20 × 2.5 g each of 8 prepared organic admixtures (4 types of admixtures in 2 forms: crushed and granulated) were prepared with the use of an electronic scale. Individual 2.5 g samples were introduced into the substrate in 20 pots, preventing contamination and excessive drying. This was replicated 9 times (9 × 20 pots = 180 pots); thus, 9 variants of seed sowing medium were prepared for testing: the substrate with no admixture (control), substrate with crushed nettle, substrate with crushed coniferous litterfall, substrate with crushed deciduous litterfall, substrate with crushed natural peat, substrate with granulated nettle, substrate with granulated coniferous litterfall, substrate with granulated deciduous litterfall and substrate with granulated peat.

On 12 May, 2016, 10 seeds of Class I Scots pine were placed in each study pot (200 seeds/variant). The seeds were sterilized in 70% ethyl alcohol (Nawrot-Chorabik, 2016) before placing in the pots.

The study pots were placed in the phytotron with a programmable automatic irrigation system to prevent seed drying (spraying intensity approx. 40 ml water/pot daily). The aggregate tank was filled with water and secured in a way preventing its contamination to avoid blockage in the washer system and uneven distribution of water.

Systematic observations were carried out for several weeks over the course of seed germination and seedling growth. Every few days, the numbers of healthy and infected seedlings in individual pots were recorded. The duration of the experiment was adjusted to the time necessary to observe the symptoms of damping-off diseases.

4. Results

Data on volumetric of the plant materials under the study is presented in Table 1.

When compared to crushed nettle, deciduous litter and peat, granulated material of these types showed 10 times higher bulk density. In the case of coniferous litterfall, bulk density was higher (almost 11.5 × higher when compared to crushed material of this type). Increased bulk density of plant material indicates that its volume was reduced.

The numbers of healthy and infected seedlings recorded during cyclic observations (from 16 May to 14 June 2016) are graphically depicted in Figures 1 and 2.

Health of Scots pine seedlings was definitely the poorest on the substrate with an admixture of organic material from common nettle. Comparatively the smallest number of seedlings with good health (only 34 out of 200) was observed in the pots with substrate mixed with crushed nettle. Nettle admixture in a granular form resulted in only a slightly higher number of healthy seedlings (44 out of 200). The number of healthy Scots pine seedlings grown on the substrate mixed with deciduous litterfall granulate was over 4 times higher (147 out of 200). Nevertheless, this result was not satisfactory in view of nursery production.

Table 1. Volumetric of plant material before and after the granulating process

Survey number	Bulk density of materials (g/cm^3)							
	Crushed admixture				Granulated admixture			
	Nettle	Hardwood litter	Softwood litter	Peat	Nettle	Hardwood litter	Softwood litter	Peat
I	0.141	0.031	0.048	0.182	1.411	0.236	0.511	1.900
III	0.133	0.024	0.041	0.189	1.321	0.247	0.529	1.940
III	0.136	0.021	0.046	0.199	1.524	0.264	0.517	1.990
Average:	**0.137**	**0.025**	**0.045**	**0.190**	**1.419**	**0.249**	**0.519**	**1.943**

Treatments with common nettle (crushed and granulated) and granulated deciduous litterfall caused a decline of the Scots pine seedling health to a level well below the control (peat-perlite substrate with no admixture). All other plant admixtures (crushed deciduous litterfall, both forms of coniferous litterfall and both forms of peat) supported healthy seedling growth at a level of almost 90% of the seed load. The differences between individual treatments ranged from 178 to 191 healthy seedlings per 200 seeds. Despite the similarity of the results obtained, none of the enriched substrates supported as good growth of Scots pine seedlings as the control substrate (194 healthy seedlings/200 seeds).

The data obtained was statistically analysed. The distribution of the variable (the number of healthy seedlings) was not normal; therefore, the non-parametric Kruskal-Wallis test was used. The obtained p value was 0.0044, which denotes the grounds to reject the null hypothesis on the equality of cumulative distribution functions in the compared groups.

5. Discussion and Conclusions

The substrates enriched with various admixtures were evaluated on the basis of two empirically examined features: the number of appropriately developing Scots pine seedlings and the number of plants infected with damping-off diseases. Scots pine seeds were sterilized before sowing, thus the occurrence of pre-emergence damping-off was excluded. Seed germination failure could be caused by damage to the seeds. Considering the fact that all the seeds under the study were obtained from the same source, the authors assumed that such a damage was merely accidental.

