

# The growth and mycorrhizal status of Scots pine seedlings planted on a outer dumping ground of the Lignite Mine in Bełchatów using different methods of seedling production

Marta Aleksandrowicz-Trzcińska<sup>1</sup>, Włodzimierz Buraczyk<sup>2</sup>

Warsaw University of Life Sciences – SGGW, Faculty of Forestry  
159 Nowoursynowska St., 02-776 Warsaw, Poland

<sup>1</sup>Department of Forest Protection and Ecology,

Phone: (+48) 0 22 59 38 173, e-mail: marta\_aleksandrowicz\_trzcinska@sggw.pl

<sup>2</sup>Department of Silviculture,

Phone: (+48) 0 22 59 38 104, e-mail: wburaczyk@wp.pl

## ■ Abstract

The study was located in a Scots pine plantation established on the outer dumping ground of the Lignite Mine in Bełchatów using different seedling production methods. The seedlings used in the study were raised in a bare-root forest nursery, a foil greenhouse and a container nursery mycorrhized and not-mycorrhized with the fungus *Hebeloma crustuliniforme*. The survival of seedlings in the first year after planting, their growth based on height and root collar diameter measurements and the degree of root mycorrhization were determined. The Scots pine seedlings growing under the outer dumping ground conditions were evaluated for their usefulness in silvicultural practice taking account of the method of their production.

## ■ Key words

Scots pine, land reclamation, mycorrhiza, seedling production methods

## ■ Introduction

The construction of the Lignite Mine in Bełchatów has led to considerable changes in the natural environment. Prior to the commencement of coal excavation works the overburden had to be removed and placed on the outer dumping ground. The overburden, and consequently the outer dumping ground contained Quaternary loose sands, loamy sands, sandy loams and cohesive soils, silt loams and clays, Tertiary loose sands, sandy loams and cohesive soils. The adopted technology did not foresee segregation of the collected material resulting in soils significantly differing in properties remaining on ground surface or within the reach of roots, for example the pH values ranged from strongly acid (pH in KCl < 3.5) to slightly alcalic. The reclamation method adopted for almost entire outer dumping ground was forest oriented. The afforestation works were started in 1984 using a variety of forest tree species mainly common birch, Scots pine, common alder, false acacia and black poplar. The results were uneven. Some plantations and thickets are of high quality, yet there are failed areas in plantations which still need restocking, so further plantings are continued (Karczmarski 1999, Łyczba and Piątkowski 1999).

The aim of the research was to evaluate the growth and the status of mycorrhizas of Scots pine seedlings planted on the outer dumping ground of the Lignite Mine in Bełchatów using different methods of seedling production. The seedlings were not only under the impact of specific soil conditions but also the decreasing yet still high sulphur and nitrogen oxide concentrations and large amounts of ash from the “Bełchatów” Electric Power Station (up to 1.5 t/ha per year).

## ■ Study site and methods

The study was conducted in a 5-year-old Scots pine plantation for four years from the moment of its establishment to the fourth year of growth. The seedlings used in the experiment were grown from the seeds collected in the stands located in the territory of the Garwolin Forest District in the bare-root nursery, foil greenhouse and container nursery mycorrhized with the fungus *Hebeloma crustuliniforme* and not mycorrhized.

The root collar diameters of seedlings were measured and the degree of root mycorrhization was assessed before planting. The seedlings produced in the bare-root nursery were almost completely mycorrhized (95%). The ectendomycorrhiza was the dominant type. The seedlings from a foil greenhouse were mycorrhized in 55%. The number of detected ectendomycorrhizas was rather high (28%) – *Thelephora* (25%) and *Laccaria* (2%). The non-mycorrhized container-grown seedlings were almost completely deprived of mycorrhizas. Ectendomycorrhizas occurred only sporadically (3%). The mycorrhized seedlings from the container nursery formed mycorrhizas with the fungus *H. crustuliniforme* in 65%. Ectendomycorrhizas were present on 10% of roots while no mycorrhizas were detected on 25% of roots.

The plantation was located on the outer dumping ground, slightly above one half of its relative height (ca 110 m) in a four random block design. The experiment had four treatments. The treatments embraced seedlings produced with four different methods. Soil preparation consisted in ploughing furrows without subsoiling. Sixteen 8 m x 27 m rectangular sample plots were established. One-year-old pine seedlings were planted at

1.5 x 0.8 m spacing. In total, 3064 seedlings were planted. Prior to the experiment, soil samples were collected from eight places uniformly distributed at the study site from a depth of 10 cm to determine its physical and chemical properties. The results of analyses are presented in Table 1.

