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Dorota Hilszczańska

Mycorrhizal status of Scots pine *Pinus sylvestris* L. seedlings grown in watered and non-watered nursery condition

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Abstract: This study describes the effect of watering on the mycorrhizal development and growth of Scots pine seedlings in a bare root nursery. Seedlings of Scots pine, grown under nursery conditions on natural soil (loamy sand) and soil + litter, were subjected to two different watering regimes for five months. During this time, measurements of soil water potential were made. Seedlings grown in natural soil and subjected to drought conditions were of significantly greater shoot height and volume and they had one mycorrhizal morphotype more than watered seedlings. However, irrigated seedlings subjected to excessive watering possessed greater mycorrhizal colonization: 46% on natural soil and 72% on soil + litter, while non-irrigated seedlings had 36% and 67% levels of mycorrhizal colonization, respectively.

Additional key words: ectomycorrhizas, Field Water Capacity, watering

Address: D. Hilszczańska, Forest Research Institute, Sękocin, 05-090 Raszyn, Poland,
e-mail: D.Hilszczanska@ibles.waw.pl

Introduction

A mycorrhiza is a mutualistic symbiosis between fungi and a plant root. The fungus receives carbohydrates from the plant, and increases resource availability and nutrient uptake for the plant in various ways. Through such increased resource availability and the alleviation of stress, mycorrhizae alter plant growth and fitness (Smith and Read1997).

Mycorrhizal development is influenced by various soil environmental conditions, e.g. pH, the availabilities of nitrogen and phosphorus, moisture and temperature. Environmental gradients, particularly in precipitation, often help explain patterns of species or community distribution across landscapes (Rosenzweig and Abramsky 1993). The distribution of ectomycorrhizal species is also potentially affected by moisture availability.

Many factors determine site conditions under which particular fungal species are likely to succeed (Molina et al. 1992). Some mycorrhizal fungi especially *Pisolithus tinctorius* (Pers.) Coker and Couch, are suited to dry habitats (Marx and Cordell 1989) while others (e.g. *Laccaria* spp., *Hebeloma* spp., *Thelephora* spp.) appear to prefer wetter locations (Stenström and Ek 1990; Stenström 1991).

Thelephora terrestris, *Laccaria* spp. and E-strain fungi are able to tolerate the unnaturally wet, fertile, and frequently disturbed nursery environment (Cottus and Nicoll 1990; Danielson and Visser 1990). Unestam and Sun (1995) found hydrophilic properties of the extrametrical hyphae (called “substrate adhesion hyphae” are most likely responsible for the uptake of water and nutrients) of *T. terrestris*, *Cenococcum geophilum*, *Laccaria laccata* and *Hebeloma crustuliniforme*.

This could be the reason why these fungi often spontaneously and successfully colonize seedlings in nurseries (Dighton and Mason 1985) According to the nursery routine, seedlings are often watered regularly: therefore to colonize the roots the mycorrhizal fungi have to withstand a prolonged or short period of high water content in soil or substrate. These fungi could, in this respect, be described as being adapted to open communities and tolerant toward moisture (Rayner and Boddy 1988).

The purpose of this study was to investigate the influence of regular irrigation vs no irrigation on mycorrhizal formation of Scots pine seedlings .

Materials and methods

The experiment was performed in a bare root nursery (in Garwolin Forest District) on weakly loamy sand and the same soil + litter (pine and spruce needles (v:v), 2m^3 litter per 100m^2 of soil surface). Amendment of litter was in order to improve the quality of soil, this nursery has been established 20 years ago. Plants were grown from seeds obtained from Poland's Forest Gene Bank (Susz, lot 99 stand 998), at the beginning of May (year 2000). Seedlings were watered in accordance with the Polish nursery routine, i.e. 2 mm every day from the beginning of May to 15 June, after that time 5 mm every second day to the end of August (Wytyczne… 1991). Seedlings of the control treatment were not watered, receiving natural precipitation only. All plants from the two treatments were subjected to the same atmospheric conditions. Precipitation and air temperature during the growing season are shown in Fig. 1 (Data are taken from Institute of Meteorology and Water Management for station in Kozienice and they are means for 10 days). Four replicates (plots about 1 m^2) were established for each treatment, each having 15 seedlings.

