

*Full Length Research Paper*

# The effect of nursery measures on mycorrhizal colonisation of Scots pine and occurrence of soil mites

Andrzej Klimek<sup>1\*</sup>, Stanisław Rolbiecki<sup>2</sup>, Roman Rolbiecki<sup>2</sup>, Dorota Hilszczańska<sup>3</sup> and Piotr Malczyk<sup>4</sup>

<sup>1</sup>Department of Zoology, 20 Kordeckiego St., University of Technology and Life Sciences, 85-225 Bydgoszcz, Poland.

<sup>2</sup>Department of Land Reclamation and Agrometeorology, 6 Bernardyńska St., University of Technology and Life Sciences, 85-029 Bydgoszcz, Poland.

<sup>3</sup>Department of Forest Ecology, Institute of Forest Research in Warszawa, Sękocin Stary, 3 Braci Leśnej St., 05-090 Raszyn, Poland.

<sup>4</sup>Department of Soil Science and Soil Protection, 6 Bernardyńska St., University of Technology and Life Sciences, 85-029 Bydgoszcz, Poland.

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**The aim of the study was to determine the influence of organic fertilization (treated sewage sludge with addition of bark or sawdust) and mulching (forest edaphon inoculation) on the vitality of Scots pine (*Pinus sylvestris* L.) seedlings and their mycorrhizal structure, as well as the occurrence of soil mites (Acari). Nursery experiments were carried out in 2005 to 2007. It was stated that the studied factors influenced seedling vigour, mycorrhizal colonization, soil properties and the occurrence of mites.**

**Key words:** *Pinus sylvestris* L., mycorrhiza, Acari, mulching, fertilization

## INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is commonly used for plantings in Poland (Olszewska and Smal, 2008; Gawroński, 2004; Koreleski, 2006a, b). One-year old seedlings of Scots pine are usually produced in forest nurseries. In some cases, production of older and larger seedlings is also justified. Such seedlings are aimed for afforestation under especially difficult conditions, for example, on post-arable grounds characterized by the strong weeding (Koreleski, 2006a, b; Kłoskowska and Niski, 1992; Klimek et al., 2008). The establishment and performance of outplanted tree seedlings may be significantly affected by ectomycorrhizal fungi, which are key players in biogeochemical cycles and contribute to host plant nutrition (Kropp and Langlois, 1990; Marx, 1980; Molina and Trappe, 1984). Land formerly used for agricultural purposes may lack ectomycorrhizal inoculum due to the absence of suitable host trees, thus, if

afforestation is to be beneficial, outplanted seedlings should have appropriate mycorrhiza on roots.

Intensive production of Scots pine seedlings in forest nurseries caused often processes of degradation connected with a decrease of biological activity of soils. High productivity of soils in forest-tree nurseries can be achieved, among others, by suitable supply of organic fertilizers, for example, composts (Klimek et al., 2008, 2009; Szołtyk and Hilszczańska, 2003). In this experiment, compost produced from sewage sludge was applied. The natural use of sewage sludge is justified from ecological point of view.

The cap humus of forest soils is inhabited by an abundant number of microarthropods. One of the most abundant groups of mesofauna is mites which play a number of important roles in forest ecosystem. Most of them as saprophages take roles in decomposition of organic matter which influences the growth of trees (Klimek et al., 2008). These organisms also provide important ecosystem functions such as CO<sub>2</sub> evolution, nutrient mobilization and soil mixing (Lindberg and Persson, 2004).

\*Corresponding author. E-mail: [klimek@utp.edu.pl](mailto:klimek@utp.edu.pl). Tel: +48 523749409.

The purpose of the study was to determine the effect of organic fertilization and mulching (edaphon inoculation) on the vitality of Scots pine (*P. sylvestris* L.) seedlings and their mycorrhizal structure, as well as the occurrence of soil mites (Acari).

In our previous experiments we investigated the influence of micro-irrigation (microjet sprinkling and drip irrigation) and organic fertilization (80% of treated sewage sludge and 20% of highmoor peat) on the vitality of white birch (*Betula pendula* Roth) and Scots pine seedlings as well as on the occurrence of soil mites (Acari) after edaphon inoculation (Klimek et al., 2008, 2009).

## MATERIALS AND METHODS

Two nursery experiments were carried out in 2005-2007 at the Forest Nursery in Białe Błota, Forest District in Bydgoszcz. The first two-year trial was conducted in 2005-2006 (2 cycles of one-year old seedling production), and the second in 2006-2007 (2 cycles of two-year old seedling production). These experiments were run in a split-plot system with four replications. Two different factors were compared. The first row factor – organic fertilization, was used in the two following treatments (main plots):  $F_1$  – treated sewage sludge ( $\frac{2}{3}$ ) + bark ( $\frac{1}{3}$ ) and  $F_2$  – treated sewage sludge ( $\frac{2}{3}$ ) + sawdust ( $\frac{1}{3}$ ). The second row factor – mulching, was used in two variants (subplots): C – without mulching (control) and M – mulching with litter. Seed origin and seeding density were similar to standard nursery practices (Kłoskowska and Niski, 1992). Scots pine seeds were obtained from the seed stand of Forest District in Bydgoszcz. The plot area was 2 m<sup>2</sup> and contained 4 rows (2 m length) of Scots pine seedlings. Total number of plots in each experiment was 16 (2 treatments × 2 variants × 4 replications).

