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ORIGINAL ARTICLE

Growth and mycorrhizal colonisation of naturally regenerating Scots pine *Pinus sylvestris* (L.) in relation to microsite conditions created by different site preparation methods

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Abstract

Mechanical site preparation (MSP) is recommended for natural regeneration of the Scots pine (*Pinus sylvestris* L.). The study aimed at comparing the effects of three MSP methods [LPz 75 double-mouldboard forest plough (FP), single-mouldboard U-162 active plough (AP) and FAO-FAR FV 4088 forest cutter (FC)] which in different ways interfere with the soil environment and influence the growth of naturally regenerating Scots pine seedlings and their mycorrhizal colonisation in the first year after self sowing.

Pine seedlings growing within and outside strips prepared with FC did not have different growth parameters and mycorrhizal colonisation, but those from ridges were bigger and less mycorrhized than seedlings growing in furrows. This difference was bigger in FP than in AP and was probably caused by more favourable trophic conditions and presence of polyphenols in ridges. Removal of the organic layer in furrows did not limit a degree of mycorrhizal colonisation. Ploughing direction had no significant impact on pine features analysed. The trend towards improved growth in furrows formed with FP can be noticed in NW-SE variant. Higher mycorrhizal colonisation was found in seedlings growing in all microsites in the NW-SE variant. However, these differences were not confirmed statistically. Growth parameters of pines located within the area the edge and in the centre of the experimental clear-cut were to a great measure influenced by light conditions; seedlings from the edge were higher, had less lateral shoots, and shorter roots than those growing in the middle of the clear-cut.

Key words

ectomycorrhiza, mechanical site preparation, microsite, natural regeneration, *Pinus sylvestris*, seedling growth

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Introduction

Mechanical site preparation (MSP) is commonly used in Poland and other countries, to enable forest regeneration of clear-cuts (Andrzejczyk and Drozdowski 2003; Hille and den Ouden 2004; Karlsson and Nillsson 2005). Clear-cutting and MSP may change diversity and abundance of soil organism communities by altering physical and chemical properties of soil as well as its structure (Mallik and Hu 1997; Bird et al. 2004). Populations of microorganisms and ectomycorrhizal fungi within clear-cuts may change in response to changes in carbon availability (decrease in litter supply) and microclimate conditions (increase of soil temperature, variation in moisture content) (Jones et al. 2003). MSP may cause reduction of a level of natural ectomycorrhizal inoculum in soil, as ectomycorrhiza density is higher in disturbed or removed soil organic layer than in mineral layer which is less affected by MSP (Brundrett and Abbott 1995; Jurgensen et al. 1997; Baier et al. 2006b). However, some authors report that inoculum levels in mineral soils from many coniferous forests or clear-cuts do not limit mycorrhizal colonisation, although ectomycorrhiza density is much higher in these soils' organic horizon of (Harvey et al. 1996; Hashimoto and Hyakumachi 1998). MSP often causes dislocation of inoculum rather than its elimination. The consequence of this dislocation may result in a temporal (approx. 2 years) reduction in the number and diversity of ectomycorrhizae (Jones et al. 2003).

Diffuse hyphae or rhizomorphs extending from living mycorrhizae and hyphae in the mantle constitute important sources of inoculum. This inoculum determines the level of colonisation of seedlings in relation to their location within the clear-cut area. Number of active ectomycorrhizas in soil volume unit is higher in peripheral parts of the clear-cut, within a few meters away from a surrounding stand. The higher number of ectomycorrhizae may result in better mycorrhization of seedlings growing in clear-cuts (Harvey et al. 1980; Parsons et al. 1994; Hagerman et al. 1999a, 1999b).

There are many potential MSP methods differing in a degree of impact on soil, as measured by the area and depth of disturbed soil (Bedford and Sutton 2000; Nordborg and Nilsson 2003). On clear-cuts, the MSP methods form microsites differing in soil properties: physical (mechanical resistance and aeration), chemical (nutrient availability) and microclimate (temperature and moisture) (MacKenzie et al. 2005; Morris et al. 2006). This leads to unequal conditions for tree growth within different microsites (MacKenzie et al. 2005).