The effects of the treatments with different types of plant material on Scots pine seed germination and seedling development, so different than expected, may be due to the fact that along with the application of the thermally unprocessed plant material, pathogenic organisms that cause damping-off could get into the substrate. For sanitary reasons, peat-perlite substrate (control treatment) obtained from the Nędza

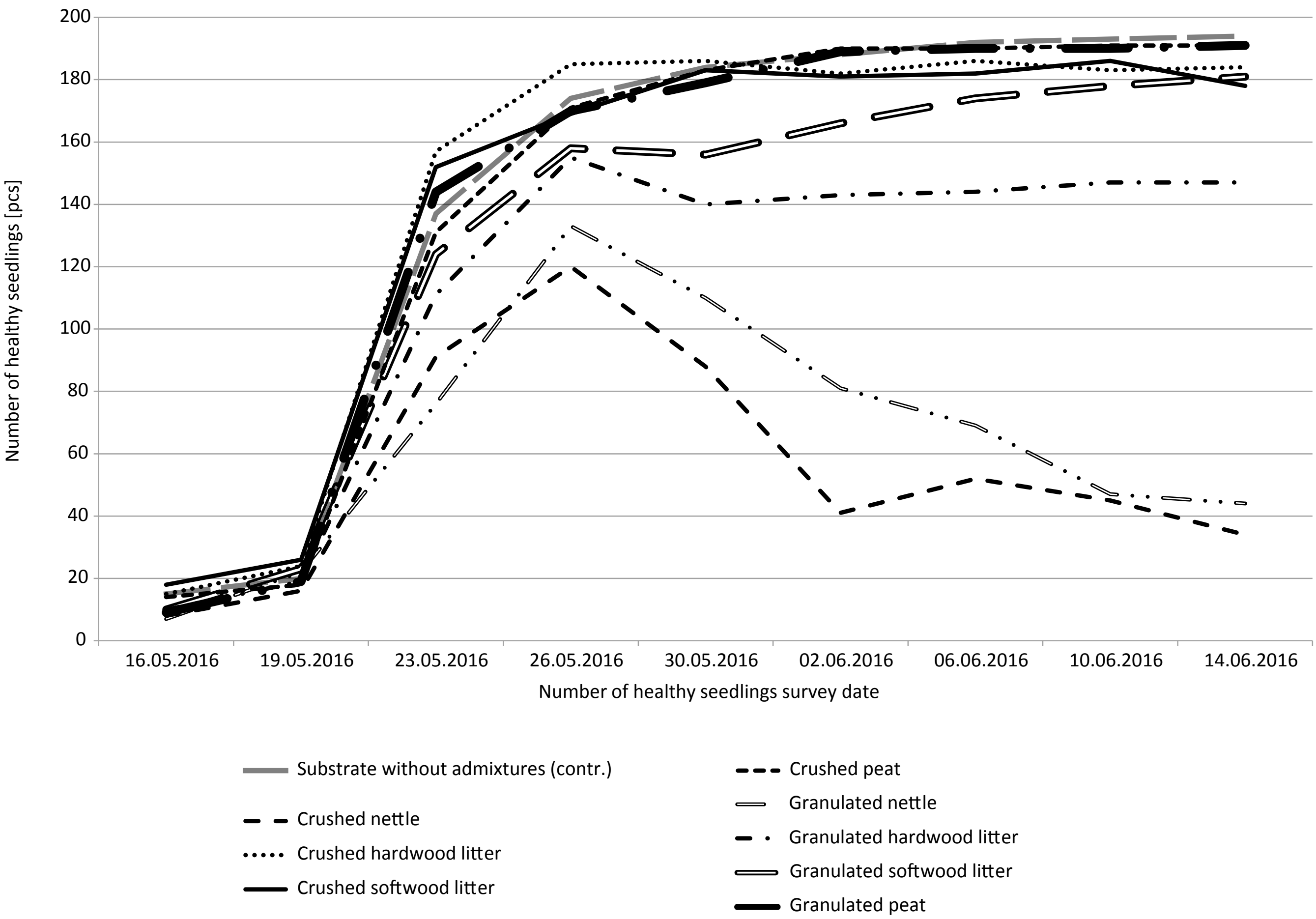

Figure 1. The number of healthy Scots pine saplings depending on the type of organic substance introduced into the ground

nursery was beforehand thermally processed at high temperatures, and thus – was free of pathogenic organisms. Consequently, it can be concluded that the differences in Scots pine seed germination and seedling development, observed under the conditions of this study, were due to the tested admixtures of plant materials added to the substrate.