Organic fertilizer was produced on the base of treated sewage sludge ( $\frac{2}{3}$ ) and Scots pine bark ( $\frac{1}{3}$ ) or sawdust ( $\frac{1}{3}$ ). This fertilizer was applied with the dose of 100 t · ha<sup>-1</sup> in spring and mixed with the topsoil (10 cm deep) before Scot pine seed time.

Mulching with litter (ectohumus) obtained from fresh coniferous forest was done with the dose of 100 m<sup>3</sup> ha<sup>-1</sup>. Introduction of edaphon consisted of mixing topsoil (2 cm deep) with organic matter obtained from the surface of fresh coniferous forest designed for tree felling. This substrate contained the living soil mesofauna which was very abundant. This measure was conducted after emergence of Scots pine seedlings in the years 2005 and 2006, respectively.

Irrigation in forest nursery was done with the use of micro-sprinklers ( $q = 80-100 \text{ dm}^3 \text{ h}^{-1}$ ) (Jeznach, 2009; Łabędzki, 2009). Terms of irrigation and water rates were established according to directives for irrigation of forest nurseries on bare areas in Poland (Pierzgalski et al., 2002).

## Soil analyses

The trial was conducted on a Cambic Arenosol (FAO, 1998) formed on fluvioglacial sand characterized by the following soil pedon: Ap-ABv-Bv-BvC-C. Soil samples were taken from the 0-15 cm surface layer of all experimental plots. Soils were air-dried at room temperature and passed through a 1 mm sieve.

The following physico-chemical and chemical properties were determined (Mocek et al., 1997):

1. pH in water and in 1M KCl (1:2.5) was measured using a pH meter (model CPC-551 Elmetron);
2. Organic carbon ( $C_{\text{org}}$ ) was determined by oxidizing the soil

sample with a mixture of potassium dichromate and sulfuric acid, diluting the suspension with water and back titrating the excess dichromate with standardized ferrous sulfate solution (Tiurin method);

3. Total nitrogen ( $N_{\text{total}}$ ) was determined by Kjeldahl method;

4. Available potassium and phosphorus was determined by Egner-Riehm metod, by extraction of soil samples in solution of calcium lactate; P was determined by colorimetric method, with the use of UV-VIS spectrometer model Marcel Media, and K was determined by emission spectrometry method with the use of spectrometer PU-9100X, Philips;

5. Available magnesium was determined by Schachtschabel method, by extraction of samples in solution of 0.0125 M CaCl<sub>2</sub>, measurement of Mg content was determined by atomic absorption spectrometry, with the use of spectrometer PU-9100 X, Philips;

6. Available microelements (zinc, copper, manganese) were determined by atomic absorption spectrometry, with the use of spectrometer PU-9100 X Philips, after their previous extraction from the soil samples in 1 M HCl;

7. Granulometric analysis was carried out according to the method of Bouyoucos in modification of Casagrande and Prószyński.

## Growth and mycorrhizal analyses

Plant growth was evaluated in late autumn (October). The height of seedlings (cm) and root-collar diameter (RCD) (mm) were measured.

Samples of plants (5 per treatment) were collected to determine the composition of ectomycorrhizal (ECM) communities. Prior to the investigation, each root system was excised from the stem and gently washed in tap water. Ectomycorrhizal tips were identified by the presence of mantle (colour, shape and surface texture), external hyphe, a slightly swollen apex and mycelial strands (Harley and Smith, 1983). Observations were done under stereomicroscope with magnification 4 to 50 x. Total numbers of vital mycorrhizal root tips per seedlings were counted, the tips being counted and distinguished using available identification manuals (Agerer, 2006; Agerer and Rambold 2007; Ingleby et al., 1990). Data were analysed using analysis of variance (ANOVA) in the statistical package STATISTICA. Differences amongst means were evaluated with the LSD-test.

## Sampling and observation of soil mites

To investigate the occurrence of mites, soil samples were taken twice a year (in June and October) in successive years. Samples of 17 cm<sup>2</sup> and 3 cm deep were taken from all plots in 3 replications (3 samples per plot). Mites were extracted during 7 days in high gradient Tullgren funnels. A total of 2672 mites (Acari) were identified to order, according to Hammen's systematics (Van der Hammen, 1972). The data were statistically processed by analysis of variance. Fisher-Snedecor test was used to determine the significance of experimental factors, and Tukey test was used to define significant differences between combinations. The data of mites were ln-transformed (x+1) prior to the analyses (Berthet and Gerard, 1965).