TABLE 1  
Soil physical and chemical properties at the experiment site

| Characteristic | pH<br>in KCl | Contents     |               |  |                  |             |
|----------------|--------------|--------------|---------------|--|------------------|-------------|
|                |              | N<br>(mg/kg) | C org.<br>(%) | P <sub>2</sub> O <sub>5</sub><br>mg/100g | K <sub>2</sub> O | Mg          |
| Mean           | 7,74         | 1,68         | 1,91          | 5,15                                     | 2,80             | 3,05        |
| Range          | 7,20–7,90    | 1,30 – 2,14  | 1,44 – 2,57   | 3,10 – 6,70                              | 1,50 – 4,00      | 2,10 – 3,60 |

The heights of seedlings were measured four times: after planting, after the first, second and fourth year of growth in the plantation and the root collar diameters – in the second and fourth year after planting. The seedling survival in the plantation was evaluated in the first year after planting.

Root samples were taken by the end of the third year of seedling growth in the plantation with a borer 3.6 cm in diameter. The borer was driven into the soil at a depth 7 cm at an approximate distance 2–3 cm from the stem of a seedling. Twenty samples were taken from each treatment, eighty samples altogether. The samples were frozen at a temperature of –18°C and then they were rinsed on a sieve under running water prior to analysis in order to select roots. Observations of root samples were made under a stereoscopic microscope at 6.3 to 40 magnification. Hundred short roots from each sample were examined and classified into autotrophic or mycorrhizal. Mycorrhizal tips were identified on the basis of the absence of capillary roots, the presence of fungal mantle, hyphae and strands branching from its surface, colour, fine root hypertrophy and their transformation into characteristic mycorrhizal forms. The mycorrhizas were categorised into morphotypes based on the appearance of the mantle. Within the morphotypes single, dichotomous, multi-dichotomously branched and coralloid forms of mycorrhizas were distinguished.

The obtained data were subject to statistical analyses using the program Statgraphics plus 1.4. The analysis of variance was applied to all the studied characteristics and the homogeneous groups were created using Duncan's test.

## ■ Results

The survival of pine seedlings raised in the container nursery (both treatments) and bare-root nursery was very high (container nursery without mycorrhization – 99.2%, with mycorrhization – 97.5%, bare-root nursery – 94.1%), whereas 22.5% of seedlings raised in the foil greenhouse did not survive in the first year after planting.

The seedling heights after planting and in successive years of growth in the plantation are given in Fig. 1. The pines raised in the bare-root nursery were statistically significantly lowest ( $p = 0.0000$ ) immediately after planting (7 cm), while they were found to be signi-

ificantly highest (67 cm,  $p = 0.0000$ ) in the fourth year of growth. The mycorrhized pines raised in the container nursery were statistically significantly highest during three successive growing seasons. In the fourth year of growth, they did not differ in height from the seedlings raised in the foil greenhouse and container nursery without mycorrhization.

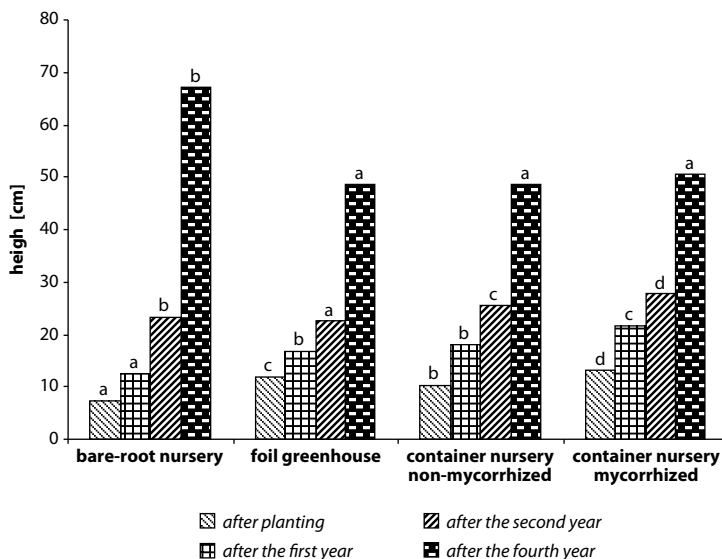


FIG. 1. Height of pines growing in the plantation established on the outer dumping ground of the Lignite Mine in Bełchatów. The same letter above the column indicating the height in a given year points to the lack of statistically significant differences between treatments; Duncan's test,  $p = 0,05$