In Poland, reforested areas are ploughed north-south (most often all along clear-cut longer side). This minimises shadowing of tree seedlings and soil (Bedford and Sutton 2000). A change in ploughing direction from north-west to south-east would mean that the ridge would put a larger part of the furrow in shadow during the most intensive insolation. Such positioning could positively influence mycorrhizal growth and colonisation due to possible lesser water loss in furrow soil. However, this has not been yet investigated.

Nowadays, in addition to the traditional double-mouldboard forest plough (FP), active plough (AP) and forest cutter (FC) are used as MSP tools in Polish forestry. The tools indicate the advantage of a lesser impact on the soil environment. The furrow and the ridge made with FP and AP, the strips prepared by FC as well as untouched soil out of the strips provide different microsite conditions for regenerating seedlings within the clear-cut (MacKenzie et al. 2005).

Our study aimed at comparing the influence of three MSP methods that in different ways interfere with the soil environment – hence create different microsite conditions, on the growth of Scots pine natural regeneration and its mycorrhizal colonisation in the first year after natural seeding. We expected that seedling growth and mycorrhization level would differ according as the following aspects (1) microsite conditions: furrow, ridge, strip, outside strip; (2) ploughing direction: N-S or NW-SE; (3) seedling location within the clear-cut (centre vs. edge).

Material and methods

Study site

The study was carried out in the Ostrów Mazowiecka Forest District (52°80' N, 21°90' E; WGS 84), located 70 km north-east of Warsaw. The mean annual temperature in this region is 8.3°C, the warmest month is July (19.2°C) and the coolest -January (–0.5°C). Vegetation period begins in mid-April and lasts on average 210 days. The mean annual precipitation is 542 mm with approx. 35% of rainfall during the vegetation period (Anonymous 2005).

Soil at the study site is podzols formed from glacial sands with mor-type humus. Before clear-cutting, the area was vegetated with aprox. 120-year-old Scots pine (*Pinus sylvestris* L.) stand with underbrush including *Sorbus aucuparia* L., *Picea abies* (L.) H. Karst, *Juniperus communis* L. and *Quercus petraea* Liebl. and ground vegetation with *Vaccinium myrtillus* L., *Hylocomium splendens* Hedw., *Convallaria majalis* L., *Solidago virga-aurea* L., *Vaccinium vitis-idea* L. and *Pleurozium schreberi* (Willd.) Mitten. The plant community was described as *Peucedano-Pinetum typicum* (Anonymous 2005). The stand was clear-cut in winter 2005 and spring 2006.

Treatments

Four clear-cuts 420 m long and 55–60 m wide were selected. Each of them was divided into six 70 m long parts. In autumn 2006, MSP was performed in two directions: from north to south (N-S) and from north-west to south-east (NW-SE) using a LPz 75 double-mouldboard forest plough (FP), a single-mouldboard U-162 active plough (AP) and a FAO-FAR FV 4088 forest cutter (FC). FP forms rectangular furrows exposing mineral soil (f1). The upper layer of the cut-out, formed ridge consists of mineral soil. Humus layer, litter and ground vegetation are located below (r1). Rotating the mouldboard of the AP gives the furrow a parabolic shape. Mineral soil is exposed and often covered with some humus that remains or falls down from the mouldboard (f2). Partial mixture of litter, humus and mineral soil takes place on the ridge (r2). FC crushes and mixes ground vegetation, litter, humus layer and mineral soil to a depth of 35 cm in strips 40 cm wide. The distance between strips with untouched soil is 110 cm (os) (Neugebauer 2008).

Seedling sampling and measurements

At the end of September 2007, 384 one-year-old naturally regenerated Scots pine seedlings were collected together with soil from: the western edge of the clear-cut (up to 5 m from the stand) and from the clear-cut centre (25–30 m from the stand), as well as furrow/strip and ridge/out of strip. From each of the 24 experiment variants (preparation method × ploughing direction × microsite × seedling location on the clear-cut), 16 seedlings were taken. Samples were put into labelled plastic bags.

In the laboratory, seedlings were kept frozen at –10°C. For each seedling we measured the diameter of the root collar and shoot length, and we determined the number of lateral shoots. After drying at 105°C, the weight of above and belowground seedling biomass was determined. Böhm's (1985) crossing method was used to assess the total length of the root system. The method is based on random positioning of the roots on a net of squares and counting the number of lines they cross. Roots length [cm] was calculated according to the following formula:

$$L = 11/14 \times n \times a$$

where:
n – the number of crossings with net of squares,
a – the length of square side (0.5 cm).

