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PRELIMINARY STUDY ON THE EFFECT OF PINE FOREST LITTER INOCULUM ON THE PLANT GROWTH, MYCORRHIZAL STATUS, AND THE OCCURRENCE OF MITES (*ACARI*) IN THE ROOT CLUMPS OF WHITE BIRCH SEEDLINGS

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Summary

The aim of the research has been to determine the effect of the inoculum (forest litter containing living edaphon mixed with peat) on selected plant growth parameters of white birch (*Betula pendula* Roth) seedlings growing with a covered root system, the mycorrhizal status as well as the abundance of the *Acari* in the root clump. The research was performed in 2013 at the container nursery of Bielawy (53°01'37.3"N 18°42'55.3"E), in the Forest Inspectorate of Dobrzejewice. The experiment started on May 10, seedlings were growing in containers placed on steel pallets in 2 treatments: C – control, L – 10% of additive of inoculate shredded pine forest litter. Seedlings of white birch cultivated in the treatment with addition of litter were significantly higher than those from control treatment. There were no significant differences between the diameter and the fresh mass of the the seedlings in the two treatments. The percentage of vital mycorrhizae increased slightly in the treatment with the addition of litter. The proportion of non-vital mycorrhizas was lower in the control treatment. Our results indicate that forest litter might improve environmental conditions rather by changing moisture-temperature relationships than by changing the availability of nutrients associated with litter. The application of the litter inoculum into the peat substrate resulted in a many-fold increase in the *Acari* abundance, especially the Oribatida. In this treatment the abundance of *Acari* groups was similar to the pattern observed in forest soils.

Key words: container nursery, seedlings, edaphon, mycorrhiza, litter

INTRODUCTION

Economic, organizational and natural conditions determined the global development and a vast application of the seedlings with a covered root system (Szabla and Pabian 2003). The technologies assumed the optimization of thermal, moisture-and-fertilisation conditions during seed sprouting and seedling growth. Benefiting from the economies of scale, such type of the plant nursery has made it possible to implement a controlled mycorrhization; the inoculation of root systems with biopreparations of ectomycorrhized fungi (Grzywacz 2009).

Among the mycorrhization methods, one can differentiate between the natural method, which involves the application of the natural inoculum in a form of pine forest litter sampled from the forest soil as well as the method of controlled mycorrhization which, at present, dominates in the nursery practice and involves the introduction of the propagules identified mycorrhized fungi to the breeding substrate (Szabla and Pabian 2003). As for the natural method, containers are introduced with, varied in terms of quantity and species composition, fungal communities, and as for the controlled method, most frequently one fungal species.

The pine forest litter applied in this experiment aimed at the inoculation of the peat culture medium with the edaphon typical of forest soils. For plant nursery practitioners the most essential component of the edaphon are fungi which can become symbionts of seedling roots. Most often one forgets or even ignores the importance of minor beneficial soil fauna, with *Acari* dominating. Let us remember that under natural conditions there exists the connection between microorganisms (fungi especially) and soil mesofauna; very strong and essential for the right functioning of the soil system. Microorganisms, including mycorrhized fungi, very often provide food for mesofauna (Remén *et al.* 2010, Schneider *et al.* 2005), however, at the same time, they make use of its occurrence: soil fauna feeding on mycorrhizae can stimulate their growth (Hanlon and Anderson 1979, 1980), it can also inoculate soil with fungal spores and hyphae through defecation or transporting on the body surface onto new substrates (Lussenhop 1992, Renker *et al.* 2005). The inoculation of the root clump of seedlings with mesofauna can be also important for the reintroduction of those animals, e.g. onto post-agricultural or degraded areas to speed up their revitalizing.Earlier research, of similar nature, reported by the authors of this paper (Klimek *et al.* 2013d) involved the use of the inoculum made up of pine forest litter in a form of 1 cm of the litter layer on the surface of the containers. Unfortunately, mulching with litter after sowing of Scots pine (*Pinus sylvestris* L.) limited the the germination of seeds considerably .

The aim of the research has been to determine the effect of the inoculum (forest litter containing living edaphon mixed with peat) on selected plant growth parameters of white birch (*Betula pendula* Roth) seedlings growing with a covered root system, the mycorrhizal status as well as the abundance of the *Acari* in the root system.

