ROLE OF MYCORRHIZAL LINKS BETWEEN PLANTS IN ESTABLISHMENT OF LIVERWORTS THALLI IN NATURAL HABITATS

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ABSTRACT

Research on the development of arbuscular fungi within thalli of *Conocephalum conicum* and *Pellia endiviifolia* was carried out on the banks of a stream ravine in the Beskid Wyspowy Mts. (Southern Poland). The links via arbuscular fungi were observed between liverworts and plants as *Dryopteris carthusiana* and *Oxalis acetosella*. *Glomus tenuis*, a fine endophyte, was the dominating mycorrhizal partner of all the plant species investigated. *Arum*-type of mycorrhiza was observed in *Oxalis acetosella* while in the fern and liverworts *Paris*-type was found. The role of plant roots in the establishment of liverworts thalli (source of fungal inoculum, important mechanical support on unstable sand banks) is considered.

KEY WORDS: arbuscular mycorrhiza, mycothalli, liverworts, *Dryopteris*, *Oxalis*.

INTRODUCTION

Liverworts often form mutualistic symbiosis with fungi. Gametophytes, which are their dominating state, associate with different groups of fungi (Boullard 1988). In thalloid hepatics of Pelliaceae, Fossombroniaceae, Lunulariaceae, Conocephalaceae and Marchantiaceae, similarly as in anthocerots (Ligrone 1988), members of *Glomales* colonize the axial zone of the thalli (Golenkin 1901; Ligrone and Lopes 1989). In leafy liverworts of Adelanthaceae, Cephaloziellaceae, Cephaloziaceae, Calypogeiaceae and Lepidoziaceae, the ericoid mycorrhizal fungus *Hymenoscyphus ericae*, a member of *Ascomycetes*, is a common symbiont (Pocock and Ducket 1985; Turnau et al. 1998) which develops within rhizoids. Basidiomycetes were reported as symbiotic partners of Jungermanniaceae, Arnelliaceae and Aneuraceae (Duckett, Read 1995). All these fungi are known to form mycorrhizas with tracheophytes, gymnosperms and angiosperms simultaneously. Mycorrhizal fungi can form close links between different plants developing near by (Newman et al. 1994; Heap and Newman 1980; Read et al. 1985). Such connections may provide a route by which organic substances and mineral nutrients are transferred between plants. Some plants may also become a source of fungal inoculum for others. This phenomenon can strongly effect the individual plants but also may alter the community structure and composition (Read et al. 1985; Newman 1988; Miller and Allen 1992).

The present paper constitutes a part of the investigation on fungal symbiosis of liverworts in Poland (Turnau et al. 1998). During observations carried out in the Beskid Wyspowy Mts several abundant populations of thalloid liverworts were found, often growing in association with certain plant species. The aim of this study was therefore to check whether links between liverworts and other plants exist and how often this phenomenon occurs in nature.

MATERIALS AND METHODS

Thalli of *Conocephalum conicum* (L.) Underw., *Pellia endiviifolia* (Dicks.) Dum. and roots of associating plants as *Dryopteris carthusiana* (Vill.) H. P. Fuchs and *Oxalis acetosella* L. were collected from stones, tree stumps or wood blocks partly or totally submerged in water, from pure sand or from forest soil (cambisol) on the banks in the vicinity of the stream. The research was carried out in the degraded *Luzulo nemorosae-Fagetum* and *Abieti-Piceetum montanum* forest of Żmiąca, Beskid Wyspowy Mts (Southern Poland) from 1996 to 1998. The soil of this area is characterised by mean pH value 3.5, a very low level of nitrogen (up to 0.3% of total N), phosphorus (average 0.65 mg of P_2O_5 in 100 g of soil), calcium (28 mg of CaO in 100 g of soil) and slightly a higher level of potassium (9.6 mg of K_2O in 100 g of soil), as estimated with atomic absorption spectrometry and by the Kjeldahl method in the case of nitrogen (Nowosielski 1968).

The thalli and roots were cleared with 7% KOH for 24 hours at room temperature, then washed in tap water and bleached with H_2O_2 containing NH_3 (10:1 v/v). Bleaching lasted a few minutes for fern roots and a little shorter in the case of liverworts and *Oxalis acetosella*. After successive washing with water the material was acidified in 5% lactic acid in water for 4-12 hours and stained for 24 hours in a 0.01% cotton blue solution in lactic acid. Following the removal of the staining solution with the filter paper, the material was stored in pure lactic acid. The material was analysed on glass slides a few days after finishing the staining procedure. In cases where links between two plants were expected, the staining was carried out by submerging in the solutions, only selected parts of the roots and thalli located in plastic boxes with holes (to avoid loosening the hyphal connections).