After washing under running water, the root systems were analysed with the use of a dissecting microscope (Nikon SMZ 645, 10–50×). The degree of mycorrhization was estimated in a random subsample form each root system. For that purpose, lateral roots were taken from the upper, centre and lower part of the root system, then cut into pieces (ca 2 cm long) and placed into Petri dishes with water. From each seedling, 100 fine roots were analysed. Ectomycorrhizal tips were identified by the presence of mantle (colour, shape and surface texture), external hyphae, rhizomorphs, a slightly swollen apex and absence of root hairs. Root branching index [pcs./cm] was calculated as the number of mycorrhizal and non-mycorrhizal root tips.

Statistical analyses

The normality of data distribution in individual variants was tested with the Shapiro-Wilk test and homogeneity of variance was tested using Levene's test. Due to differences in the homogeneity of variance and deviance from data normal distribution within samples, the non-parametric Mann-Whitney Z test ($N > 20$) was used to prove significant differences of two study variants or the Kruskal-Wallis H test for multiple comparisons were used. The post-hoc multiple comparison of average rank test (Siegel and Castellan 1988) was used to detect homogenous groups of experiment variants. For relationships between the number of mycorrhizal tips and seedlings growth features we used Spearman rank correlation analysis. All statistical tests were performed with STATISTICA 9.0 software (StatSoft, Inc., USA).

Results

Trees from FP-made furrows (f1) had almost all biometric parameters significantly smaller than seedlings growing on the ridges (r1). The only exception was the root branching index on the basis of which no significant differences were observed. Smaller differences concerning only 3 out of 7 growth parameters were found for seedlings growing in the furrows (f2) and on the ridges (r2) prepared using AP. Pines from the latter variant were higher, thicker in root collar and characteristic of a lower root branching index value. Only the root branching index differed seedlings growing on FC strips from those growing out of FC strips. The analysis of pines from all MSP variants revealed that FP-furrow seedlings were the smallest, while FP-ridge seedlings were the largest (Tabs. 1, 2).

Ploughing direction had little influence on the growth parameters analysed. In seedlings growing under same microsite conditions in N-S and NW-SE variants no statistically significant differences in biometric features were found. However, some tendencies of changes in growth parameters can be noted, especially for FP furrows and ridges. The change of ploughing direction from N-S to NW-SE caused improvement of growth conditions in the furrow and their worsening on the ridge (Tabs. 3, 4).

Pines growing on the edge of the clear-cut were significantly higher, and had fewer lateral shoots as well as lower dry root biomass. The seedlings did not differ in terms of other investigated features (Tab. 5).

No difference was noted in pine seedlings growing in or out of the strips made with FC. Greater variability was observed in pines growing in FP furrows and ridges than in the case of AP treatment. The highest

Tab. 1. Growth parameters (mean and standard error in parentheses) of aboveground part of one-year-old Scots pine seedlings from natural regeneration in relation to microsite conditions created by different site preparation methods

Treatment		Shoot length (cm)	Root collar diameter (mm)	Aboveground dry mass (g)	Number of lateral shoots (pcs)
Forest plough	f1	5.72(0.15) x a	1.39(0.05) x a	0.306(0.03) x a	1.7(0.19) x a
	r1	6.78(0.22) y b	1.79(0.06) y c	0.543(0.03) y c	2.3(0.16) y a
Activ plough	f2	6.04(0.18) x a	1.43(0.04) x ab	0.339(0.02) x ab	2.0(0.18) x a
	r2	6.79(0.21) y b	1.56(0.04) y bc	0.411(0.03) x b	2.0(0.17) x a
Forest cutter	s	6.37(0.15) x a	1.52(0.04) x ab	0.430(0.03) x b	2.3(0.23) x a
	os	6.58(0.23) x b	1.64 (0.05) x c	0.421(0.03) x bc	2.2(0.19) x a

Explanations: f1 – furrow formed by the forest plough, s1 – ridge formed by the forest plough, f2 – furrow formed by the active plough, s2 – ridge formed by the active plough, s – strip made by the forest cutter, os – untouched soil, outside the strip; x, y – various letters indicate significant difference in the Mann-Whitney Z test, $p \leq 0.05$; a, b, c – various letters indicate significant difference in the Kruskal-Wallis test, $p \leq 0.05$.