MATERIALS AND METHODS

The research was performed in 2013 at the container nursery of Bielawy (53°01'37.3"N 18°42'55.3"E), of the Forest Inspectorate of Dobrzejewice. The experiment started on May 10 was set up in multi-plates (containers) placed on steel pallets in 2 treatments: C – the control, L – 10% of additive of inoculate shredded pine forest litter. In the containers there was used the peat substrate (pH = 5.0), mixed with the Osmocote fertiliser granulate (N – 14%, P – 7%, K – 9%, MgO – 2%) at the rate of 2 kg · m^{-3}. In both variants 1 m^3 of peat substrate was added with 24.5 dm^3 of peat-vermiculite substrate with the mycelium of *Hebeloma crustuliniforme* (Bull.) Quél. The experiment variant involved the steel palette with 27 containers (block containers HIKO V-250 manufactured by BCC; 18 containers in the cassette with each container 250 cm^3). For about 1 month the containers were located in the foil tent ensuring the microclimate conditions optimal for the seedlings development. The seedlings, after a month of an intensive growth, in strictly controlled environmental conditions, were transferred onto the sprinkling field where they remained to the end of the production cycle (Photo 1). Throughout the growth period the seedlings were irrigated applying the sprinkling ramp (Photo 2).

The material for the inoculation of peat substrate with edaphon was acquired on the day preceding the establishment of the experiment from the layer of ectohumus of mature pine forest in the Forest District of Białe Błota (the Forest Inspectorate of Bydgoszcz) – 53°05'31.3"N 17°55'03.5"E. The material was twice shredded using the VIKING GE 250 garden shredder.

Plant growth was evaluated in late autumn (17 October 2013). The height of the seedlings (cm), the diameter (mm) and the fresh mass of the above-ground parts of the seedlings (g) were measured.

The seedlings growing in two treatments C and L were collected (5 per treatment) to determine the percentage of mycorrhizae on roots (October 17, 2013). Prior to the investigation, each root system was excised from the stems and gently washed in tap water. Root samples were then placed in a Petri dish with tap water and observed under a dissecting microscope. Mycorrhizal tips from each soil sample were sorted in morphotypes for further morphological screening, counted, and placed in 1.5-ml tubes containing distilled water. All autotrophic roots were also counted. Mycorrhizal tips were identified by the presence of mantle (colour, shape and surface texture), external hyphae, a slightly swollen apex and mycelial strands (Agerer 1987-2006; Agerer and Rambold 2004-2007).

To investigate the occurrence of mites, soil samples were taken three times: June 6, August 1 and October 17, 2013. Ten samples were taken for the each treatment at the successive dates (a total of 60 samples from the whole experiment). Sections of the substrate were taken from the top and bottom of the substrate prills (100 cm^3) with the root system of a plant; after cutting the seedlings at root crown. Mites were extracted for 7 days in Tullgren funnels and then they were preserved in 70% ethanol and dissected. All the mites were identified to order; a total of 1,106 mites were determined. The average density (N) of the mites was expressed per 100 cm^3 of substrate, and the group of mites dominance index value (D) was given in percentage.

RESULTS AND DISCUSSION

Assessment of plant growth.

The seedlings of white birch cultivated as treatment L showed theirsignificantly increased height (63.33 cm) as compared to those in treatment C (58.56 cm) (Table 1). In the study of Klimek *et al.* (2009) which was conducted in 2003-2005 at the Forest Nursery in Białe Błota, irrigation significantly increased the height of one-year-old seedlings of white birch from 32.6 cm in control plots (without irrigation) to 67.8 and 73.2 cm, for drip irrigation and micro-jet sprinkling, respectively. In another experiment carried out in 2008-2009 by Klimek *et al.* (2011b) at Forest Nursery in Bielawy, one-year-old white birch seedlings demonstrated the height from 31.1 cm to 37.4 cm.

There were no significant differences between the diameter of the seedlings in C and L treatments. The mean value of diameter, for both the examined treatments, was 4.98 mm. In the study of Klimek *et al.* (2009), white birch seedlings cultivated in the treatments with organic fertilization were significantly higher in diameter (8.2 mm) as compared to the control (6.0 mm) with mineral fertilization only. For comparison, the diameter of one-year-old white birch seedlings in the second study reported by Klimek *et al.* (2011) ranged from 4.6 mm to 4.9 mm.