Mycorrhizal frequency (F%), mycorrhizal root length (M%) and arbuscular richness (A%) in the analysed material were assessed (Trouvelot et al. 1986).

Preparation for scanning electron microscopy (SEM) was also carried out in closed, plastic boxes with holes. The material was fixed in a 2% glutaraldehyde solution in cacodylate buffer for 2 hours at room temperature, washed with the same buffer, dehydrated in an increasing ethanol concentration series, critical-point dried, fixed on carbon stubs and observed with a scanning electron microscope (SEM) at 20 kV.

RESULTS

Mycothalli of liverworts

The thalli of *Conocephalum conicum* and *Pellia endiviifolia* from the Żmiąca Valley in Beskid Wyspowy Mts. were collected from sites differing especially in moisture level. The first species was often found completely submerged in water, growing on stones in the stream. It was often accompanied by algae, mosses and *Cardamine amara* L. Neither of these plants formed symbiosis with members of Glomales. Instead of them, the hyphae typical for *Pythium* sp. (Oomycetes) were observed within hepatic thalli (Fig. 2). The infection was not always restricted to the central midrib of the thalli and the branching of the hyphae did not result in the formation of coils or fan-shaped structures within plant cells. Of 60 samples of both species collected near the stream (Fig. 1) almost all were colonized by *Glomus tenuis* (Glomales), the fine endophyte with hyphae of 0.8-1.3 μm in diameter growing on the surface of the thalli, intracellular hyphae of 0.4-0.7 μm in diameter, typical fan-shaped structures (Fig. 3) and small vesicles of 4-7 μm (Fig. 4). Strongly stained mycelium with abundant branching and vesicles was very well developed within smooth rhizoids of *Conocephalum conicum* (exceptional colonization was only found within tuberculate rhizoids) and rhizoids of *Pellia endiviifolia*. From the rhizoids, the mycelium spread intracellularly into the midrib of both liverworts. The hyphae sometimes formed there coil-like structures. The very delicate arbuscules were developed abundantly (Fig. 5). No intercellular growth of hyphae was observed. In 10% of the studied thalli, apart from *Glomus tenuis*, the thicker mycelia (1.5-2.4 μm) forming abundant arbuscules and vesicles (which were however much bigger than in case of *G. tenuis*) were also observed (Fig. 6).

Mycorrhiza of plants associating with liverworts

Except for one case, all the liverworts in the investigated ravine were accompanied by strongly mycorrhizal *Dryopteris carthusiana* or *Oxalis acetosella* (Figs 1, 7). In the roots of these plants *Glomus tenuis* was again the most frequent endophytic fungus. In fern roots the growth of the fungus was, in many aspects, similar to above described colonization within the hepatics. Very characteristic fan-shaped structures and small vesicles were often observed (Fig. 8). Within root hairs, branched mycelium with small vesicles was present. The colonization of the cortex was almost totally intracellular. The arbuscules were more clearly visible than in the hepatics and always accompanied by well developed mycelium, forming coils within the same root cells (Fig. 9). This symbiotic system was often complemented by the abundant presence of cyanobacteria colonies in its vicinity (Fig. 10).

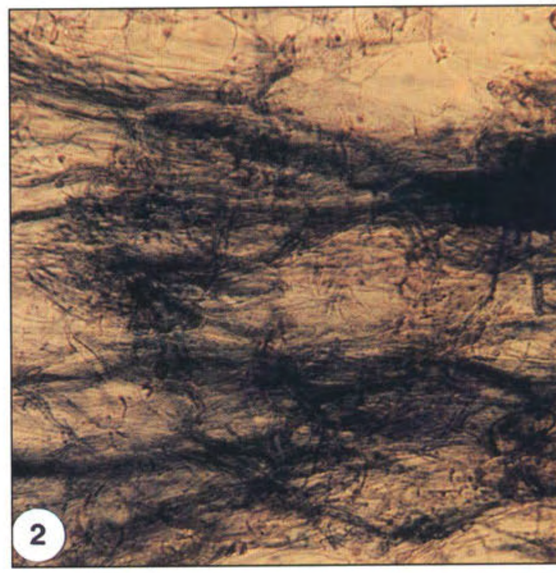
A different pathway of colonization was observed within *Oxalis acetosella* roots. In this case the mycelium growth followed mainly intercellular spaces. The arbuscules were formed terminally on the hyphae penetrating root cells (Fig. 11). Abundant vesicles were presents in root hairs and within the cortex (Fig. 12). Despite the differences of the growth in both plant species the frequency (F) of the mycorrhiza ranged from 40% to 100%, mycorrhizal colonization (M) from 20% to 75% and arbuscule richness (A) from 20% to 75%.