## RESULTS

### Climatic conditions and irrigation

Mean air temperature during the vegetation period (April-September) in the years 2005-2007 was 14.7°C, ranging from 14.2 to 15.1°C, in 2005 and 2006, respectively

**Table 1.** Average air temperature during the period of the study (°C).

Year	Decade	Month						IV-IX
		IV	V	VI	VII	VIII	IX	
2005	1	6.9	10.0	12.3	19.5	15.3	18.1	-
	2	9.1	9.1	15.2	20.2	16.0	13.6	-
	3	6.2	17.1	17.3	18.5	17.4	12.9	-
	1-3	7.4	12.2	14.9	19.4	16.3	14.8	14.2
2006	1	5.3	12.9	11.8	22.7	17.6	15.2	-
	2	7.3	13.1	18.9	21.8	17.4	15.7	-
	3	8.7	11.4	19.7	22.7	15.0	14.6	-
	1-3	7.1	12.5	16.8	22.4	16.6	15.2	15.1
2007	1	5.9	9.3	18.8	15.7	18.6	12.6	-
	2	9.3	12.7	19.5	21.1	18.6	11.3	-
	3	10.2	19.0	16.2	17.3	16.4	13.2	-
	1-3	8.5	13.8	18.2	18.0	17.8	12.4	14.8
2005-2007		7.7	12.8	16.6	19.9	16.9	14.1	14.7
Norm		7.7	13.1	16.2	18.2	17.8	13.0	14.3

**Table 2.** Rainfall during the period of the study (mm).

Year	Decade	Month						$\Sigma_{IV-IX}$
		IV	V	VI	VII	VIII	IX	
2005	1	3.8	46.6	20.9	0	19.3	0	-
	2	1.5	13.2	6.1	2.5	1.6	7.9	-
	3	18.5	9.7	3.7	37.7	0	10.0	-
	$\Sigma_{1-3}$	23.8	69.5	30.7	40.2	20.9	17.9	203.0
2006	1	0	9.6	6.6	0	74.6	37.3	-
	2	0	20.0	15.2	25.9	23.4	0	-
	3	45.0	33.9	0	4.5	16.5	4.2	-
	$\Sigma_{1-3}$	45.0	63.5	21.8	30.4	114.5	41.5	316.7
2007	1	5.3	21.2	42.8	79.1	2.7	17.5	-
	2	0	23.3	24.2	3.7	11.0	5.3	-
	3	2.7	4.6	36.4	28.5	45.5	12.8	-
	$\Sigma_{1-3}$	8.0	49.1	103.4	111.3	59.2	35.6	366.6
$\Sigma_{2005-2007}$		25.6	60.7	52.0	60.6	64.9	31.7	295.4
Norm		26.6	40.7	54.8	65.4	51.4	44.3	283.2

(Table 1). Mean monthly values of air temperature in the years of the study varied from 7.7°C in April to 19.9°C in July. The last month was especially warm in 2006 (22.4°C). Total rainfall from 1 April to 30 September, on average for 2005-2007, amounted to 295.4 mm, ranging from 203.0 to 366.6 mm, in 2005 and 2007, respectively (Table 2).

Among the months of the vegetation period, May, July

and August were characterized by the highest rainfall amount (more than 60 mm), and April by the lowest (25.6 mm). Rainfall amounts of particular months were very differentiated in the period of the study, e.g. the amounts of rainfall ranged widely from 21.8 mm (2006) to 103.4 mm (2007), or from 30.4 mm (2006) to 111.3 mm (2007) as well as from 20.9 mm (2005) to 114.5 mm (2006) in June, July and August, respectively. There were three

**Table 3.** Water use from the soil layer of controlled moisture and irrigation requirements at forest nursery Białe Błota near Bydgoszcz, acc. to Pierzgalski et al. (2002) (mm).

Year	Months of the vegetation period (mm)						$\sum_{IV-IX}$
	IV	V	VI	VII	VIII	IX	
<b>Water use (mm)</b>							
2005	51	81	88	125	89	60	494
2006	48	82	97	153	92	61	533
2007	54	84	99	112	93	55	497
Mean	51	82	95	130	91	59	508
<b>Irrigation requirements (mm)</b>							
2005	27.2	11.5	57.3	84.8	68.1	42.1	291.0
2006	3	18.5	75.2	122.6	-22.5	19.5	216.3
2007	46	34.9	-4.4	0.7	33.8	19.4	130.4
Mean	25.4	21.6	42.7	69.4	26.5	27.0	212.6

**Table 4.** Seasonal irrigation rates as dependent on the studied year and the seedling production cycle.

Specification	One-year old seedlings		Two-year old seedlings	
Year	2005	2006	2006	2007
Seasonal irrigation rate (mm)	185	210	88	65

three rainfall-free decades in the vegetation period 2005 (1/VII, 3/VIII i 1/IX), five – in 2006 (1/IV, 2/IV, 3/VI, 1/VII i 2/IX), and only one (2/IV) in 2007.

Water use from the soil layer of controlled moisture during the vegetation period (1 April – 30 September), determined as evapotranspiration (Et) according to method of Pierzgalski et al. (2002), amounted to 508 mm, ranging from 494 to 533 mm, in 2005 and 2006, respectively. Mean monthly amount of water needs varied from 51 mm in April to 130 mm in July.

Irrigation needs of the Scots pine nursery during the vegetation period (April- September), on average for 2005-07, amounted to 212.6 mm, ranging from 130.4 to 291 mm, in 2007 and 2005, respectively (Table 3).

