



FUNGAL ECOLOGY

(sometimes with special regard to macromycetes)

- Fungi and their environment • Life strategies and interactions of fungi
- Ecological groups of fungi, saprotrophs (terrestrial fungi, litter and plant debris, wood substrate, etc.) • **Fungal symbioses** (ectomycorrhiza, **endomycorrhiza**, **endophytism**, lichenism, bacteria, animal relationships) • Parasitism (parasites of animals and fungi, phytopathogenic fungi, types of parasitic relations)
- Fungi in various habitats (coniferous forests, broadleaf forests, birch stands and non-forest habitats, fungal communities)
- Fungal dispersal and distribution • Threat and protection of fungi

(the study material has not been corrected by native speaker)

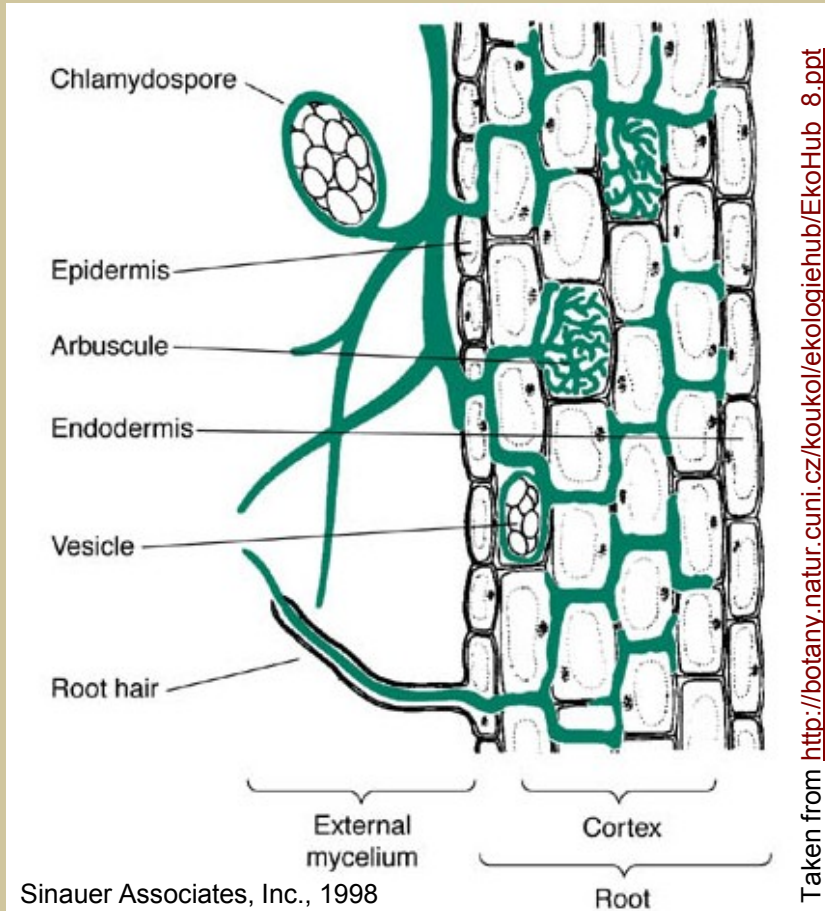
ENDOMYCORRHIZA

The vast majority of plants develop **endotrophic mycorrhiza**; about 75% of species are reported, including ferns, lycophytes and some bryophytes.

The most common type is **arbuscular mycorrhiza** (AM); in this case, the mycorrhizated roots do not differ from the others, the root hairs remain here.