Tab. 2. Growth parameters (mean and standard error in parentheses) of one-year-old Scots pine seedlings roots from natural regeneration in relation to microsite conditions created by different site preparation methods

Treatment		Root length (cm)	Root dry mass (g)	Root branching index (pcs/cm)
Forest plough	f1	60.5(3.4) x a	0.052(0.004) x a	5.3(0.22) x ab
	r1	79.6(4.9) y b	0.078(0.005) y b	4.9(0.20) x ab
Activ plough	f2	74.3(3.9) x ab	0.060(0.004) x ab	5.3(0.14) y b
	r2	87.2(5.5) x b	0.061(0.003) x ab	4.4(0.14) x a
Forest cutter	s	75.6(5.2) x ab	0.064(0.005) x ab	5.5(0.19) y b
	os	65.4(4.2) x ab	0.058(0.004) x a	4.7(0.17) x a

Explanations: see Tab. 1.

Tab. 3. Growth parameters (mean and standard error in parentheses) of aboveground part of one-year-old Scots pine seedlings from natural regeneration in relation to ploughing direction

Treatment		Shoot length (cm)	Root collar diameter (mm)	Aboveground dry mass (g)	Number of lateral shoots (pcs)
FP f1	N-S	5.54(0.17) x a	1.30(0.04) x a	0.269(0.02) x a	1.6(0.29) x a
	NW-SE	5.89(0.25) x ab	1.48(0.08) x a	0.343(0.04) x ab	1.8(0.24) x a
FP r1	N-S	7.12(0.34) x b	1.83(0.09) x b	0.554(0.05) x c	2.3(0.22) x a
	NW-SE	6.44(0.27) x ab	1.75(0.08) x b	0.531(0.04) x c	2.4(0.22) x a
AP f2	N-S	5.91(0.23) x ab	1.47(0.06) x a	0.369(0.03) x ab	2.2(0.22) x a
	NW-SE	6.16(0.28) x ab	1.39(0.06) x a	0.309(0.02) x ab	1.8(0.29) x a
AP r2	N-S	7.04(0.28) x b	1.59(0.06) x ab	0.428(0.04) x abc	1.9(0.25) x a
	NW-SE	6.53(0.31) x ab	1.54(0.06) x ab	0.395(0.04) x abc	2.2(0.23) x a
FC s	N-S	6.54(0.21) x ab	1.57(0.07) x ab	0.497(0.05) x bc	2.7(0.39) x a
	NW-SE	6.20(0.20) x ab	1.48(0.05) x ab	0.363(0.02) x ab	1.9(0.22) x a
FC os	N-S	6.42(0.29) x ab	1.61(0.07) x b	0.381(0.03) x abc	2.0(0.25) x a
	NW-SE	6.74(0.34) x ab	1.68(0.07) x b	0.460(0.04) x bc	2.5(0.29) x a

Explanations: FP – double-mouldboard forest plough, AP – active plough, FC – forest cutter, N-S, NW-SE – ploughing direction. For further explanations see Tab. 1.

Tab. 4. Growth parameters (mean and standard error in parentheses) of one-year-old Scots pine seedlings roots from natural regeneration in relation to ploughing direction

Treatment		Root length (cm)	Root dry mass (g)	Root branching index (pcs/cm)
FP f1	N-S	57.5(4.8) x a	0.047(0.004) x a	5.8(0.38) y b
	NW-SE	63.4(4.9) x ab	0.058(0.007) x ab	4.8(0.16) x ab
FP r1	N-S	87.6(7.4) x b	0.081(0.007) x b	4.5(0.25) x ab
	NW-SE	71.6(6.3) x ab	0.076(0.007) x b	5.3(0.31) x ab
AP f2	N-S	76.2(5.3) x ab	0.064(0.005) x ab	5.3(0.21) x ab
	NW-SE	72.5(5.8) x ab	0.056(0.005) x ab	5.4(0.19) x ab
AP r2	N-S	90.7(9.5) x b	0.063(0.005) x ab	4.4(0.21) x a
	NW-SE	83.7(5.6) x b	0.059(0.004) x ab	4.5(0.18) x ab
FC s	N-S	76.4(8.7) x ab	0.068(0.008) x ab	5.5(0.25) x b
	NW-SE	74.9(5.9) x ab	0.059(0.005) x ab	5.5(0.29) x b
FC os	N-S	66.0(6.7) x ab	0.056(0.006) x ab	5.1(0.28) x ab
	NW-SE	64.9(5.1) x ab	0.061(0.006) x ab	4.4(0.20) x a