Water deficits were noted in all months of the vegetation period excepting August 2006 and June 2007. Among the months, July was characterized – on average for 2005-2007 – by the highest irrigation needs (69.4 mm) ranging from 0.7 to 122.6 mm. The variability of irrigation requirements was also great in June and August.

Seasonal irrigation rates were dependent above all on rainfall amounts – the lowest water dose (65 mm) was applied in 2007 (Table 4).

### Soil characteristics

In this experiment the pH values were in most cases neutral (Table 5). Higher pH values were noted in control treatments – without mulching (C). The measure of mulching with ectohumus decreased pH below 7.0

(treatments S). Mulched plots were also characterized by increased contents of  $C_{org.}$  and  $N_{total}$  as compared to those on control plots. A similar tendency was noted in case of available K and P contents. No major differences of Mg content were noted in the experimental treatments. Application of ectohumus (mulching) caused an increase of Zn, Cu and Mn.

### Growth of seedlings

The height of seedlings ranged from 10.35 to 11.87 cm and from 34.12 to 36.42 cm in case of one-year old seedlings and two-year old seedlings, respectively (Table 6).

Fertilization influenced significantly only the height of one-year old seedlings. The effect of fertilization on the growth of two-year old seedlings was not significant. The diameter of seedlings ranged from 2.9 to 3.3 mm and from 6.6 to 8.3 mm, for one-year old seedlings and two-year old seedlings, respectively. There were no significant differences in diameter between seedlings fertilized with treated sewage sludge and bark ( $F_1$ ) and treated sewage sludge and sawdust ( $F_2$ ).

Measure of mulching with ectohumus (forest edaphon inoculation) significantly increased the height of seedlings from 10.83 to 11.21 cm and from 34.77 to 35.27 cm, for one-year-old and two-year-old seedlings, respectively. There were no significant differences in diameter between seedlings grown on control plots (C) and those on mulched plots (treatments M), although seedlings

**Table 5.** Characteristics of some physico-chemical properties of the soils.

Treatment	Soil characteristics									
	pH H <sub>2</sub> O	pH KCl	C <sub>org.</sub> (%)	N <sub>total</sub> (%)	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Mg	Zn	Cu	Mn
					mg-100 g <sup>-1</sup>			mg-kg <sup>-1</sup>		
CF <sub>1</sub>	7.11	7.02	1.16	0.08	2.05	4.22	2.15	0.52	0.36	18.56
CF <sub>2</sub>	7.23	7.05	1.38	0.09	1.88	4.28	2.61	0.62	0.25	14.26
MF <sub>1</sub>	6.85	6.64	2.11	0.15	3.15	6.14	2.62	0.91	0.94	34.21
MF <sub>2</sub>	6.90	6.73	1.64	0.12	2.84	5.45	2.14	0.94	0.81	25.11
Mean	7.02	6.86	1.57	0.11	2.48	5.02	2.38	0.75	0.59	23.03
Organic fertilization										
F <sub>1</sub>	6.98	6.83	1.63	0.11	2.60	5.18	2.38	0.71	0.65	26.38
F <sub>2</sub>	7.06	6.89	1.51	0.10	2.36	4.86	2.37	0.78	0.53	19.68
Mulching										
C	7.17	7.03	1.27	0.08	1.96	4.25	2.38	0.57	0.30	16.41
M	6.87	6.68	1.87	0.13	2.99	5.79	2.38	0.92	0.87	19.66

F<sub>1</sub>, Treated sewage sludge (2/3) + bark (1/3); F<sub>2</sub>, treated sewage sludge (2/3) + sawdust (1/3); C, without mulching (control); M, mulching with litter.

**Table 6.** Effect of organic fertilization and mulching on the Scots pine seedling height and diameter.

Organic fertilization	Mulching	One-year old seedling*		Two-year old seedling**	
		Height (cm)	diameter (mm)	Height (cm)	diameter (mm)
F <sub>1</sub>	C	11.31	3.11	35.02	7.10
	M	11.87	3.30	34.12	7.90
F <sub>2</sub>	C	10.35	2.93	34.52	6.60
	M	10.56	3.12	36.42	8.30
Influence of fertilization (I)					
F <sub>1</sub>	-	11.59 <sup>a</sup>	3.16 <sup>a</sup>	34.57 <sup>a</sup>	7.50 <sup>a</sup>
F <sub>2</sub>	-	10.45 <sup>b</sup>	3.03 <sup>a</sup>	35.47 <sup>a</sup>	7.40 <sup>a</sup>
Influence of mulching (II)					
-	C	10.83 <sup>a</sup>	3.02 <sup>a</sup>	34.77 <sup>a</sup>	6.90 <sup>a</sup>
-	M	11.21 <sup>b</sup>	3.21 <sup>a</sup>	35.27 <sup>b</sup>	8.10 <sup>a</sup>

F<sub>1</sub>, Treated sewage sludge (2/3) + bark (1/3); F<sub>2</sub>, treated sewage sludge (2/3) + sawdust (1/3); C, without mulching (control); M, mulching with litter.  
; \*, \*\* – mean for 2005-2006 and 2006-2007, respectively. Means in a column followed by the same letter do not differ at 5% level of significance.

cultivated on mulched plots were characterized by an increased diameter as compared to that of seedlings grown on control plots.