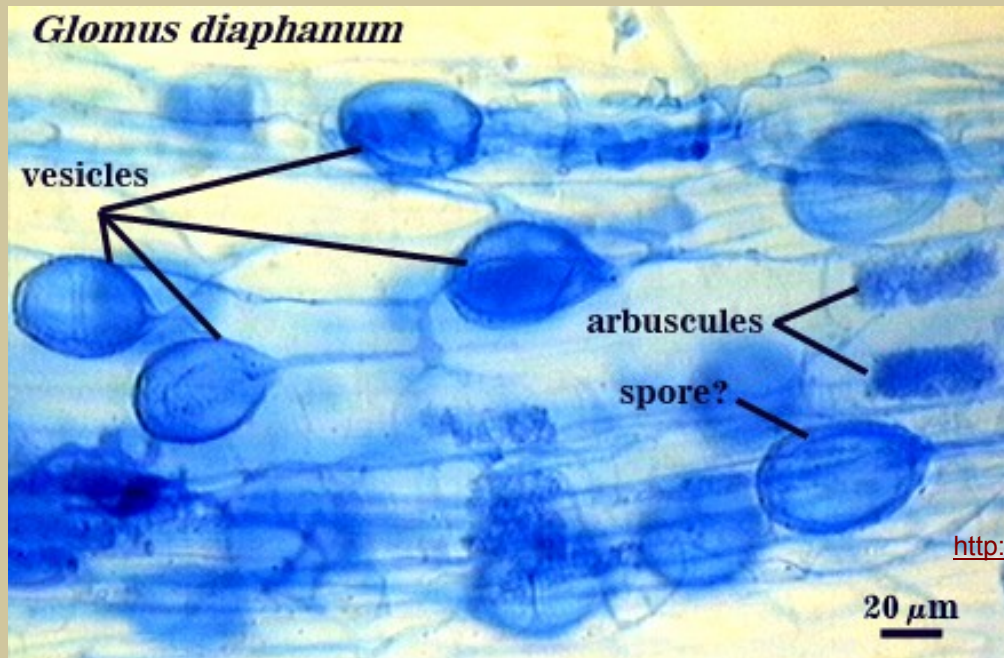
The extraradical mycelium extends outside the rhizosphere, the hyphae are forming appressoria on the root surface and penetrate the cells of the inner cortex (on the other hand, they never penetrate the endodermis, vascular bundles or root caps).

- At first the hyphae form branched **arbuscules** – it is a metabolically active part of the fungus, there is a bilateral transfer of substances across the membranes (initially there is no penetration of the plasmalemma of the plant cell, but invagination – the transfer of substances is still realised across two membranes).



Taken from http://botany.natur.cuni.cz/koukol/ekologiejhub/ElkoHub_8.ppt

Presence of the fungus affects life of the cell – a solid cell wall is not formed in the place of contact, or remains in the form of a fibrillar net; there is also evidence that the fungus may cause limited lysis of the pectin component of the cell wall. After a certain time (days to weeks), the arbuscules are „digested“ by the cell.



- Later, spherical **vesicles** are formed on terminal or intercalary positions of intraradical hyphae; these are long-lasting structures, which do not have absorbent but storage function – there are lipid particles inside and solid chitinous wall on the surface.