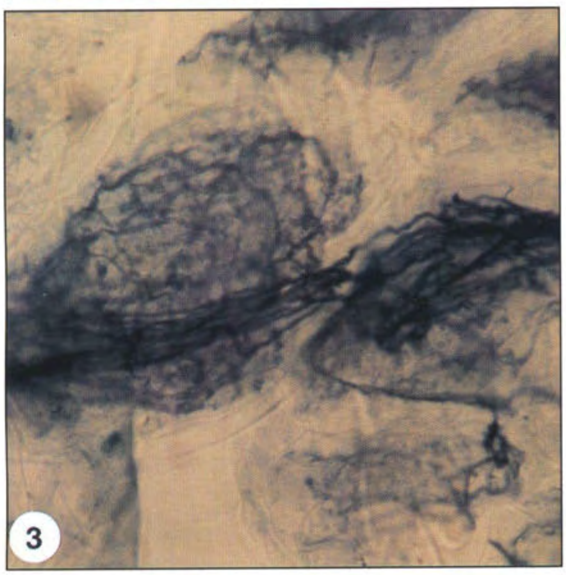
Links between vascular plants and liverworts

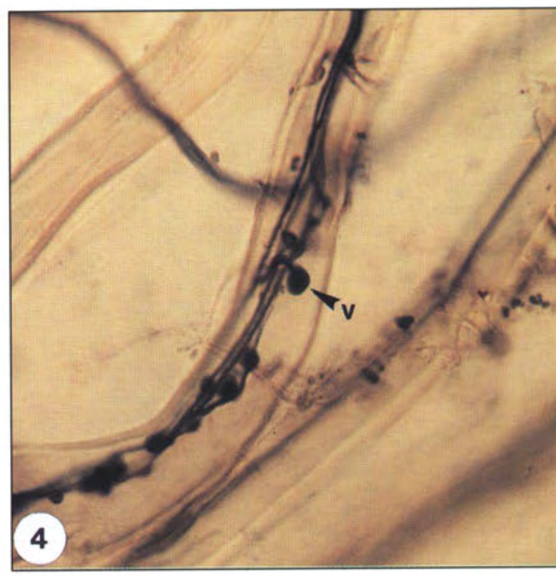
Conocephalum conicum was mostly found in the vicinity of *Oxalis acetosella*. The connection between these two plants were not easy to observe due to the long, thin roots of the plant hardly visible between pieces of plant debris and mosses usually present in the substrate. The thalli, which in the investigated area abundantly formed sexual structures, often covered areas up to 0.5 m^2. The only cases where the liverworts were growing without the company of arbuscular mycorrhizal plants was found in very old thalli growing in several layers one on another, where lower thalli were already dead. The younger parts were connected to them and the rhizoids were growing across old layers, strongly attaching them to the surface of tree roots and mosses covering the surface of the stones. The roots of trees were, however forming typical ectomycorrhiza. In the case of *Pellia endiviifolia*, the thalli sometimes covered areas of a few square meters. No specimens were found totally submerged in water. They were most frequent on the moist footpaths often associated with plants as *Oxalis acetosella* growing near the borders of the path, from where the liverworts started to grow during the moist period. More stable growth conditions were found on the moist forest soil, where the litter was removed by foresters. In such places over 20 specimens of *Dryopteris carthusiana* were found. The roots of the fern were growing partly on the surface of the soil and then they were easily followed due to the presence of liverworts, always growing in close connection to them. The most spectacular situation was found on the steep sandy banks of the stream, where *D. carthusiana* roots were growing down towards the water, accompanied by young thalli of *P. endiviifolia*. Microscopic observations of both plants left no doubts as to the existence of links between them via arbuscular fungi. Scanning electron microscopy study revealed the abundant development of AMF mycelium in places where rhizoids were attached to the fern root (Figs 13, 14). The mycelium usually penetrated the rhizoid tissue after a short distance growth on its surface.