Best results, especially for two-year-old seedlings, were obtained at treatments MF<sub>2</sub> – the seedling height was 36.42 cm and its diameter, on average equaled 8.3 mm.

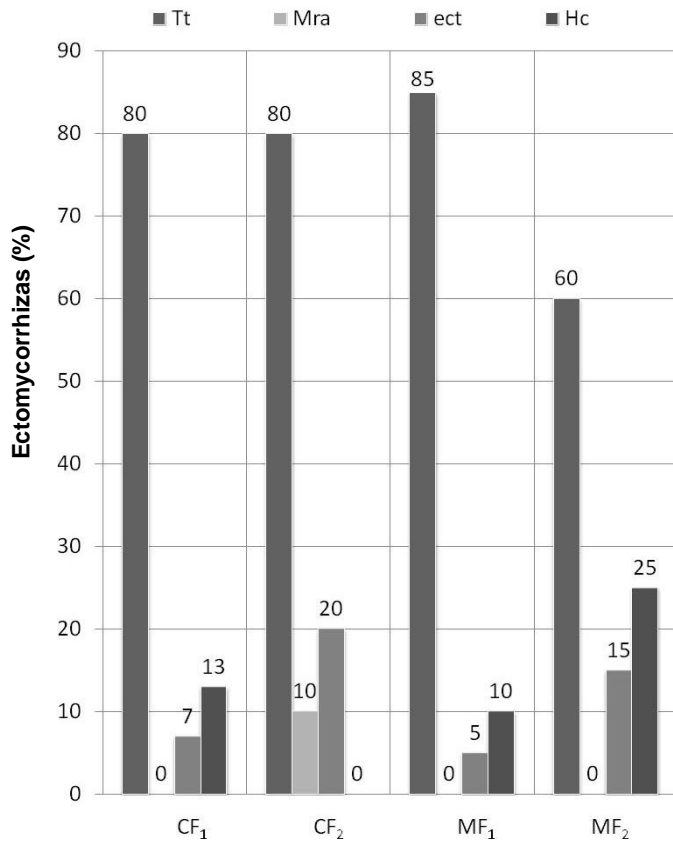
### Mycorrhizal colonisation

In all treatments dominated mycorrhizas of *Thelephora terrestris* Ehrh (Figures 1 and 2). Share of *Hebeloma*

*crustuliniforme* (Bull.) mycorrhizas was the highest in case of seedlings growing in treatment MF<sub>2</sub>, whereas on seedlings from treatment CF<sub>2</sub> (treated sewage sludge + sawdust) these mycorrhizas were not found. Percentage of ectendomycorrhizas was higher on seedlings from treatments CF<sub>2</sub> and MF<sub>2</sub> than treatments CF<sub>1</sub> i MF<sub>1</sub>.

Mycorrhizas of *Mycelium radialis atrovirens* Melin were found only on the seedlings from treatment CF<sub>2</sub>.

Two-year-old seedlings had been colonized by at least six different fungi, that is, *T. terrestris*, *Suillus* sp. Theleporaceae, *Lactarius* sp., *Wilcoxina mikolae* (Yang and Wilcox), *Wilcoxina* sp. All treatments were dominated



**Figure 1.** Percentage of mycorrhizal morphotype of one-year old Scots pine seedlings (Tt – *Thelephora terrestris*, Mra – *Mycelium radicis atrovirens*, ect – ectendomycorrhizas, Hc – *Hebeloma crustuliniforme*).

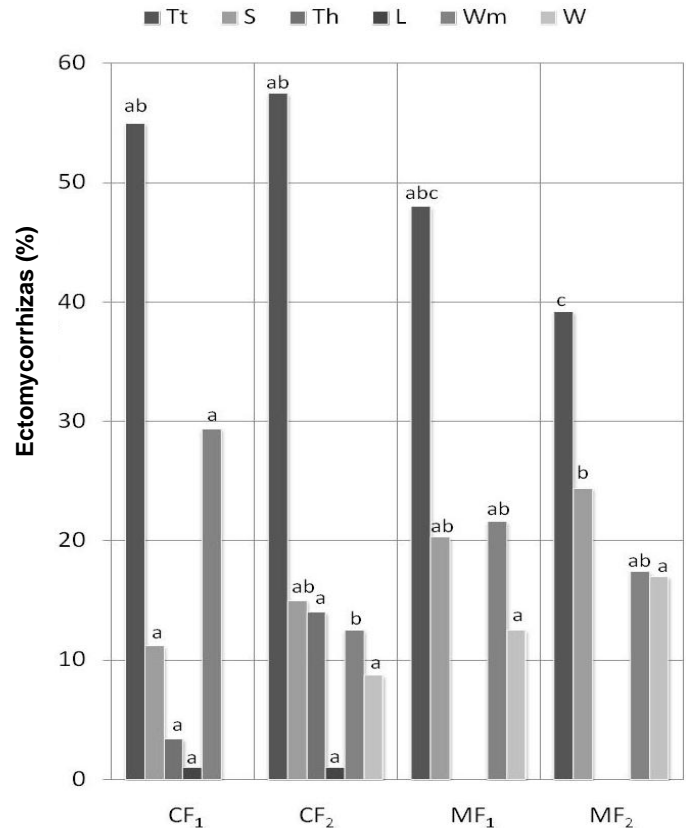
irrespective of the treatment type, mycorrhizas of *T. terrestris* dominated on roots of Scots pine seedlings. It was true in case of one- and two-year old Scots pine seedlings as well.