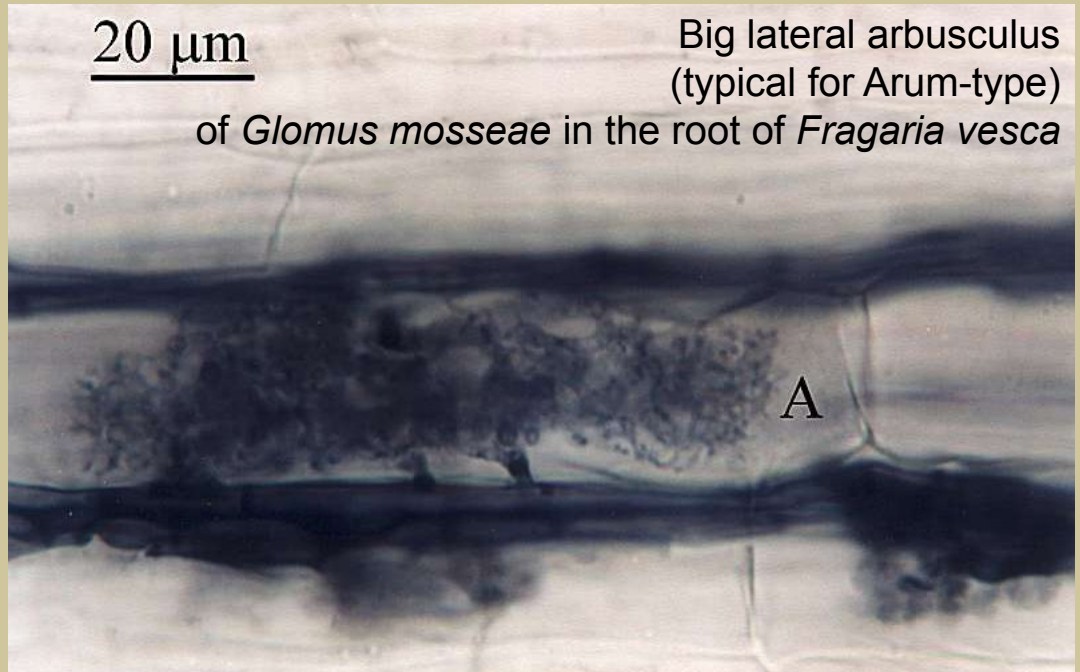
<http://invam.caf.wvu.edu/fungi/taxonomy/Glomineae/glomves.JPG>

In some plant species, vesicles are not created (family feature in some cases – it is also used in classification, in addition to molecular analyses), therefore, the abbreviated term „arbuscular mycorrhiza“ is recently preferred instead of the well-established „vesicular-arbuscular mycorrhiza“ (VAM).

In plants of the families *Zingiberaceae* and *Burmanniaceae*, arbuscular structures are also formed in the tissues of leaves, rhizomes or xylem.

Two basic morphological types of arbuscular mycorrhiza:

– Arum-type (linear AM): in the intercellular spaces of the root cortex, a hyphal network grows, the lateral protrusions of which invaginate cortical cells and form arbuscules (usually „digested“ in 4–20 days); vesicles are usually intercellular;