Probably, drying at temperatures above 38°C would sterilize the plant material under the study from pathogenic organisms, but it could inhibit the desired activity of mycorrhizal fungi.

The negative effect of nettle on Scots pine seed germination and seedling growth was to some extent confirmed by the results of research conducted in Spain by Garmendia et al. (2018) on the possibility of enriching the substrate for cultivation of potato *Solanum tuberosum* L. in organic farming with the use of liquid nettle suspension. The study showed no positive effects of nettle treatment either on yield or chlorophyll contents in the plant aboveground parts or the pests and diseases in the organic potato crops.

An attempt to enrich the nursery substrate with litterfall does not always bring results as expected. This was demonstrated by Nyathi and Campbell (1995) in the study on the effects of litterfall of Zimbabwe native tree species *Brachystegia spiciformis* Benth. (miombo tree) and *Leucaena leucocephala* Lam. de Witt (mimosoid tree) on growth and development of maize *Zea mays*. Litterfall was added to the soil either with no additional admixtures or mixed with NPK fertilizer and manure, in various combinations. When compared to the control, treatment with *L. leucocephala*, litterfall caused a clear decrease in dry matter of plant aboveground parts, as well as grain yields. Slightly better results were obtained in consequence of treatment with *B. spiciformis* litterfall mixed with fertilizer as well as treatment with sole fertilizer; however, when compared to the control, the results were not considerably different. Hence, there was found no positive effect of litterfall on the site productivity in the case of maize crops (Nyathi and Campbell 1995).

Different results were obtained by Sarkar et al. (2010) in the study on the effects of various litterfall types on enrichment of the substrates used in agricultural production in Bangladesh. The test plant was red amaranth *Amaranthus*