As for the fresh mas of the above-ground parts of the seedlings, the difference between treatment C and treatment L was non-significant. There were also no significant differences between C and L treatments in the fresh mass of seedling the roots. Similar results were reported in the previous experiment of Klimek *et al.* (2011b).

Photo 1. Containers with white birch seedlings at the external field (photo by A. Klimek)

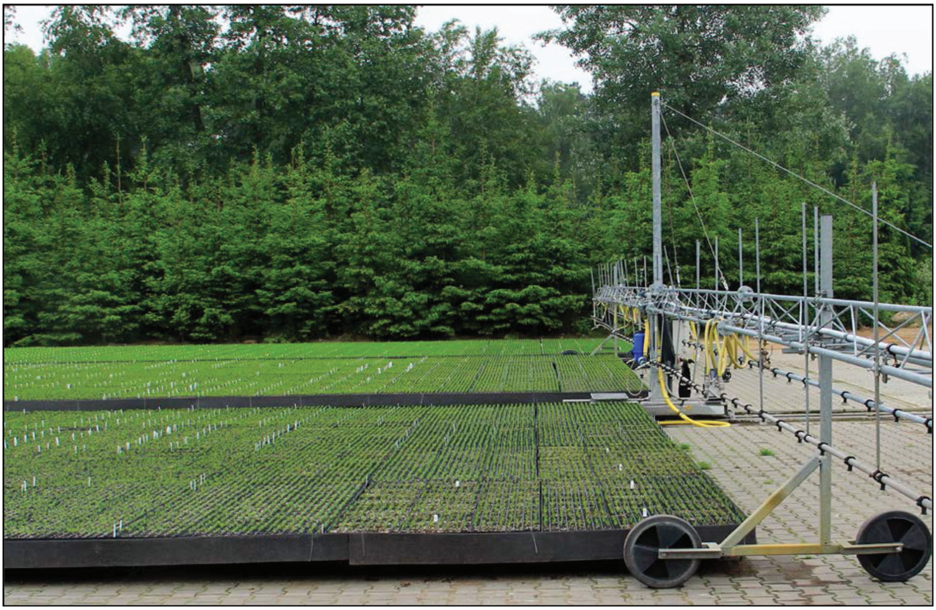


Photo 2. The external field of sprinkling and the sprinkling ramp (photo by A. Klimek)

Table 1. Selected growth parameters in white birch seedlings

Treatment	Variant of the experiment			$LSD_{0.05}$
	C	L	Mean	
Height (in cm)	58.56	63.33	60.95	3.714
Diameter (in mm)	5.08	4.88	4.98	ns
FM of above-ground parts (in g)	4.35	4.43	4.39	ns
FM of roots (in g)	24.60	27.08	25.84	ns

Source: own research

Assessment of mycorrhizal status.

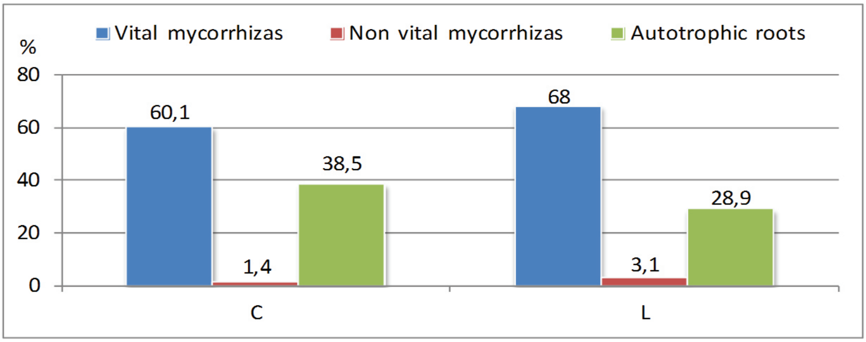
The percentage of vital mycorrhizae increased slightly from 60.1 in control to 68.0 in the treatment with addition of litter. The proportion of non-vital mycorrhizas was lower in the control treatment. Following litter addition the share of autotrophic roots decreased from 38.5 to 28.9 (Figure 1).

Increasing soil nutrients through litter manipulation, pollution, or fertilization can adversely affect mycorrhizal communities by inhibiting fungal growth (Cullings *et al.* 2003, Cullings *et al.* 2010). The authors found that after litter addition the number of mycorrhizal roots increased significantly, and the increase occurred only in the upper soil layer, directly adjacent to the litter added . Our findings coincide with these results.