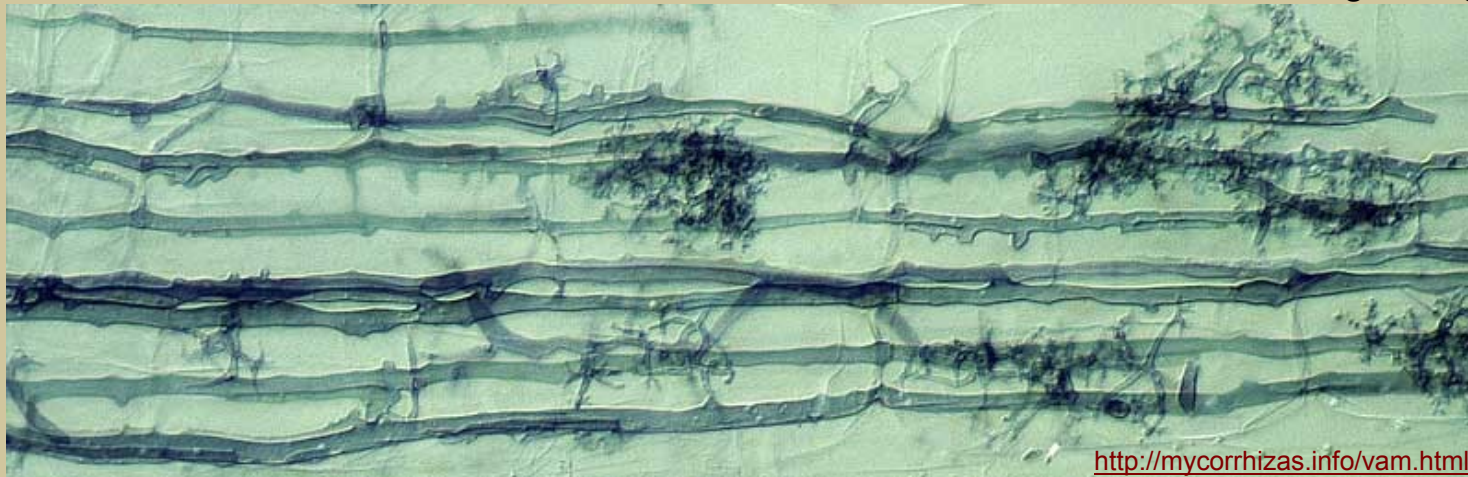
20 μm

Big lateral arbusculus
(typical for Arum-type)

of *Glomus mosseae* in the root of *Fragaria vesca*

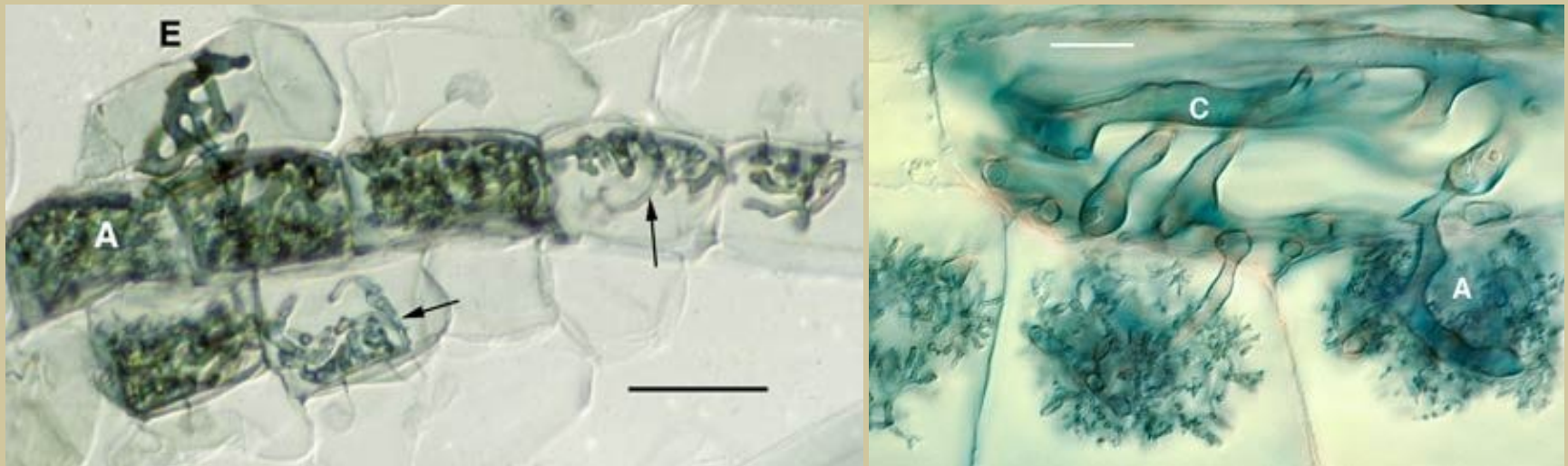
A

Bottom: Longitudinal growth of *Glomus versiforme* hyphae <http://www.sci.muni.cz/~mykorri/html/arbuscule.htm> with formation of arbuscules; their number increases with distance from the growing hyphal apex.



<http://mycorrhizas.info/vam.html>

– Paris-type (coiling AM; type widespread even in non-green plants): extensive development of intracellular hyphae in the cortex, which are surrounded by plasmalemma of the plant cells; they often form coils (different size and density in different plants), which can branch inside the cells and form lateral arbuscules.



Left: Growth of hyphae in the root cortex of *Erythronium americanum* (E – entry into the root, A – arbuscules, arrows show coiled hyphae growing through the cells; scale bar 100 μm).