The statistically significantly smaller root collar diameters ( $p = 0.0000$ ) were detected in the pines raised in the foil greenhouse (2.1 mm) and bare-root nursery (2.3 mm) before planting, while the greatest diameters were found in non-mycorrhized seedlings from the container nursery (3.6 mm). After the second year of growth in the plantation, significantly greater ( $p = 0.0000$ ) root collar diameters were detected in the seedlings from the bare-root nursery (6.2 mm) and mycorrhized seedlings from the container nursery (5.9 mm) in comparison with the pines from the other two experimental variants (5 mm). The seedlings raised in the bare-root nursery had the significantly greatest ( $p = 0.0000$ ) root collar diameters (19 mm) after the fourth year of growth (Fig. 2.).

Six mycorrhizal morphotypes were identified on seedling roots:

1. Ectendomycorrhizas – brown, sporadically with pale tips, frequently with a thin smooth mantle, sporadically with white single strands. Single and dichotomously branched mycorrhizas.
2. *Suillus* type – from light to dark brown. Absorption white, grey or greyish brown mycelium emanating from mantle. The mycelium infrequently found, very abundant, gluing the mycorrhizal tips growing close to each other, in other places the mantle was less abundant or sparse occurring only at a mycorrhizal base. Rhizomorphs thick, well developed white or concolorous with mycorrhizi-

- zas. All mycorrhizal forms were observed: single, dichotomously branched and coralloid.
3. *Hebeloma* type – light elongated mycorrhizas with a quite abundant white mycelium. Mycelium with infrequent crystals. Single and dichotomously branched mycorrhizas were observed.
  4. *Thelephora* type – mycorrhizas with smooth mantle. Occurred on two root samples taken from the seedlings grown in a bare-root nursery and a foil greenhouse.
  5. Black mycorrhizas with a black mycelium from very abundant to single hyphae emanating from mantle. This morphotype was found in two root samples taken from the seedlings raised in the bare-root nursery and container nursery without mycorrhization.
  6. Light mycorrhizas with white-pinkish mycelium, fluffy and very abundant. Rhizomorphs abundant, very thick with frequently emanating mycelium. Coralloid and cluster type mycorrhizas dominated. This morphotype was observed in two root samples taken from the seedlings raised in a bare-root nursery.

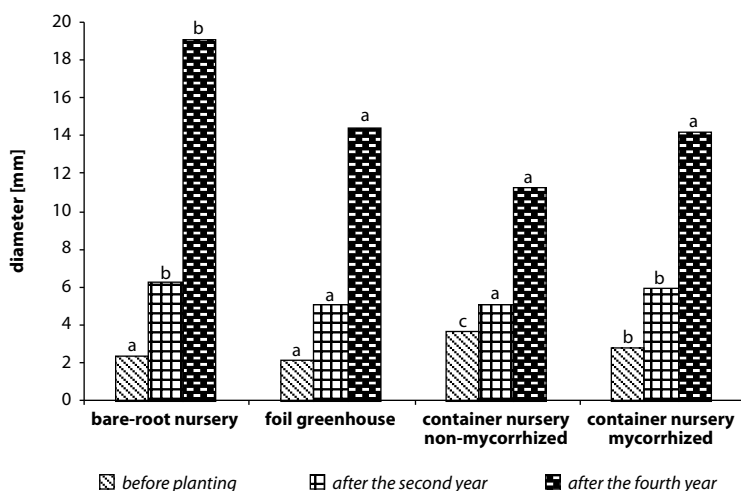


FIG. 2. Root collar diameter of pines growing in the plantation established on the outer dumping ground Lignite Mine in Bełchatów. The same letter above the column indicating the diameter in a given year points to the lack of statistically significant differences between treatments; Duncan's test,  $p = 0,05$

All six mycorrhizal morphotypes occurred in the seedlings grown in the bare-root nursery. Four morphotypes were identified on pine roots from the container nursery without mycorrhization and three each in the foil greenhouse and container nursery with mycorrhization.