**Occurrence of mites**

Large differences in density of mites were noted in the study – the lowest density was found in the first year on the plot CF<sub>1</sub>, and the highest one – in the second year on the mulched plot MF<sub>1</sub> (Table 7).

Mulching was a factor influencing distinctly and positively the density of these arthropods. After treatment of mulching, the density of Acari increased 4 times in the first year, and in the second year – on the variant MF<sub>1</sub> – even 8 times as compared to that in non-mulched plots. Differences between variants C and M were significant in the first and in the second year.

Actinedida were the predominant order of mites on non-mulched plots – 62 to 69% of all Acari. On mulched plots saprophage Oribatida were the predominant order of mites (81 to 84%); predacious Gamasida as well as



**Figure 2.** Frequencies of mycorrhizal morphotypes of two-year old Scots pine seedlings (Tt – *Thelephora terrestris*, S – *Suillus* sp., Th – *Thelephoraceae*, L – *Lactarius* sp., Wm – *Wilcoxina mikolae*, W – *Wilcoxina* sp.).

Tarsonemida were less numerous.

**DISCUSSION**

Temperatures of May and August for the investigated period were lower than long-term values for these months. Żarski et al. (2010), on the base of the multi-year research, showed that in the vicinity of Bydgoszcz during the period 1949-2008 the mean air temperature of May and August were characterized by an increase with time. On the other hand, in opinion of Żarski et al. (2010), a statement that the climate in the vicinity of Bydgoszcz is getting warmer should be treated with caution due to – among others - different trends of linear equations that are dominated by insignificant equations. Similar results were noted for the Zielonka Forest (central part of Wielkopolska region) in the period from 1848 to 2008 year – the mean temperature time series for the investigated vegetation periods expressed statistically significant positive trends changes for March and May (Miller and Okoński, 2011).

It was stated that the irrigation requirements were

**Table 7.** Abundance ( $10^3$  individuals  $\cdot$  m $^{-2}$ ) of mites under differentiated organic fertilization and mulching.

Group of mites	Year	Treatments				M	M×F
		CF <sub>1</sub>	CF <sub>2</sub>	MF <sub>1</sub>	MF <sub>2</sub>		
Actinedida	I	1.43 <sup>a</sup>	2.58 <sup>a</sup>	2.03 <sup>a</sup>	2.53 <sup>a</sup>	ns	ns
	II	1.30 <sup>a</sup>	2.21 <sup>b</sup>	1.73 <sup>a</sup>	1.45 <sup>a</sup>	ns	ns
	Mean	1.37 <sup>a</sup>	2.40 <sup>b</sup>	1.88 <sup>a</sup>	1.99 <sup>a</sup>	ns	ns
Gamasida	I	0.28 <sup>a</sup>	-	0.63 <sup>a</sup>	0.60 <sup>a</sup>	<0.001	ns
	II	0.08 <sup>a</sup>	0.15 <sup>a</sup>	0.20 <sup>ac*</sup>	0.43 <sup>bc</sup>	0.026	ns
	Mean	0.18 <sup>a</sup>	0.08 <sup>a</sup>	0.41 <sup>b</sup>	0.51 <sup>b</sup>	<0.001	ns
Oribatida	I	0.03 <sup>a</sup>	0.10 <sup>a</sup>	4.16 <sup>b</sup>	6.65 <sup>b</sup>	<0.001	ns
	II	1.28 <sup>ax</sup>	1.9 <sup>ax</sup>	20.47 <sup>bx</sup>	14.67 <sup>b</sup>	<0.001	ns
	Mean	0.65 <sup>a</sup>	1.00 <sup>a</sup>	12.32 <sup>b</sup>	10.66 <sup>b</sup>	<0.001	ns
Tarsonemida	I	-	-	0.05 <sup>a</sup>	0.03 <sup>a</sup>	ns	ns
	II	-	-	0.05	-	ns	ns
	Mean	-	-	0.05 <sup>a</sup>	0.01 <sup>a</sup>	ns	ns
Acari total	I	1.73 <sup>a</sup>	2.68 <sup>a</sup>	6.87 <sup>b</sup>	9.81 <sup>b</sup>	<0.001	ns
	II	2.66 <sup>a</sup>	4.26 <sup>ax</sup>	22.45 <sup>bx</sup>	16.56 <sup>b</sup>	<0.001	0.032
	Mean	2.19 <sup>a</sup>	3.47 <sup>a</sup>	14.66 <sup>b</sup>	13.18 <sup>b</sup>	<0.001	ns

<sup>a</sup>, The same letter means lack of significant differences at  $p < 0.05$ ; \*, significant between I and II year,  $p < 0.05$ ; M, mulching effect (p), F, fertilization effect (p).

inversely proportional to rainfall amount in particular years. The higher were rainfall amounts, the lower were irrigation needs. Water deficits in the same forest nursery noted in 2003-2005 (Klimek et al., 2008, 2009) were higher (230-348 mm) than those during 2005-2007. The difference can be explained among others by lower rainfall (April 1 to September 30) which amounted from 177 to 244 mm, in 2003 and 2004, respectively. In comparison, average irrigation requirements of forest nurseries in 2000-2009 amounted 183 mm in the vicinity of Bydgoszcz but 116 and 170 mm in vicinities of Chojnice and Toruń, respectively (Rolbiecki et al., 2010). It should be mentioned that the region of Bydgoszcz is characterized by high irrigation needs (Żarski and Dudek, 2009; Żarski, 2011).