Right: Arbuscules (A) growing from irregularly coiled hyphae (C) into the inner cortex cells of the *Asarum canadense* root (scale bar 10 μm).

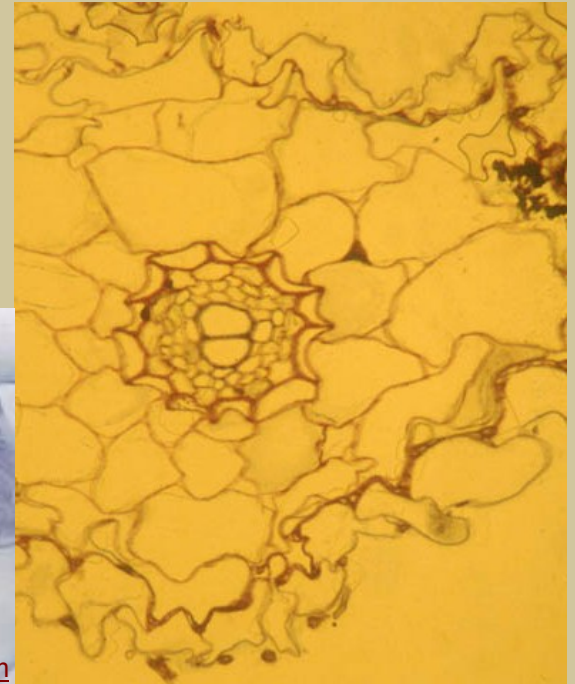
<http://mycorrhizas.info/vam.html>

Some *Gentianaceae* (*Centaurium*) have a specific type: a single plant is not able to germinate, but needs to be accompanied by other individuals, with which it is connected by fungal hyphae, through which it draws nutrients from the other ones; in addition, the cytoplasm of fungal cells is „digested“ within a few days, hence the plant needs to be repeatedly colonised for successful germination.

Another example of an atypical mycorrhiza is the tropical *Thysanotus* (*Anthericaceae*) – the fungi also connect newly germinating plants with the older ones as in the previous type; in addition, the fungal tissue forms a „sheath“ between the cortex and epidermis, which resembles mantle of ectomycorrhizal fungi after detachment of the epidermis.

http://bugs.bio.usyd.edu.au/learning/resources/Mycology/Plant_Interactions/Mycorrhizas/Atypical/atypical.shtml

Thysanotus root, cross section: distinct darker line between cells of the epidermis and the outer cortex; hyphal growth is limited to this layer only.



The process of **fungal root colonisation** can be primary and secondary.

Primary phase: hyphae germinate from the individual diaspore, grow along the root and form appressoria when contacting its surface => usually the penetration hypha (growing between cells or into rhizodermis cells) grows from only one appressorium, while the others die.

Subsequent colonisation depends on a positive response from the host plant => the hyphae penetrate through the hypodermis cells (through „short“ cells, while „long“ cells are suberised) or through the intercellular spaces.

In the Arum-type, hyphae spread through the intercellular spaces => at first the arbuscules are formed near the point of penetration into the root, then further => as the colony grows, many arbuscules are formed, but their formation decreases over time; little is known about the course of their formation in the Paris-type.

Vesicles are usually formed at certain stage of the life cycle at certain places in the root – this is likely due to the genetic information of the fungus and the plant.

Secondary colonisation: from the outer hypha (outside the root surface) a lateral protrusion grows near the appressorium => the hypha grows along the root and after a certain time (10–14 days) it colonises (again appressorium => penetration) the same root or it can grow through soil to another root. The rate of secondary colonization depends on the ability of the fungus to spread (it depends on environmental conditions and available sources, but fungi with rapid growth of hyphae forming large colonies have an advantage over species spreading mainly by individual spores).

In terms of time (if fungal diaspores are present in the soil), colonisation occurs within a few days after root formation; initially it proceeds quite quickly until it reaches a certain „coverage“ of the root with the fungus and stabilises the state. The course and rate of colonisation depends on the fungal species, number of diaspores and their distribution in the soil profile.