The seedlings produced with four different methods significantly differed in mycorrhization level and share of individual mycorrhizal morphotypes (Fig. 3). The proportion of autotrophic root tips of pine seedling ( $p = 0.0000$ ) from the bare-root nursery and foil greenhouse was twofold lower (27.6% and 30.6% respectively), in comparison with the

container-grown pines both mycorrhized (64.3%) and non-mycorrhized (66.7%). More ectendomycorrhizas ( $p = 0.0000$ ) and dead mycorrhizas ( $p = 0.0091$ ) were found on the roots of seedlings grown in the bare-root nursery and foil greenhouse in comparison with other treatments. *Hebeloma* mycorrhizas were absent only in the seedlings raised in the foil greenhouse. The significantly greatest number of this type of mycorrhizas (12.2%,  $p = 0.0000$ ) was detected on the mycorrhized seedlings from container nursery. The share of *Suillus* mycorrhizas was similar in all experimental treatments accounting for about 5%. However, the percentage of individual mycorrhizal forms differed between seedlings (Table 2). The most favourable structure was observed in the mycorrhized pine seedlings in which coralloid form represented nearly half (46.7%) of all mycorrhizas. The percentage of individual mycorrhizal forms in the seedlings raised in the foil greenhouse was comparable. The highest percentage of single mycorrhizas was detected in pines from the bare-root nursery (55.8%) and container nursery without mycorrhization (92%).

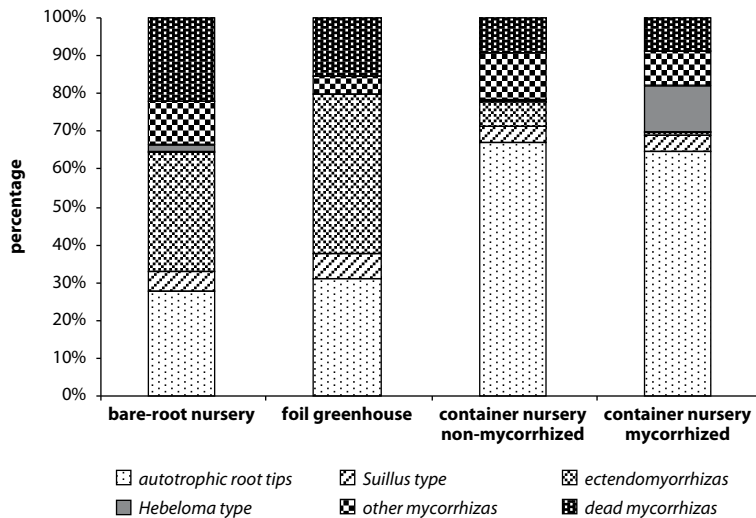


FIG. 3. Percentage of mycorrhizal morphotypes in pine seedlings growing in the plantation established on the outer dumping ground of the Lignite Mine in Bełchatów

TABLE 2  
Percentage share of mycorrhizal forms in the *Suillus* type

| Treatment                         | Share of mycorrhizas (%) |             |                              |           |
|-----------------------------------|--------------------------|-------------|------------------------------|-----------|
|                                   | single                   | dichotomous | multi-dichotomously branched | coralloid |
| Bare-root nursery                 | 55,8                     | 19,8        | 5,8                          | 18,6      |
| Foil greenhouse                   | 28,9                     | 26,4        | 25,6                         | 19,1      |
| Container nursery non-mycorrhized | 92,0                     | 2,3         | 2,3                          | 3,4       |
| Container nursery mycorrhized     | 10,4                     | 31,2        | 11,7                         | 46,7      |

## ■ Discussion

The pines planted on the outer dumping ground of the Lignite Mine in Bełchatów grew on soils with very unfavourable, slightly alkaline pH. Soil chemical analyses showed a high content of organic carbon, a satisfactory content of phosphorus and manganese and a low content of nitrogen and potassium (Table 1) (Baule and Fricker 1973, Walendzik and Szołtyk 1993). In such conditions, the pines raised in the bare-root nursery demonstrated a high survival rate and significantly best height and diameter growth after four years from planting. The seedlings from this treatment were significantly lowest after planting and exhibited the greatest annual growth after the first growing season expressed in percent values compared to the height after planting, and also in absolute values for successive years. The pines raised in the foil greenhouse showed the significantly poorest growth on the outer dumping ground. The survival and height growth of pines from this treatment after the first growing season was found to be lowest. It could be that the seedlings raised under favourable greenhouse conditions (compared to other treatments) went through a transplant shock, hence their high mortality in the plantation. The seedlings from this treatment featuring the smallest root collar diameters, after planting showed intensive diameter growth and slower height growth (in comparison with other treatments).