Explanations: see Tab. 1, 3.

mycorrhizal colonisation level (90.2%) was observed in the seedlings from FP furrows and the lowest was found for those growing on FP ridges (42.5%) and AP ridges (52.5%) (Fig. 1).

Ploughing direction had no significant effect on the analysed features. Although pines from NW-SE furrows or strips were a little more mycorrhized than those growing in N-S variants, statistically significant differences were found only for seedlings growing in AP-furrows (Fig. 2). No significant differences were observed in mycorrhizal colonisation of pines growing on the edge (67.4%) and in the centre (65.5%) of the clear-cut. The seedlings from individual microsites that grew on the edge of the clear-cut exhibited a higher level of mycorrhizal colonization than those in the clear-cut centre, except for the seedlings on FP ridges. However, significant differences were observed only among pines out of strips, in FC experimental variant (Fig. 3).

Tab. 5. Growth parameters (mean and standard error in parentheses) of one-year-old Scots pine seedlings from natural regeneration in relation to location on the clear-cut area

Feature	Location on the clear-cut area	
	edge	centre
Shoot length (cm)	6.64(0.12) y	6.12(0.10) x
Root collar diameter (mm)	1.55(0.03) x	1.56(0.03) x
Aboveground dry mass (g)	0.397(0.02) x	0.419(0.02) x
Number of lateral shoots (pcs)	1.9(0.10) x	2.3(0.11) y
Root length (cm)	70.9(2.5) x	76.6(2.9) x
Root dry mass (g)	0.058(0.002) x	0.066(0.003) y
Root branching index (pcs/cm)	5.1(0.11) x	4.9(0.10) x

Explanations: see Tab. 1.

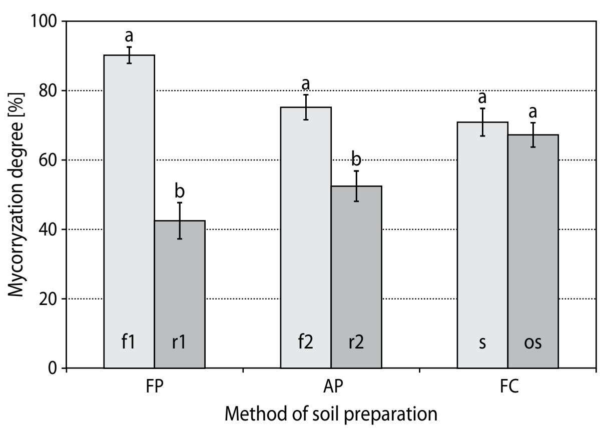

Fig. 1. Mycorrhization degree (mean ±SE) of one-year-old Scots pine seedlings from natural regeneration in relation to microsite conditions created by site preparation method

Explanations: FP – double-mouldboard forest plough, AP – active plough, FC – forest cutter, f – furrow, r – ridge, s – strip, os – outside the strip; the same letters indicate lack of significant differences in the Mann-Whitney Z test, p ≤ 0.05.

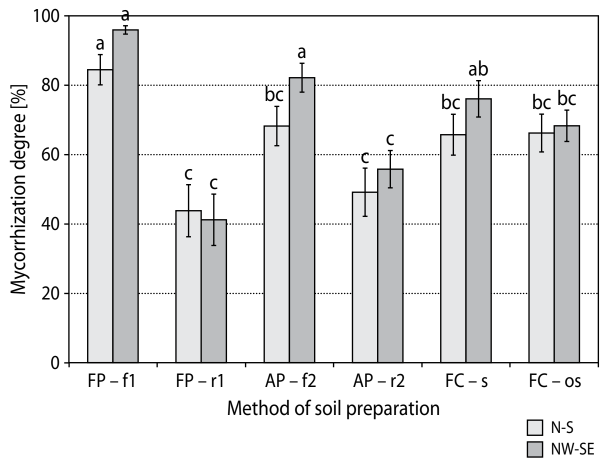

Fig. 2. Mycorrhization degree (mean ±SE) of one-year-old Scots pine seedlings from natural regeneration in relation to microsite conditions and ploughing direction

Explanations: N-S, NW-SE – ploughing direction. The same letters indicate lack of significant differences in the Kruskal-Wallis test, p ≤ 0.05. For further explanations see Fig. 1.