In annuals, the death of mycorrhiza foreshadows the plant death; in perennials it depends on the condition of individual roots – new roots are quickly colonised, while in old ones the fungus retreats, what is connected with the depletion of organic carbon reserves in the dying root.

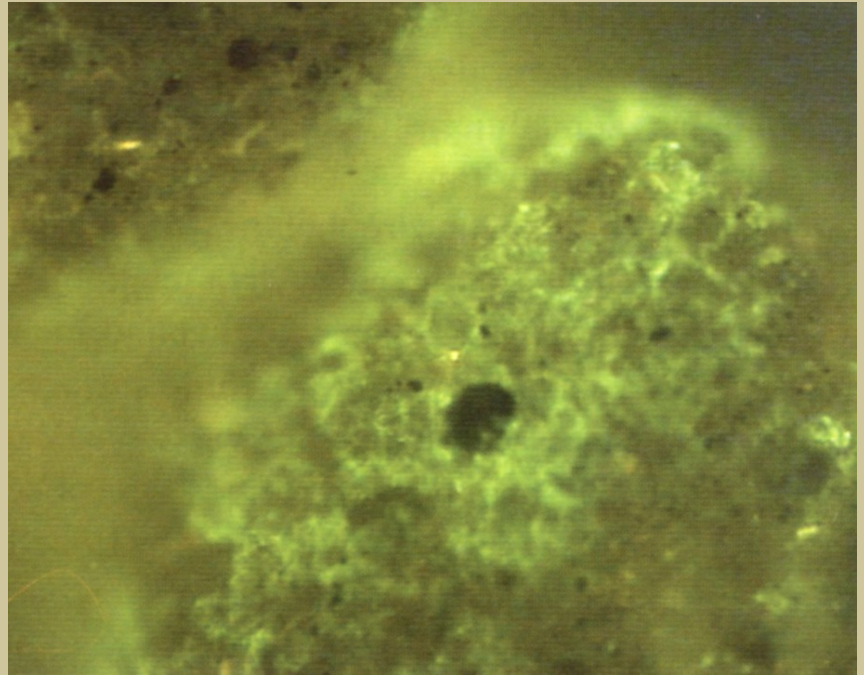
The success and extent of colonisation are influenced by environmental factors:

- amount of phosphorus in the soil: generally an indirect proportion (if there is enough available phosphorus, the plants do not need fungi), but at a certain level the mycorrhizal relationship is maintained even in phosphorus-rich soils;
- light: at low light (PhAR) the loss of organic carbon in favour of the fungus exceeds its gain from photosynthesis => plants attenuate the mycorrhizal relationship to reduce losses (this works well experimentally, but in nature, where different fungi can interconnect the roots of different plants and mutual provision of substances occurs, the results are not so clear);
- soil structure/texture and associated oxygen availability: the fungi need oxygen, so hyphae grow well only in oxygenated layer of the soil; in anoxic soil (deeper layers, very compact, clayey or flooded soil) they are able to grow only close to the roots from which they can obtain oxygen.

Other factors (water availability, pH, temperature) rather affect the mycorrhizal relationship indirectly through the condition of the plant.

Endomycorrhizal fungi release the glycoprotein **glomalin** (in tropical soil up to 5% of nitrogen and carbon can be present in this compound), which forms a hydrophobic layer on the surface of soil particles and can connect them => together with a network of hyphae and plant roots it can connect soil micro-aggregates (<250 μm) to macroaggregates (> 250 μm) => in some cases a „conglomerate“ is formed with an anaerobic environment inside, while the outer surface is aerobic => thus creating an environment for both aerobic and anaerobic microbes.