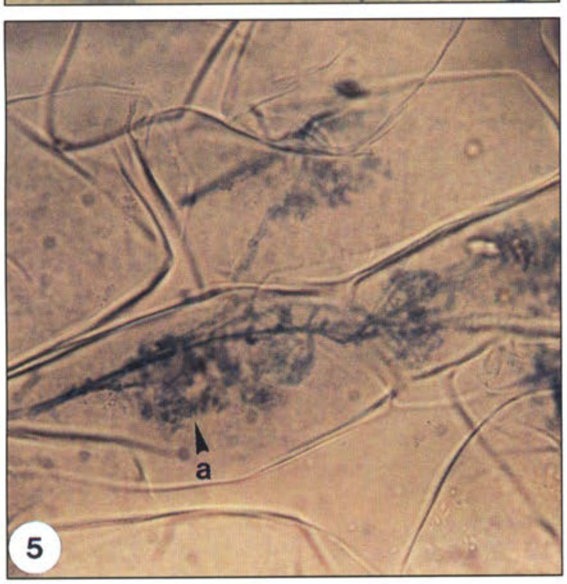
DISCUSSION

Forest communities of the temperate region are dominated by ectomycorrhizal trees which however are accompanied by understories plants forming extensive ericoid and arbuscular mycorrhizas. The community structure and diversity is greatly effected by the fungal diversity and links formed by the fungi between populations and between individual specimens. Liverworts are often overlooked or neglected when the community structure is investigated. Duckett and Read (1995) suggested that mats of leafy liverworts developing on acidic organic substrate may provide sources of inoculum for ericaceous seedlings. In the Beskid Wyspowy Mts. such an interaction was observed between leafy liverworts which were the first inhabitants on tree stumps and *Vaccinium myrtillus* seedlings (Turnau et al. 1998). In case of thalloid liverworts on









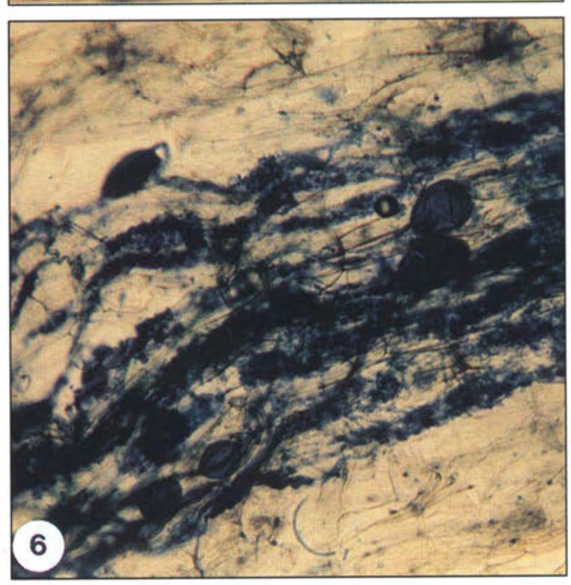


Fig. 1. *Pellia endiviifolia* growing on a stump in association with *Oxalis acetosella* and ferns.

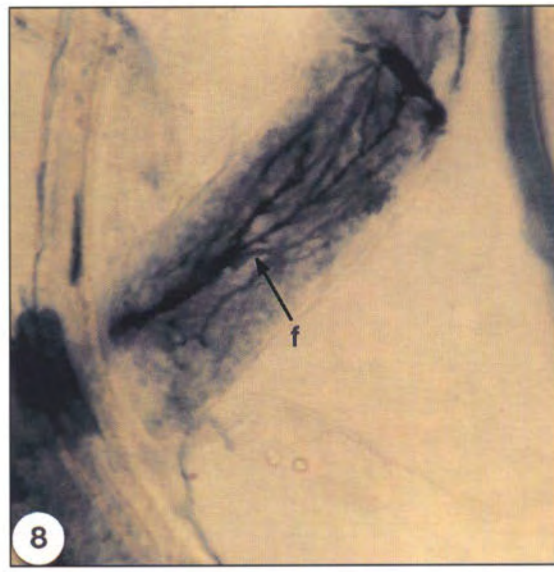
Fig. 2. Mycelium of *Pythium* sp. (Oomycetes) within *Conocephalum conicum* thalli collected from the stream.

Fig. 3. Cells of *Pellia endiviifolia* colonized by *Glomus tenuis* with fan-shaped structures.

Fig. 4. Vesicles (v) of *Glomus tenuis* within the rhizoids of liverworts.