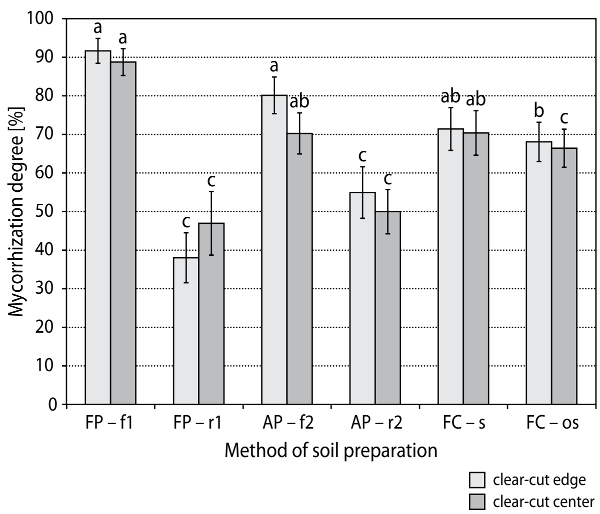

Fig. 3. Mycorrhization degree (mean ±SE) of one-year-old Scots pine seedlings from natural regeneration in relation to microsite conditions and location on the clear-cut (edge, centre)

Explanations: see Fig. 1, 2.

Spearman rank correlation coefficients, between numbers of mycorrhized root tips in 100 analysed tips per seedling and growth parameters, are presented in Tab. 6. Shoot length, diameter of the root collar and dry aboveground biomass were significantly negatively correlated with the number of mycorrhizal root tips. On the other hand, the root branching index was positively correlated with the number of mycorrhizal root tips. The number of side shoots, root system length and biomass were not significantly correlated with the number of mycorrhizal root tips.

Tab. 6. Spearman rank correlation coefficients between frequency of mycorrhizal root tips and growth parameters of one-year-old Scots pine seedlings from natural regeneration

Feature	Number of mycorrhizal root tips in sample of 100 analysed root tips per seedling
Shoot length (cm)	–0.194*
Root collar diameter (mm)	–0.196*
Aboveground dry mass (g)	–0.232*
Number of lateral shoots (pcs)	-0.098^{ns}
Root length (cm)	-0.082^{ns}
Root dry mass (g)	-0.081^{ns}
Root branching index (pcs/cm)	0.315*

Explanations: n = 384; *$p \leq 0.0001$; ns – not significant $p > 0.05$.

Discussion

Various trophic conditions characterise different microsites (MacKenzie et al. 2005; Baier et al. 2006a). In the present study, the highest values of growth parameters were observed for pine seedlings growing on FP ridges. The trees utilised nutrients abundant in the humus layer, lying under a thin layer of mineral soil or under the double organic layer, in places where there was no ground vegetation. According to Andrzejczyk 2000 the poorest trophic conditions are observed in FP furrows. On podzols, after removal of the thin humus layer, seedlings grow in an exposed eluvial horizon with little nutrient content (Andrzejczyk 2000; Andrzejczyk and Drozdowski 2003). This may be the reason for the smallest values of the growth parameters observed in pines on FP furrows. Slightly better conditions characterised AP furrows. They are shallower and humus remains on their surfaces in small portions, which results in better growth parameters achieved by pines growing in AP furrows when compared with FP furrows (Andrzejczyk 2000).

In turn, inside AP ridges, the organic layer is partly mixed with mineral soil, which create similar conditions to those in FC strips. A microclimate develops which is favourable for activity of microorganisms participating in the processes of mineralisation or decomposition of organic matter. Nutrient availability for seedlings growing in mixed soil is better than in furrows (Lundmark-Thelin and Johansson 1997; Mallik and Hu 1997). Growth parameters of pines growing in AP ridges and FC strips were on the one hand worse when compared with seedlings from FP ridges, but better than growth parameters of the seedlings from FP and AP furrows. A 10-year trial with *Pinus contorta* Dougl. Ex Loud. proved that the trees grew poorer when planted in furrows in comparison with those growing on the ridges and untouched soil (Bedford and Sutton 2000).