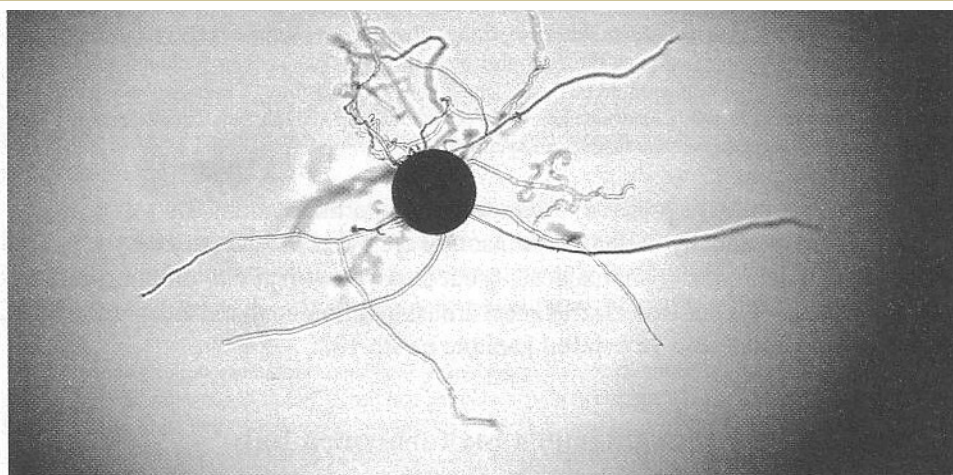
There is a direct relationship between the stability of macroaggregates and the amount of hyphae of mycorrhizal fungi in the soil (from 0.5 m/g in cultivated soils /it corresponds to about 3 m of hyphae per 1 cm of root/ to about 5 m/g in stable stands, in extreme up to 20 meters of hyphae per gram of soil). Due to the mechanical binding of soil particles and the production of exudates, there is higher stability in soils under stable stands than in disturbed or cultivated stands; if the soil is degraded, recovery of the stability is a rather slow process.



Soil „conglomerate“ with hyphae on the surface.

Source: Ritz & Young 2004; taken from http://botany.natur.cuni.cz/koukol/ekologiehUB/EkoHub_4.ppt

In the soil, fungi survive and spread through hyphae or spores – they either grow directly on the hyphae of the extraradical mycelium (terminally or subterminally) as blastospores or are formed in sporocarps. Simple sporocarps are only a tangle of hyphae surrounding the spores, complex ones have a distinct external peridium and possibly a stem with which they can be attached to the surface of soil particles; the number of spores in the sporocarps varies from units to thousands. Individual blastospores are mostly formed by species growing in disturbed soils, sporocarps mostly by species of undisturbed soils.



Obr. 10 Klíčící spora arbuskulární mykorrhizní houby *Glomus claroideum*. Spora má v průměru zhruba 150 μm a klíčí v kapce sterilního inkubačního média. Klíčící hyfy se rozrůstají všemi směry, a pokud se náhodně setkají s kořenem vhodného hostitele, kolonizují ho.

Gryndler et al.: Mykorrhizní symbióza, Praha, 2004



Glomus microcarpus, sporocarps

<http://tartufi-online.com/2008/01>

J. Trappe

<http://www.wynboer.co.za/recentarticles/200705miko.php3>

Mature spores of *Gigaspora gigantea*

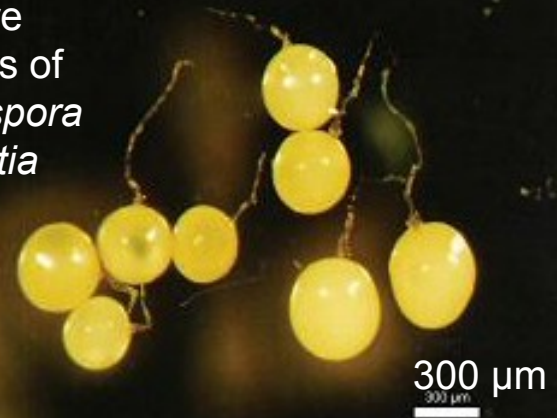


Photo André Meyer

While spore formation depends mainly on the amount of available storage substances and energy, and root colonisation is roughly the same throughout the soil profile, the density of hyphae growing freely in the soil is highest just below the surface and decreases downward – as already mentioned, the hyphae prefer less compact and more oxygenated soil.