Fig. 5. Midrib cells of *Conocephalum conicum* with arbuscules (a).

Fig. 6. Heavy colonization by thicker AM mycelium within midrib of *Conocephalum conicum*.



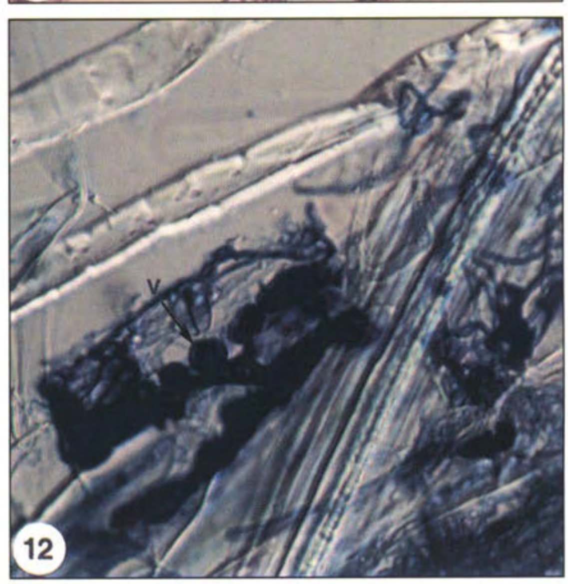
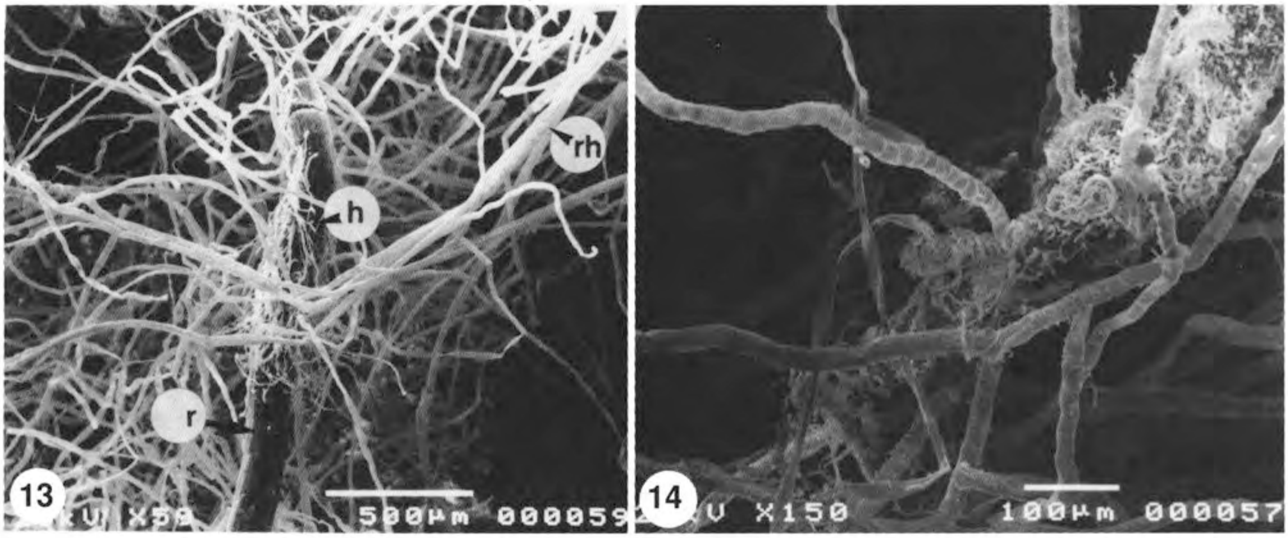


Fig. 7. Thalli of *Pellia endiviifolia* on the roots of *Dryopteris carthusiana*.
Fig. 8. Typical fan-shaped mycelium (f) of *Glomus tenuis* within cortical cells of *Dryopteris carthusiana*.
Fig. 9. Mycorrhiza of *Dryopteris carthusiana* with abundant arbuscules (a).
Fig. 10. Cyanobacteria (c) on the surface of *Dryopteris carthusiana* roots.
Fig. 11. Mycorrhiza of *Oxalis acetosella* with intra- and intercellular hyphae and abundant arbuscules.
Fig. 12. Abundant vesicles (v) within root cells of *Oxalis acetosella*.