In our study, the ploughing direction had no significant influence on the growth parameters observed and mycorrhizal colonisation. According to Bedford and Sutton (2000) a switch in direction from N-S to NW-SE causes changes in light conditions and soil moisture. However, inthe present study ploughing direction change was probably too little for pine seedlings to respond with faster growth and increased mycorrhizal colonisation – possible to be confirmed statistically. Notwithstanding, some tendencies may be recognised in the change of growth parameters values, especially as far as FP furrows and ridges are concerned. The switch in ploughing direction applied resulted in better growth conditions in the furrow and worse growth conditions on the ridge. Higher colonisation degrees were likewise observed in pines from NW-SE ploughing treatment. However, the difference was not statistically significant.

Ecological conditions of seedling growth are different on the edge and in the centre of the clear-cut. Within the area of some meters away from the stand, light conditions are worse in comparison to the more distant part of the clear-cut (Andrzejczyk 2000; Walters et al. 2006). The range of the root system may vary from 5 up to even 16 m from the stem (Brockway and Outcalt 1998; McGuire et al. 2001). Presence of tree roots in soil (Taskien et al. 2003; Walters et al. 2006) as well as a limited amount of precipitation reaching the ground, so called 'rain shadow' (Andrzejczyk 2000), restrict availability of soil water. A decrease in moisture in this area may slow down decomposition of dead organic matter and nitrification of nitrogen compounds, which may in turn lead to limitation of N in soil (Prescott et al. 2003; Walters et al. 2006). According to our results, in the first year of seedling growth, light conditions seem to have the greatest impact on growth parameters. Faster height growth, smaller numbers of side shoots and lower root dry biomass of pines growing on the edge of the clear-cut than those of trees from the centre, support this idea. Worsening of trophic conditions within the edge zone of the clear cut probably takes place during more than just one vegetation period.

Beside trophic, moisture and light conditions, a level of mycorrhizal colonisation is an important factor shaping the growth of seedlings in the beginning of their life. It is thought that from 10 to 30% of tree photosynthesis products are located in the mycorrhiza mycelium depending on plant and fungus species, bedrock type, nutrient content and weather conditions (Fogel and Hunt 1979; Vogt et al. 1982; Högberg and Högberg 2002; Högberg et al. 2008). This may result in restrictions in seedlings growth (Stenström and Ek 1990). In our research the above results were confirmed by obtained negative correlation between biometric features of the aboveground part of seedlings and the number of mycorrhizal root tips. Both ecological conditions and degree of mycorrhization shaped growth parameters of the seedlings observed.

The best conditions for mycorrhiza formation were in furrows and the poorest on the ridges prepared with FP. Conditions of mycorrhiza establishment and development varied on individual microsites according as inoculum availability (Lazaruk et al. 2008), soil moisture (Andrzejczyk 2000) and abundance of polyphenols (Conn and Dighton 2000; Jonsson et al. 2006). Many authors report that density of both sclerotia and ectomycorrhizal root tips are highest in the organic soil horizon (Visser 1995; Jurgensen et al. 1997; Hashimoto and Hyakumachi 1998; Baier et al. 2006b). Decaying wood is especially important source of ectomycorrhizal inoculum (Kropp 1982; Väre 1989; Tedersoo et al. 2003). That is why removal of organic horizon in furrows may cause decreased in inoculum availability, at least in the case of some fungal species (Mah et al. 2001). However, roots of seedlings growing in furrows may develop mycorrhiza with species whose sclerotia or ectomycorrhizal root tips occur in mineral soil (Rosling et al. 2003; Tedersoo et al. 2003; Baier et al. 2006b). Some fungi colonise seedlings in regenerating forest stands from spores (Jones et al. 2003). Pioneer fungi such as *Suillus*, *Scleroderma* or *Geastrum* formed the most common ectomycorrhiza on naturally regenerating seedlings growing in clear-cuts (Ingleby et al. 1998). According to Redecker et al. 2001 similar dispersal strategy characterises some ectomycorrhizal fungi from mature stands (e.g. *Amanita francheti* (Boudier) Fayod, *Russula cremoricolor* Earle, *Lactarius xanthogalactus* Peck). The highest degree of mycorrhizal colonisation exhibited by pines growing in furrows proves that removal of organic horizon does not restrict colonisation process in mineral soil. Our results are consistent with those presented by Hashimoto and Hyakumachi (1998) and Harvey et al. (1996).