The pines raised in the container nursery (both treatments) had the best (compared with other treatments) height growth parameters when planted, and the container system helped reduce the transplant shock. These parameters decided about the very high survival rate after the first year of growth in the plantation and high growth during the first two years. As regards height parameters, after four years of the study, the container-grown seedlings, both mycorrhized and non-mycorrhizal, were clearly inferior when compared with the bare-root seedlings. This might be the effect of the uptake of nutrients accumulated in the root-ball by seedlings in the first year of growth and, possibly, a limited downward growth of roots into the soil.

The mycorrhized pines from the container nursery showed the best mycorrhization level prior to planting. They formed mycorrhizas with the inoculated fungus *H. crustuliniforme* in 65%. These are effective mycorrhizal associations (Marmeisse *et al.* 1999), well developing in a broad range of soil pH, including the neutral one. This was confirmed in the *in vitro* studies by Pachlewski (1993). The share of *Hebeloma* type of mycorrhizas was greatest after three years of growth in the plantation in comparison with other morphotypes, yet it accounted for only 12.2% with 35.7% of total root mycorrhization. This indicates the withdrawal of the mycorrhizas transferred from the nursery (*H. crustuliniforme* and ectendomycorrhizas) which are taken over, to a small extent, by autochthonous species, e.g. *Suillus*. The container-grown seedlings not subject to mycorrhization showed a similar mycorrhization level after three years of growth in the plantation (33.3%). However, the mycorrhizas of these pines were formed by local species. The studies conducted by Hilszczańska (2005) also on the outer dumping ground demonstrated that the container-grown seedlings, which at the moment of planting had 90% of *Thelephora terrestris* mycorrhizas and 10% of ectendomycorrhizas, formed 75% of mycorrhizas with the fungus *Suillus luteus* after the growing season. In the presented research the percentage of the suilloid mycorrhizas was minor (ca 5%) and comparable to that in all other variants. These types of mycorrhizas are favourable for pine at young age and are functioning quite well not only in typical forest habitats but also on post-agricultural lands and in areas dama-

ged by industrial activity (Kieliszewska-Rokicka et al. 1997, Aleksandrowicz-Trzcńska 2006).

The share of ectendomycorrhizas, detected on pine roots in all variants before planting ranged from 3% for the seedlings from the container nursery without mycorrhization to 97% for the pines from the bare-root nursery. The predominance of ectendomycorrhizas on the roots of pines from the bare-root nursery and foil greenhouse and the presence of this morphotype in the seedlings from both container nursery treatments after three years of growth are not surprising. The studies by Mikola (1965) and Pachlewski (1983) show that the plants infected in the nursery by the ectendomycorrhizal fungus after transplanting in forest conditions formed, in a short time, typical ectomycorrhizas, whereas after transplanting onto the post-agricultural or degraded lands the ectendomycorrhizal status still prevailed. The fungi forming ectendomycorrhizas, showing high variability and tolerance to changes in the soil environment, make symbiotic associations with plants being under physiological stress or extreme environmental conditions but also in forest nurseries in which soil fertility, high pH and moisture content favour ectendomycorrhizal formation (Kowalski 1987, Pachlewski 1993, Werner and Chojnicki 1994, Werner *et al.* 2000). Moreover, these fungi are little competitive, especially in the forest environment (Mikola 1965, Pachlewski 1983). Under the outer dumping ground conditions presumably favouring the ectendomycorrhizal development and with a low competition from ectomycorrhizal fungi, the ectendomycorrhizas increased their number on the roots of seedlings raised in the foil greenhouse and container nursery without mycorrhization, while they decreased their number in pines from the bare-root nursery and container nursery with mycorrhization. The ectendomycorrhizas in the seedlings from the bare-root nursery were, to some extent, taken over by more favourable mycorrhizas formed by local taxa.

The percentage of dead mycorrhizas was higher in the treatments in which pine roots were better mycorrhized (bare-root nursery and foil greenhouse) in comparison with two other treatments. The relatively high proportion of dead mycorrhizas on all seedling roots points to adverse conditions for both the plants and their fungal partners. Presumably, the more intensely the new mycorrhizal associations are formed the greater is the number of dead mycorrhizas.