Soil water measurement

Measurement of water contents of soil was carried out with time-domain reflectometry (TDR) equipment (Malicki et al. 1996). TDR is a method for the direct and non-destructive measurement of volumetric water content (θ) of a soil; it averages moisture content along the length of a pair of connected rods. The technique is based on measuring the velocity of a pulse, which travels along an electromagnetic transmission line as a guided wave. The pulse velocity is used to calculate the dielectric constant of soil, which is dominated by the contribution from soil water. Free water has a dielectric constant about 20 times greater than that of mineral matter, so effect of mineral matter on the pulse velocity is small (Whalley 1993). To characterize the soil water content a permanent array of probes was installed in 10 cm depth. Measurements were made once a day. Field water capacity (FWC) values for soils were computed according to method described by Brogowski and Czerwiński (1985). Volumetric soil moisture at FWC was defined as 100%, all measured values of soil water are related FWC and have been performed as percentage of FWC (Fig. 2)

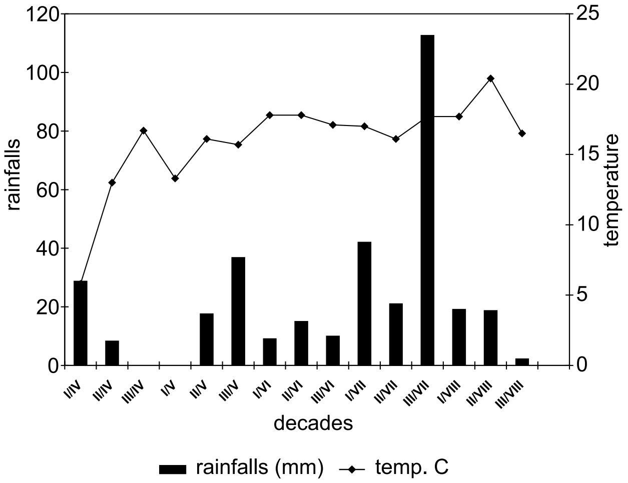

Fig. 1. Seasonal precipitation and air temperatures in a nursery. Term decades do mean ten days, so are three decades in each month, for examples I/IV determine first ten days of April

Sampling

At the beginning of September, 10 seedlings were taken at random from each treatment and root-collar diameter and height of the seedlings were measured Shoot volume was approximated as shoot height × root-collar diameter^2 (Stenström 1990; Marx 1991).

The root systems of the seedlings were studied using a stereomicroscope (magnification 10–50 ×). To standardize the sample, lateral roots were taken from

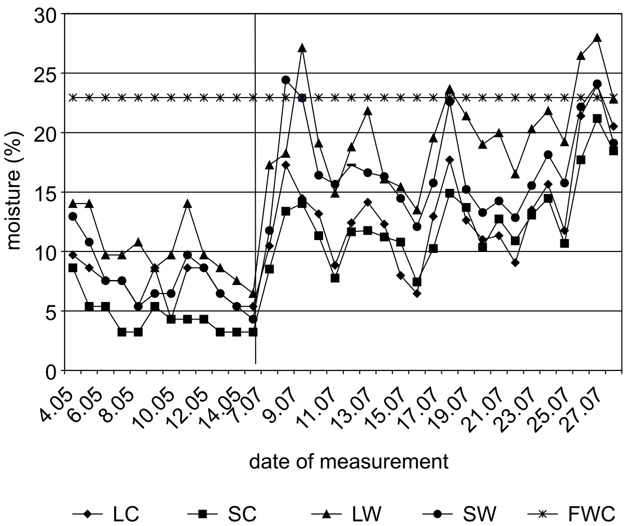

Fig. 2. Soil moisture changes in first (May) and second (July) period of watering. LC-litter control, SC-soil control, LW-litter watered, SW-soil watered, FWC-Field Water Capacity

Table 1. Characteristic of mycorrhizas formed by indigenous fungi on Scots pine seedlings in nursery

Mycorrhizal Morphotypes	Key morphological features
Type 1 ectendomycorrhiza	monopodial to dichotomous to multiple-dichtomous, strong orange yellow to dark reddish brown to brownish black in older portions, mantle usually one layer of hyphe 2–3 μm wide, rhizomorphs not observed
Type 2 – *Thelephora*	single to multiple dichotomous, strong orange yellow to deep to dark brown, mantle three to six layered 15–30 μm, strands undifferentiated and often attached to mantle
Type 3	single, white, floccose, short, stubby and blunt-ended mycorrhizae, cream mantle10–20 μm wide, no strands
Type 4	monopodial, rarely branched, pale yellow-brown, mantle 10–20 μm thick, smooth and shiny with slightly granular hyphae, club-shaped tip, strands present
Type 5 – *Cenococcum*	monopodial, rarely pinnate, black, covering the apex, mantle 20–40 μm thick, robust hyphe emanating from mantle, rhizomorphs not observed

three different levels of the entire root system from each plant (top, middle and bottom), cut into small pieces (ca. 1cm), and mixed in a Petri dish containing water. The percentage of mycorrhizal short roots for each plant was assessed by counting at least 200 short roots from the mixture of segmented roots under the stereomicroscope (Parlade et al. 1996). Following the guidelines of Agerer (1987–1997), and Ingleby et al. (1990) morphotype data were recorded on overall morphology and colour. The description of the types is presented in Table 1.