Beside inoculum availability, soil moisture is the factor that influences the mycorrhization level of roots. Seedlings growing in ridges formed with the use of FP or AP method may have suffered from water shortage (Agestam et al. 2003). More intensive drying of analysed ridges may be a result of relatively low precipitation in the study area. In the case of FP ridges, intensive drying is additionally caused by the lack of water oozing caused by ‘air bubble’ that occurs in places where ground vegetation is intensively developed or thick layers of poorly decomposed mor-type humus occurs (Andrzejczyk 2000). Seedlings in furrows had better moisture conditions because of water oozing (Andrzejczyk 2000). Low soil moisture on the ridges may restrict mycorrhizal colonisation (Erland and Taylor 2003). Root length and the root branching index may prove that such phenomenon did not occur in this study. Greenhouse experiments show that drought periods favour formation of wide-spread root system, while irrigation stimulates roots lengthening (Feil et al. 1988). Also, Kottke and Agerer (1983) found higher density of fine roots on sites where soil was regularly dried, in comparison with places with sufficient amount of moisture. The longest and less spread roots of seedlings from FP and AP ridges point out that these microsites had good moisture conditions. These results could be due to the fact that soil was prepared in the autumn of the year prior to the natural seeding. Soil preparation in autumn enabled accumulation of moisture in the organic horizon in ridges. This especially relates to microsites on FP ridges (Strzelecki and Sobczak 1972).

Polyphenols present in litter and humus influence the structure of ectomycorrhizal fungi communities (Conn and Dighton 2000; Souto et al. 2000; Jonsson et al. 2006). Baar et al. (1994) reported that growth of the majority of tested ectomycorrhizal fungi was inhibited, although water extracts from litter formed of Scots pine needles and shoots stimulated this process in some tree species. Remains of ericaceous plants, rich in phenolic compounds that are present in litter and humus (Mallik and Pellissier 2000; Mróz and Demczuk 2010), caused inhibition of growth and respiration of mycorrhizal fungi (Boufalis and Pellissier 1994; Boufalis et al. 1994; Souto et al. 2000). Hence, the litter and humus, containing leaves of *V. myrtillus* in furrows formed with FP and AP ploughs as well as in strips prepared following the FC method, could be the reason of the poorer mycorrhization of seedlings.

The degree of mycorrhization of pines growing on the edge and in the centre of the clear-cut did not differ significantly. Probably, during the short period of time that had passed since stand removal loss of inoculum in the central parts of the area did not occur. Moreover, it did not concern only inoculum in the form of sclerotia or spores that are able to survive some years in post-clear-cut soil (Hagerman et al. 1999b), but first of all – extraradical hyphae extending from metabolically active ectomycorrhizas. Harvey et al. (1980) as well as Parsons et al. (1994) proved that in the case of Douglas-fir, western larch and lodgepole pine forest, the total loss of active ectomycorrhizas took place in two vegetation periods after stand removal.

Conclusions

Various pine growth and mycorrhiza colonisation conditions characterised individual microsites (furrow, ridge, strip, unscarified soil out of the strip). Pines in furrows were smaller but had a higher degree of mycorrhizal colonisation than on the ridges. No difference in growth parameters and mycorrhization level was observed between seedlings, regarding location on or out of the strip. The same applies to the degree of mycorrhizal colonisation of seedlings from different microsites within variants with the same soil preparation tool.

Ploughing direction had no significant influence on pine growth parameters and mycorrhizal colonisation. Worse light conditions on the edge of the clear-cut may result in high growing of trees near the stand. These trees were higher but had less lateral shoots and shorter roots than those from the clear-cut centre. There was a lack of differences in mycorrhizal colonisation of pines from the edge and centre of the clear-cut. This lack was probably caused by the short period that had passed since stand removal and the remaining inoculum in type of sclerotia and spores, but mainly because of all the metabolically active mycorrhiza roots in the soil.

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