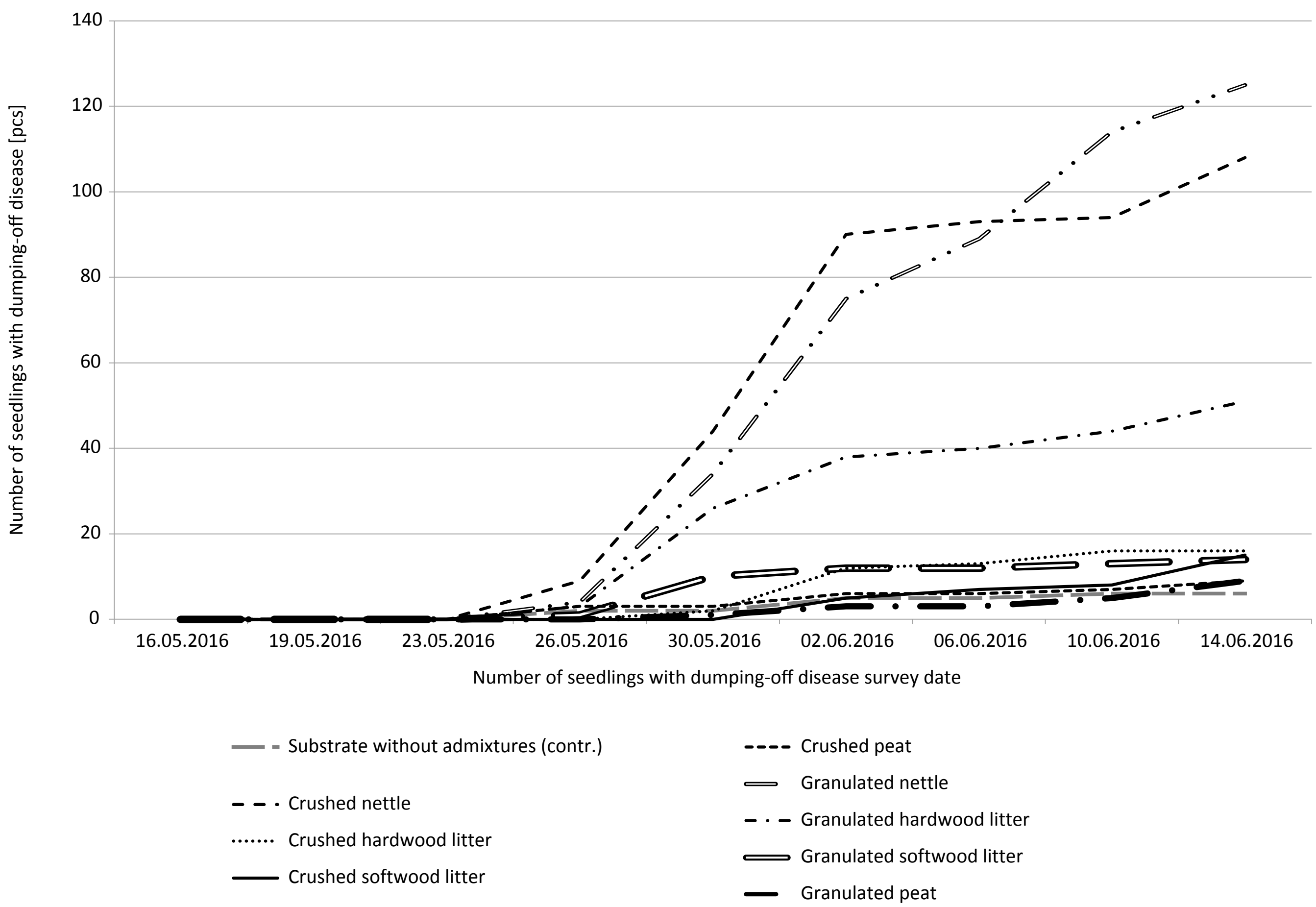

Figure 2. The number of Scots pine saplings infected with dumping-off depending on the type of organic substance introduced into the ground

cruentus L. grown as leaf vegetable throughout South-East Asia. The study results showed a significant effect of litterfall on the reduction of substrate acidity, as well as an unambiguous increase in NPK contents in the substrate, which was reflected in the increase in the height of plant aboveground parts. Although the effects of using litterfall admixtures were far from those observed when mineral fertilizers were used, the values obtained were considerably higher than those achieved in the control treatment. Nonetheless, the authors of the study suggested that litter could be successfully used as an ecological substitute for chemical fertilizers used in the cultivation of leafy vegetables.

The results obtained in the present study do not allow to explicitly conclude on the effectiveness of specific forms of admixture material – crushed or granulated. In most cases, treatments with both forms had similar effects. This indicates that biomass exposure to high temperature and pressure during the granulation process does not necessarily affect the quality of plant material used for improving the substrates.

The exception to this rule was deciduous litterfall, as in this case, much worse results were obtained in treatments with granulated material when compared to those with crushed material. This is confusing in view of the fact that the granulated and crushed materials were derived from the same litterfall sample. On the other hand, granulated material was subjected to specific physical processes (pressure pressing and high temperature). This case shows that understanding the relationship between plant material and the loss of its biological properties during processing requires separate studies. For the purposes of this study, however, this is not of much importance due to the fact that treatments with both forms of deciduous litter were ineffective in terms of improving properties of the nursery substrate. The biomass granulation process increased litterfall bulk density almost tenfold, which came to the reduction of biomass volume. If the effectiveness of granulated admixtures was shown, substrate storage in forest nurseries could be much improved. Granulated substrates occupy comparatively small storage spaces, which practically translates into cost-effectiveness of storage room operations (smaller storage space is directly associated with savings at the investment stage, building administration costs, property tax, etc.).

Based on the results obtained, it can be concluded that:

None of the four substrate admixtures examined, regardless of the application form, had a positive effect on Scots pine seed germination and seedling development.

The highest number of healthy and properly developing seedlings was recorded in the control treatment (peat-perlite substrate).

In all the experimental variants, the first symptoms of damping-off diseases in Scots pine seedlings were revealed not earlier than 14 days after seeding.

The granulation process led to a 10-fold increase in the bulk density of plant material, and thus – a reduction in its volume.

Conflict of interest

The authors declare no potential conflicts.

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Authors' contribution

D.K. – literature review, data review, statistical analysis, interpretation of the results, manuscript writing/preparation/revision; K.S. – concept, assumptions, methods, research coordination, manuscript revision; J.K. – experimental works, equipment operation, data verification.

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