Statistics

Data were analysed by ANOVA and t-tests using *Statistica 98* (StatSoft), wise mean separation was determined using Tukey's Honestly Significant Difference Test at $p< 0.05$. Prior to analysis, arcsine transformations were performed on percentages of mycorrhizae. Indication of significance is given against untransformed values.

Results

Approximate values of soil water potential were: 9.7% (44% Field Water Capacity- FWC) on control soil (without watering) and 11.3% (51% FWC) on watered soil, 13.8% (66% FWC) on control soil + litter and 16.6% (75% FWC) on watered soil + litter (Fig. 2). Seedlings growing on soil were higher and had greater shoot volumes than those growing on soil + litter, irrespective of watering (Fig. 3). The growth of plant on watered soil was greater than growth of plants in the control treatment. No difference was recorded in the growth characteristic of seedlings from all the soil + litter plots.

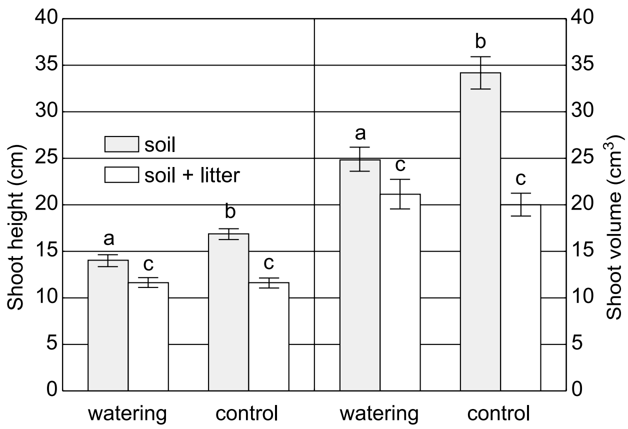

Fig. 3. Mean shoot height (left side) and volume (right side) for Scots pine seedlings growing in nursery, *watering* – plants watered according to nursery routine, *control* – plants receiving precipitation only. Values followed by the same letter are not significantly different, n=10, I=SE

Mycorrhizal colonization was greatest in seedlings growing on soil + litter: 72% in the watered and 67% in the non-watered treatments. The same trend was noted on soil without litter, where mycorrhizal colonization was 46% in case of watered seedlings and 36% in case of non-watered ones (Fig. 4).

The dominant mycorrhizal morphotype on all experimental plots, irrespective of moisture conditions was type 2 –*Thelephora terrestris*. The greatest percentage of those ectomycorrhizas was noted for seedlings from the watered soil without litter – 40.0% of all ectomycorrhizas while 30.0% was noted on seedlings from the control soil + litter (Table 2). Mycorrhizas of types 3 and 4 were more frequent on the plots with the watering treatment, though the differences between watered and control plots in case of soil were

Table 2. Frequencies of mycorrhizal morphotypes in soil and soil + litter on Scots pine seedlings, data are means (%) ± SD (n = 10). Values followed by different letters within a treatment indicate significant differences at P < 0.05 using t-tests

Treatment	Irrigated status	Type 1	Type 2	Type 3	Type 4	Type5
Soil	watered	$10±3.20^a$	$40±6.30^a$	$20±6.62^a$	$30±5.89^a$	–
	non-watered	$15±3.27^b$	$38±6.63^a$	$15±3.80^a$	$25±5.69^b$	7±2.40
Soil + litter	watered	$7±1.83^a$	$36±3.71^a$	$30±4.94^a$	$27±3.86^a$	–
	non-watered	$11±3.17^b$	$30±6.36^b$	$25±4.97^b$	$28±2.83^a$	–

