THELEPHORA TERRESTRIS (EHRH.) FR. IN DEVELOPMENT OF SCOTS PINE OUTPLANTED ON POST-AGRICULTURAL LAND

THELEPHORA TERRESTRIS (EHRH.) FR. W ROZWOJU SOSNY ZWYČZAJNEJ NA GRUNCIE POROLNYM

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The overall aim of the work was to study root-related mycological aspects of afforestation of former agricultural land with Scots pine seedlings. The ubiquitous nature of Thelephora terrestris fungus and its numerous ectomycorrhizae on seedlings in Polish nurseries were the main factors in order to: (1) investigate the share of T. terrestris ectomycorrhizae on inoculated and non-inoculated seedlings after their outplanting, (2) assess how inoculation with chosen mycobionts and different levels of fertilizer use change the concentration of nitrogen in seedling needles, biometric parameters as well as the mycorrhizal structure within four years after outplanting, (3) investigate the enzymatic activity of T. terrestris mycelium in pure cultures and in ectomycorrhizae of the fungus.

The investigation described in paper I revealed that the extent of mycorrhizal colonization with T. terrestris depends on the cultivation system. Five months after outplanting, the seedlings from the nursery outdoor bare root system had higher percentage of T. terrestris mycorrhizae than seedlings from greenhouse cultivation system. Moreover, this work revealed that changes of mycorrhizal structure on roots to a large extent depend on mycorrhizal status inherited from nurseries. Hence, it is important to select a proper cultivation system in forest nurseries which may improve ectomycorrhizal species richness and abundance. The latter factors are essential to increase seedlings vitality, and consequently their survival and growth after replanting (paper V).

The mycorrhizal community structure in roots of Pinus sylvestris L. seedlings was studied (paper II). Seedlings grown in sandy soil on post-agricultural land were subjected to inoculation with three mycorrhizal species: Suillus luteus, Boletus

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pinicola and Hygrophorus olivaceoalbus. Five months after inoculation the highest percentage of ectomycorrhizal short roots were present in seedlings inoculated with B. pinicola. The inoculated seedlings had lower percentage of T. terrestris mycorrhizae as compared to the non-inoculated ones. Identification of mycorrhizae on the basis of morphotyping and PCR RFLP methods revealed that seven ectomycorrhizal species were engaged in the colonisation of roots. Percentage of roots colonised by inoculated fungi in all treatments was about 20%, what indicates that the success of mycorrhizal inoculation in the field largely depends on the fungus, host tree, and ecological conditions of the soil which to a great extent regulate mycorrhizal colonisation at a given site.

The investigation of the effect of N-fertilized substrata on biometrics parameters and nitrogen content of seedlings with T. terrestris (paper III) revealed that seedlings growing in soil with lower nitrogen content possessed higher values of parameters as well as a higher number of mycorrhizae. On substrata enriched with nitrogen, the proportion of fine roots decreased dramatically. The results showed that, irrespective of the investigated type of substratum, the examined strain of T. terrestris used as an inoculum reacted negatively to the higher content of nitrogen.

After two growing seasons following plantation establishment (paper IV), mycorrhizal abundance in Scots pine seedlings was higher in the inoculated plants than in non-inoculated ones. PCR RFLP analysis confirmed a share of two different isolates of T. terrestris engaged in mycorrhizal symbiosis. A part of mycorrhizae had the same pattern of RFLP as the isolate used for inoculation. Similar results were obtained in the second year of experimental study in the field, confirming the persistence of artificially introduced T. terrestris in post-agricultural soil as an important component of the ECM community.

The investigation of mycorrhizal structure within four seasons of seedlings’ growth (paper V) showed statistically significant differences in N concentration in needles in two- and four-year-old seedlings inoculated with T. terrestris. The number of ectomycorrhizae and concentration of N in needles were negatively correlated. After four growing seasons the greatest numbers of ectomycorrhizae were present on inoculated seedlings growing with high dose of nitrogen. The smallest number of ectomycorrhizae occurred on seedlings growing with high dose of nitrogen but non-inoculated with T. terrestris. Two-year-old seedlings had been colonized by at least six different fungal taxa, i.e. Cenococcum geophilum, Hebeloma crustuliniforme, Rhizopogon sp., Suillus bovinus, T. terrestris and a non-identified one. Irrespective of the dose of nitrogen, the inoculated seedlings’ roots were dominated by T. terrestris ectomycorrhizae whose number was significantly greater than in the case of non-inoculated seedlings. On four-year-old seedlings ectomycorrhizae of T. terrestris were dominating on seedlings from all treatments of inoculation and fertilization, while ectomycorrhizae of Hebeloma sp. were not found. This may suggest that the latter fungus is a poor competitor that can only thrive in species-poor communities.

In paper VI, the activities of enzymes such as cellulase, pectinase, proteinase, chitinase and acid phosphatase produced in vitro by T. terrestris and H. crustuliniforme, as well as the activity of β-glucosidase in roots of inoculated Scots pine seed-
lings, are described. In pure culture an isolate of *T. terrestris* produced nine out of ten enzymes, whereas an isolate of *H. crustuliniforme* produced only five enzymes. The activity of enzymes was higher in *T. terrestris* cultures than in *H. crustuliniforme*, the only exception being neutral proteinase. It was observed that in roots of seedling inoculated with *T. terrestris* level of β-glucosidase was about 107% higher than in roots of non-inoculated seedlings. The enzyme level in roots inoculated with *H. crustuliniforme* was 44% lower than in those inoculated with *T. terrestris*. Given that high activity of β-glucosidase is one of the factors which are responsible for protection of host tree against pathogens, *T. terrestris* mycorrhizae seems to play a positive role in the colonization of roots following planting-out of seedlings on former agricultural land.

### Research papers constituting the habilitation thesis


*The habilitation colloquium took place with the Scientific Council of the Forest Research Institute in Sękocin Stary, on June 15, 2010. The degree of doctor habilitatus was confirmed on the same day.*

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