Figs 13, 14. SEM micrographs of fern root (r) and liverwort rhizoids (rh) accompanied by abundant fungal hyphae (h).

the same area the links were formed by arbuscular fungi and the ferns or angiosperms were the donors of the inoculum. The consequent presence of liverworts outside the stream almost always in the company of arbuscular mycorrhizal plants, suggests the importance of this phenomenon for the establishment of these plants. Both plants are photosynthetically active, so the exchange of carbon metabolites probably have little (if any) role. The liverwort certainly may use the plant root system as an inoculum source. It may also share the nutrients absorbed from the soil by the hyphal net established by the plant, which has already been present before the appearance of the hepatics. In places such as sandy, steep banks the root system may also provide the mechanical support for the developing thalli. *Glomus tenuis* is known as a very efficient coloniser of many plant species (Hall 1977; Wilson 1984). Certainly, in the investigated area *Glomus tenuis* was the dominating mycorrhizal partner. This is the first report of the fine endophyte presence in liverworts. The research carried out on mycorrhiza of *Dryopteris carthusiana* from over 30 stands in Southern Poland (Unrug 1998, mskr.) suggests that, in general, *Glomus tenuis* is the most common symbiotic fungus in the moist places. The presence of this species was established in almost 70% of the analysed stands. Only a small percentage of the material was devoid of arbuscular fungi (on the stands rich in nutrients). *G. tenuis* was accompanied by another AM fungi in 20% of stands.

The restriction of the mycelium growth to intracellular spaces in hepatics and ferns has already been reported in these plants (Ligrone and Lopes 1989; Cooper 1976; Smith and Smith 1996). Generally, mycorrhiza of both liverwort and fern species described above, fit the characteristics of *Paris*-type mycorrhiza class, where the intracellular growth prevails, coils are very extensive and the arbuscules develop as lateral branches from the coils (Smith and Smith 1996). Unlikely, as in *Paris*-type however was the abundance of arbuscules. There were no differences in arbuscule richness in liverworts, ferns and *Oxalis acetosella* in which the *Arum*-type, with prevailing intercellular growth of mycelium and the formation of arbuscules terminally on hyphal branches, was found. The formation of two different types of mycorrhiza by the same fungal species, is a known feature explained by the differences in the anatomy of the root or other absorbing organ and remains under genetical control of the plant (Gerdemann 1965; Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983).

In July 1997 a serious flood occurred in Poland. The investigated area belonged to those, which suffered the most. No liverwort population was left there as all the plants of the ravine disappeared. In 1998 the first plants, as *Oxalis acetosella*, were found in places which could be good habitats for liverwort development. The observations on the succession of these plants and associating liverworts will be carried out.

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ROLA MIKORYZOWYCH POŁĄCZEŃ POMIĘDZY ROŚLINAMI W OSIEDLANIU SIĘ PLECH WĄTROBOWCÓW NA NATURALNYCH SIEDLISKACH

STRESZCZENIE

Badania nad obecnością i rozwojem kolonizacji plech wątrobowców *Conocephalum conicum* i *Pellia endiviifolia* przez grzyby arbuskularne prowadzone były na okazach pochodzących z naturalnych siedlisk na stromych zboczach doliny potoku w Beskidzie Wyspowym. Analiza zebranych materiałów pozwoliła na potwierdzenie kolonizacji plech wątrobowców przez grzyby mikoryzowe, włącznie z wytwarzaniem przez nie typowych struktur symbiotycznych (arbuskule, pęcherzyki). Zaobserwowano również połączenia pomiędzy wątrobowcami a obecnymi w ich sąsiedztwie roślinami mikoryzowymi (*Dryopteris carthusiana* i *Oxalis acetosella*) za pośrednictwem strzępek grzybów symbiotycznych. U wszystkich badanych roślin, dominującym gatunkiem grzyba był *Glomus tenuis*. U paproci i wątrobowców kolonizacja mikoryzowa przejawiała cechy typu *Paris*, podczas gdy *Oxalis acetosella* cechował się rozwojem mikoryzy typu *Arum*. U wszystkich badanych roślin *Glomus tenuis* wytwarzał charakterystyczne, delikatne struktury wachlarzykowate i drobne pęcherzyki. Przedstawione obserwacje wskazują na istotną rolę, jaką odgrywają w rozwoju wątrobowców towarzyszące im mikoryzowe rośliny naczyniowe (obfite źródło inokulum grzybów mikoryzowych, stałe podłoże na niestabilnych zboczach).

SŁOWA KLUCZOWE: mikoryza arbuskularna, mikoplechy, wątrobowce, *Dryopteris*, *Oxalis*.