Total amounts of irrigation water were higher in case of one-year old seedling production cycle (2005-2006) than those for the two-year old seedlings (2006-2007). It resulted to a great extent from the different irrigation needs of the seedlings during their first or second growing season as well as from differentiated irrigation control (Pierzgalski et al., 2002; McDonald, 1984).

Mineral nutrient availability in soils of forest nurseries depends among others on a proper pH value. Acid or slightly acid pH value is needed for a suitable assimilability of nutrients by forest tree seedlings (Szołtyk and Hilszczańska, 2003). The measure of mulching with ectohumus decreased the pH value below 7.0. Decrease

of pH is favourable for seedlings. According to Szołtyk and Hilszczańska (2003), the  $pH_{KCl}$  optimum in forest nurseries should be in the range of 4.3 to 5.5 and 4.5 to 5.7, for the light soils and the heavy soils, respectively. In our previous experiment on Scots pine seedlings carried out in the same forest nursery soil pH values which were measured in H<sub>2</sub>O and 1-molar KCl, ranged from 6.95 to 7.23 and from 6.70 to 7.09, respectively (Klimek et al., 2008). Similar reaction (pH values neutral to alkaline) were also noted in trials on white birch seedlings, Conducted simultaneously at this nursery (Klimek et al., 2009).

In experiments on Scots pine seedlings which were carried out previously at the same forest nursery (Klimek et al., 2008), the studied soils were characterized by a considerable shortage of these elements (Zn, Cu and Mn). In this investigation the application of ectohumus (mulching) caused an increase of Zn, Cu and Mn.

In our experiments the height of seedlings was in the range 10.35 to 11.87 and 34.12 to 36.42 cm in case of one-year old seedlings and two-year old seedlings, accordingly. For comparison, in experiments on Scots pine seedlings which were carried out previously at the same forest nursery, the height of one-year old Scots pine seedlings was 8.9 cm on control plots, and this parameter was significantly increased by irrigation to 13.2 and 13.7 cm for micro-jet sprinkling and drip irrigation, respectively. In case of seedlings during their second

growing season (two-year old seedlings) in the above-mentioned trials, irrigation significantly increased the height from 26.4 to 33.6 and 34.8 cm for micro-jet sprinkling and drip irrigation, respectively.

The height of seedlings grown on plots mulched with ectohumus was significantly increased from 10.83 to 11.21 cm and from 34.77 to 35.27 cm, for one-year-old and two-year-old seedlings, respectively. These data are partly in agreement with results obtained in previous experiments conducted in 2003-2005 in nursery Białe Błota with Scots pine and white birch (Klimek et al., 2008, 2009). However, in the above-mentioned trials the introduction of edaphon (organic matter obtained from the surface of partial cutting in habitat of fresh coniferous forest) was carried out early directly before sowing in every year (2003 and 2004) on the whole area of experiments (all the investigated plots). In case of these experiments the measure of mulching was conducted later after emergence of Scots pine seedlings (in the years 2005 and 2006, respectively). For comparison, this growth parameter of Scots pine seedlings on plots fertilized with compost (sewage sludge 80% and highmoor peat 20%) under conditions of irrigation ranging from 14.4 to 15.2 and 38.4 to 39.4 cm, for one-year old seedlings and two-year old seedlings, respectively.

It is well known that irrigation influenced on the woody plant growth (for example, Senyigt and Ozdemir, 2011). Best results of the plant growth, especially for two-year-old seedlings, were noted at treatments MF<sub>2</sub> – the seedling height was 36.42 cm and its diameter, on average equaled 8.3 mm. In this experiment irrigation of the whole investigated area was done with the use of micro-sprinklers (Pierzgalski et al., 2002). These results corroborate those of Lamhamedi and Gagnon (2003) who showed that to produce quality seedlings, forest nursery irrigation and fertilization management must be optimized as well as those from experiments on Scots pine seedlings conducted previously at forest nursery Białe Błota (Klimek et al., 2008), a significant interaction of organic fertilization with irrigation was found. In case of the last mentioned trials, irrigated seedlings grown on plots fertilized with compost (sewage sludge and highmoor peat) were taller than plants cultivated under control conditions.