Aucina *et al.* (2015) showed that the addition of pine, oak and spruce forest litter in bare-root forest nursery enhanced pine seedling height and root-collar diameter. Each forest litter type increased also the total number of mycorrhizal tips significantly. Our results indicate that forest litter improves environmental conditions by changing moisture-temperature relationships rather than nutrients associated with litter.

Mite occurrence.

Post-agricultural and degraded soils, in general, show low biological activity. For the purpose of afforestation in those areas and their revitalising, birch seedlings with a covered root system provided with mycorrhizal fungi are perfect (Szabla and Pabian 2003). Mycorrhizae are to ensure the seedling growth in difficult habitat conditions. In earlier research it was found that the root clumps of seedlings with a covered root system provide good conditions for the occurrence of *Acari* (Klimek 2013, Klimek *et al.* 2013d). There is then a potential to use root clumps of seedlings for the reintroduction of the forest edaphon, including beneficial Acari, onto post-agricultural or degraded areas. The authors of this paper in many experiments performed earlier in field nurseries recorded a positive effect of soil inoculation with mesofauna with the use of forest litter (Klimek *et al.* 2008, 2009, 2013a,b,c).



Source: own research data

Figure 1. Mycorrhizas and autotrophic roots of birch seedlings in the studied variants of the experiment

Applied in this experiment, the 10% additive to the nursery substrate of the forest litter inoculate 4-fold increased the *Acari* density (Table 2). In the control variant the most abundant *Acari* were *Actinedida* – N = 3.30 individuals per 100 cm^3 and D = 46%, with predatory *Mesostigmata* coming second in the hierarchy of that grouping and, representing saprophages; the *Oribatida* came only third. The application of litter inoculate for the peat substrate resulted in a 13-fold increase in the abundance of the Oribatida in birch root clumps. Those *Acari* in variant L came first in the hierarchy of groupings of *Acari* – D = 61%. To determine the stability of the ecosystems, one can use the abundance proportion of *Oribatida* to *Actinedida* (Gulvik 2007). According to Werner and Dindal (1990), the values below 1.0 are characteristic for arable fields, and above 1 for the soils of more stable ecosystems (e.g. semi-natural meadows or forests). That indicator in variant C was 0.4, and in L it increased up to 3.4. Considering a high bioindicative value of the *Oribatida* (Behan-Pelletier 1999, 2003; Gulvik 2007), one can state that in the rhizosphere of birch seedlings, after adding litter inoculate, there increased the biological activity and balance and the pattern of abundance of the groups of *Acari* got similar to the structure typical for forest soils (Klimek 2000).

The results reported in the present experiment confirm the earlier observations (Klimek 2013, Klimek *et al.* 2013d) which point to good conditions of the *Acari* development, especially in the rhizosphere of the seedlings of deciduous species, whichgives a chance to use seedlings with a covered root system for the reintroduction of beneficial mesofauna into post-agricultural and degraded areas.

Table 2. Average density of mites *N* (in 100 cm^3 of substrate) and dominance index *D* (in %) of order in the gatherings of mites in the variants of the experiment

Taxon	Variant of the experiment			
	C		L	
	Index			
	N	*D*	*N*	*D*
Actinedida	3.30	46	5.37	18
Mesostigmata	2.00	28	5.53	19
Oribatida	1.40	20	18.20	61
Tarsonemida	0.43	6	0.63	2
Acari (Total)	7.13	100	29.73	100

Source: own research

SUMMARY

The seedlings of white birch cultivated in the treatment with the addition of litter were significantly higher than those in the control. However, there were no significant differences between the diameter and the fresh mass of the seedlings in the two treatments tested.

The percentage of vital mycorrhizae increased slightly in the treatment with addition of litter. The proportion of non-vital mycorrhizas was lower in the control treatment. Our results indicate that forest litter improves environmental conditions by changing moisture-temperature relationships rather than by increasing nutrients associated with litter.

The application of the litter inoculate into the peat substrate resulted in a many-fold increase in the abundance of *Acari*, especially *Oribatida*. In seedlings' rhizosphere after litter addition the pattern of Acari groups abundance was similar to the one typical for forest soil

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