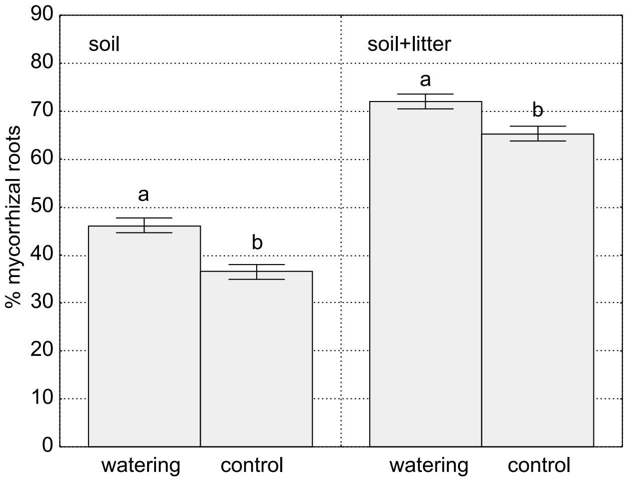

Fig. 4. Mycorrhizal colonization on Scots pine seedlings in different moisture of soils. Values followed by different letter are significantly different (in soil p=0,0003 and in soil + litter p=0,0015), n=10

not statistically significant. In both kinds of soils a significantly greater number of ectendomycorrhizas (type 1) were found on seedlings in the control treatments. Ectomycorrhizas formed by *Cenoccocum geophilum* (type 5) were only observed on seedlings in the control treatment in the case of the soil without litter – 7% of all mycorrhizas.

Discussion

The results have shown that ectomycorrhizal community may vary with moisture conditions. Occurrence of *T. terrestris* ectomycorrhizas was found to stimulated by higher soil moisture, although this species was generally frequent in all plots, irrespective of soil type and soil moisture regime. These data support view that specific nursery conditions (high nutrition and moisture) increased development of mycorrhizal hyphe of *T. terresrtis* (Villeneuve 1991; Garbaye and Churin 1997). Ubiquitous character of *T. terrestris* makes that this fungus is a strong competitor in nurseries' soil (Tyminska et al. 1986; Perry et al.1989; Guehl and Garbaye 1990).

Mycorrhizal species vary in effectiveness for tree growth (Dell et al. 1994). It is dependent on the amount and differentiation of the extrametrical mycelium which is a very important ecological factor for tree performance apart of abundance of ectomycorrhizas (Thomson et al. 1994). *T. terrestris,* a representative of the medium-distance smooth exploration type, reduced concentration of nitrogen, phosphorus and potassium in fermentation horizon organic matter to a considerably lower degree than for instance *Suillus bovinus,* a representative of the long distance exploration type (Bending and Read 1995) Hence, presence of *T. terrestris* could explain why the seedlings from the watered treatment had lower values of growth parameters than these from control treatments.

Occurrence of *C. geophilum* mycorrhizas in Scots pine seedlings grown on plots with soil moisture of 44% FWC confirms findings that this species is particularly noted for its drought resistance (Worley and Hacskalyo 1959; Pigott 1982; Coleman et al. 1989. However, this fungus is not limited to dry sites as it has been observed in poorly drained, wet soils (Trappe 1962). Unestam and Sun (1995) found that in pure culture hyphae of *C. geophilum* tended towards the water alike as the hyphe of other hydrophilic mycorrhizal fungi. Probably *C. geophilum* belongs to the fungi which have wide habitat range and is a representative of short-distance exploration type (Agerer 2001). The effectiveness of these ectomycorrhizas for tree growth may be low because they does not form rhizomorfs which are extension of roots. Result from this work appears to confirm such finding.

Irrespective of watering numerous ectendomycorrhizas were found, although greater number were presented on non-watered seedlings. This fact may suggest that these mycorrhizas are hydrophobic or (what is well documented view) prefer soils with high level of nitrogen (Laiho 1988; Lehto1989; Rudawska et al. 2001). Because of watering many of soil's nutrients is leached, it is highly likely that watered soil had lower content of nitrogen then non-watered. Irrespective of the soil-moisture regime, a higher percentage of mycorrhizal infection of seedlings was obtained on soil with litter, thereby confirming the findings of Barr and de Vries (1995). The authors showed significant effects of leaf litter and humus on the increase of mycorrhizal community structure of Scots pine.

With the exception of the non-watered seedlings growing on natural soil plots, all seedlings of Scots pine possessed similar shoot heights and volume (Fig. 3). One of the reason may be mycorrhizal diversity of seedlings. The results of this study demonstrate that mycorrhizal fungi show greater efficiency in colonising roots of Scots pine seedlings when the soil moisture remains above 50% Field Water Capacity.

Amendment of soil by litter was associated with greater soil moisture, though it is difficult to confirm whether the immediate cause of the greater number of mycorrhizal roots was litter or higher moisture.

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