Dominance of *T. terrestris* might be resulted from abilities of this species to grown even in poor arable soils with high moisture as well as high nitrogen concentration (Castellano and Molina, 1989; Hilszczańska et al., 2008). A few authors is of the opinion that *T. terrestris* mycorrhizas are not optimal type of mycorrhizas since they cannot promote seedlings' growth (Stenstrom and Ek, 1990) as for instance other mycorrhizal fungi, for example, *Suillus bovinus* (L. ex Fr.) O. Kuntze (Bending and Read, 1995). Nevertheless, Lehto (1992) in short time experiment found that mycorrhizas of *T. terrestris* supported development and growth of *Picea abies* (L.) H. Karst seedlings affected by drought. A positive role of

*T. terrestris* in the colonization of Scots pine roots following the planting-out of seedlings on former farmland was found by Hilszczańska et al. (2008). The second, most abundant mycorrhizas on roots of two-year-old seedlings were these created by *W. mikolae* i *Wilcoxina* sp. Share of *H. crustuliniforme* mycorrhizas confirms hydrophilic character of the fungus, these mycorrhizas were very abundant on seedlings which grown in nurseries with high soil moisture (Hilszczańska, 2001).

Presence of ectendomycorrhizas, belonging to *Wilcoxina* (Ascomycota) indicates high concentration of nitrogen in the soil. Ectendomycorrhizas on conifer seedlings are especially abundant in nurseries where high doses of nitrogen are being used (Danielson, 1991). The latter mycorrhizas were found also on *Picea abies* seedlings growing in nurseries (Menkis et al., 2005; Rudawska et al., 2006). After seedling's outplanting from nurseries ectendomycorrhizas were observed to readily replace by other mycorrhizal fungi. The fungi can thrive on roots only when soil's environment is lack of competitive species (Danielson and Prudel, 1990).

Low number of *Lactarius* sp. mycorrhizas may be a reflection of weak competitiveness that indicates the fungus can only thrive in species – poor communities. Fungi belonging to genera *Lactarius* are thought to be late-stage fungi, being colonized older trees (Termorshuizen and Ket, 1991; Visser, 1995). However, fruiting bodies of *Lactarius rufus* (Scop.) Fr. had being found in multi-aged stands of *P. sylvestris* in Finland, Estonia and Netherlands (Termorshuizen and Ket, 1991; Hintikka, 1988; Kalamees and Silver, 1988). It seems that addition of litter to the nursery soil might be the cause of the presence of *Lactarius* mycorrhizas although those mycorrhizas were also found on seedlings from treatments with no addition of the litter. Hence, it is not so easy to answer why mycorrhizas of *Lactarius* emerged on young seedlings. One of probable scenarios is that spores of the fungus could come with the wind from nearby forests.

Mulching was the factor influencing positively the abundance of Acari; their density increased 4 times in the first year, and in the second year on the variant MF<sub>1</sub>; even 8 times as compared to that in non-mulched plots. In experiments on two-year old Scots pine seedlings which were carried out previously in the same forest nursery, the edaphon introduction after treatment of earlier mulching in comparison to this trial was less felicitous because the density of mites was about 50% lower (Klimek et al., 2008).

Oribatid mites composed about 70% of all mites in typical forest soils. The density of these mites in Scots pine forests is distinctly higher than that in the studied nursery, for example in Scots pine greenwood the density of Oribatida ranged 49,220 to 258,810 individuals m<sup>-2</sup> (Seniczak et al., 1997). The increased density of oribatid mites after treatment of mulching can be recognized as a very positive result of this measure because this confirm



that the edaphon introduction was well-timed and that the gatherings of mites were advantageously reconstructed and adapted to the structure which is specific for forest soils in that oribatid mites play a very important role. These mites can feed on ectomycorrhizal fungi (Schneider et al., 2004, 2005). On the other hand, edaphon can effect on fungal growth by grazing (Hanlon and Anderson, 1979, 1980), which also may split the hyphal connections. Soil animals are also able to relocate directly nutrients by defecation, and transport microbial propagules into fresh substrates (Lussenhop, 1992; Lussenhop and Wicklow, 1984).

Literature confirms that soil saprophages (including Oribatida) are regarded as good bioindicators (Andrews et al., 1989; Butovsky, 1996). The increase in density of Oribatida can indicate that biological properties of the investigated soils were improved (in some measure) due to mulching. It seems that mulching in a forest nursery influenced positively on a biological activity of the soil that immediately resulted in measured features of Scots pine seedlings.

## Conclusions

The soils of mulched plots in comparison to the soils without mulching were characterized by the following changes: decreased pH values, increased contents of  $C_{org}$  and  $N_{total}$  as well as increased concentrations of K, P, Zn, Cu and Mn.

Measure of mulching with ectohumus (forest edaphon inoculation) significantly increased the height of one-year-old seedlings and two-year-old seedlings. Best results in the case of two-year-old seedlings were observed on the plots mulched with ectohumus and fertilized with treated sewage sludge with sawdust additive.

Mycorrhizal structure is well developed. This factor is promising regarding improvement of seedlings' adaptation on post-agricultural land. Sewage's sediments used along with sawdust and pine bark did not influence negatively on ectomycorrhizae development of juvenile seedlings.

Mulching distinctly increased the density of mites, especially saprophage Oribatida which can prove about the positive influence of this measure on the increase of the bioactivity in the investigated soils.

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