The experiment does not clearly point out to the treatment which has the best mycorrhized seedlings. Although the pines raised in the bare-root nursery and foil greenhouse featured twice as high as degree of root mycorrhization in comparison with the two remaining treatments, they still had many more relatively low effective ectendomycorrhizas and significantly more dead mycorrhizas. The analysis of annual height and diameter growth of seedlings in individual treatments permit suggesting that the range of morphotypes and their highest biodiversity (6 morphotypes) on bare-rooted seedling are most favourable for the pines growing under outer dumping ground conditions.

## ■ Conclusions

1. The pines raised in the bare-root nursery had the best growth parameters (height and root collar diameter) compared with pines raised in the foil greenhouse and container nursery with and without mycorrhization after four years of growth in



- the plantation established on the outer dumping ground of the Lignite Mine in Bełchatów.
2. The seedlings from the bare-root nursery and foil greenhouse after three years of growth in the plantation were better mycorrhized and had more ectendomycorrhizas and dead mycorrhizas in comparison with the seedlings raised in both container nursery variants.
  3. The seedlings raised in the bare-root nursery growing under the outer dumping ground conditions proved to be most useful in silvicultural practice, while the pines raised in the foil greenhouse, especially due to their low survival rate were least useful.

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## ■ Streszczenie (Summary)

### Wzrost i stan mikoryz sadzonek sosny zwyczajnej, wyprodukowanych różnymi technologiami, w uprawie na zwałowisku Kopalni Węgla Brunatnego w Bełchatowie

Obiektem badań była uprawa sosny zwyczajnej założona na zwałowisku zewnętrznym Kopalni Węgla Brunatnego w Bełchatowie, z sadzonek wyprodukowanych czterema technologiami: w szkółce otwartej, namiocie foliowym oraz w szkółce kontenerowej – mikoryzowanych grzybem *Hebeloma crustuliniforme* i niemikoryzowanych. Celem pracy była ocena wzrostu i stanu mikoryz w czterech kolejnych latach hodowli. Wzrost sadzonek oceniono na podstawie wysokości i grubości w szyi korzeniowej. Po pierwszym roku hodowli w uprawie określono przeżywalność sadzonek, a po trzecim stopień zmikoryzowania.

Przeżywalność sadzonek wyhodowanych w szkółce kontenerowej (oba warianty) i w szkółce otwartej była bardzo wysoka (powyżej 90%), znacznie niższą przeżywalnością charakteryzowały się sosny wyprodukowane w namiocie foliowym (77,5%). Po czterech latach wzrostu w uprawie istotnie najlepsze parametry wzrostowe osiągnęły sadzonki pochodzące ze szkółki otwartej w porównaniu z trzema pozostałymi wariantami.

Na korzeniach sadzonek stwierdzono występowanie 6 morfotypów mikoryz. Istotnie lepiej zmikoryzowane były sosny pochodzące ze szkółki otwartej (72,4%) i namiotu foliowego (69,4%) w porównaniu z sadzonkami ze szkółki kontenerowej mikoryzowanymi (35,7%) i niemikoryzowanymi (33,3%). Mikoryzy utworzone przez *H. crustuliniforme* na korzeniach inokulowanych sadzonek ze szkółki kontenerowej, w momencie wysadzenia na uprawie stanowiły 65% udziału. Po trzech latach wzrostu typ *Hebeloma* był dominującym morfotypem sosen tego wariantu, lecz jego udział wyniósł jedynie 12,2%. Ektendomikoryzy dominowały u sadzonek ze szkółki otwartej (31,7%) i namiotu foliowego (41,9%). Istotnie więcej mikoryz tego morfotypu jak i mikoryz martwych stwierdzono na korzeniach sadzonek pochodzących ze szkółki otwartej i namiotu foliowego w porównaniu z dwoma pozostałymi wariantami. Udział mikoryz typu *Suillus* był niewielki i zbliżony we wszystkich wariantach (ok. 5%).

W warunkach zwałowiska zewnętrznego najlepszą przydatnością do hodowli charakteryzowały się sadzonki wyprodukowane w szkółce otwartej, a najmniejszą sosny pochodzące z namiotu foliowego.

Received on December 12, 2006