Mycorrhizal fungi have a different ability to survive in the soil even if the plant symbiont dies (some do not survive, others up to several years); they survive best in undisturbed and dry soils (in moist soil there is higher probability that the fungus germinates and wastes its reserves in the absence of any symbiont).

Internal dormancy (several months, it does not allow germination in unfavourable periods) facilitates spore survival in some genera (mainly of the family *Acaulo-sporaceae*), while in fungi adapted to extreme temperatures, external dormancy (developed as adaptation to environmental conditions) is applied in high heat or cold (when no symbiont is available).

The possibility of survival in the form of mycelium is reduced by influences such as soil disturbance, invertebrate eating, bacteria; the hyphae survive better in soil full of organic residues than in the mineral fraction. Although arbuscular species have long been considered fungi with minimal saprotrophic abilities, dependent on their hosts, a nitrogen transport (^{15}N isotope from decaying leaves; Hodge et al., Nature, 2006) has been experimentally demonstrated in *Glomus hoi* – the question remains whether it is able to participate in the leaf decay (saprotrophy in principle) or just took the decomposition products from other microorganisms.

(Vesiculo-)arbuscular mycorrhiza is formed exclusively by fungi of the order **Glomerales** s. l. (formerly *Zygomycota*, now **Glomeromycota**; recently this order has been split into up to four orders) – conversely, *Glomerales* s. l. are solely mycorrhizal fungi (except for *Geosiphon pyriforme*, which has symbiosis with cyanobacteria).

There is a very low specificity – species of several fungal genera (about 150 species are morphologically recognised; the question is how much the number of species changes based on DNA studies) form arbuscular mycorrhiza with thousands of different plant species including ruderal (*Chenopodium*), aquatic (*Isoëtes*) or salt marsh plants (*Spartina cynosuroides*).

Compared to ectomycorrhiza, we find arbuscular representatives predominantly in herbal communities and abundantly in tropical regions (moreover there are, and will be, considerable reserves in exploration for a long time to come).

Ecto- and endomycorrhizae do not exclude each other – for example, in acacias, a connection with fungi of the genera *Thelephora* or *Pisolithus* (*Basidiomycota*) and the order *Glomerales* has been found (bacteria can also stimulate the success of a mycorrhizal relationship here, see below).

Ericoid mycorrhiza is formed by imperfect fungi (*Oidiodendron*) or ascomycetes (probably only of the orders *Helotiales* and *Leotiales*, chiefly *Pezoloma ericae*, also classified in the genera *Pezizella*, *Rhizoscyphus* or *Hymenoscyphus*) or some basidiomycetes (*Clavaria*, *Tulasnella*, *Sebacinales*). These fungi have a wide range of enzymes, including proteases, chitinases and phenol oxidases, which allow them to decompose organic matter in humus at low pH and draw nutrients from complex organic sources inaccessible to plants (peptides, proteins, chitin from fungal mycelia or dead insects, etc.).

Their partners are plants of the families *Ericaceae* and *Epacridaceae* (family from the southern hemisphere, recently also included in *Ericaceae*), usually growing on strongly acidic and nutrient-poor soils (heath vegetation, tundra and undergrowth of some northern hemisphere forests; in the southern hemisphere they occur in Australia and South Africa – simply put, plants with ericoid mycorrhiza are distributed more to the north/south and also in higher elevations than ectomycorrhizal or arbuscular plants).

The greatest problem of plants, which symbiosis with fungi helps to solve, is the difficult availability of nitrogen and phosphorus – the latter is bound mainly in organic form, often in complexes with iron and aluminum. The fungi must be tolerant to these metals – the mechanism of tolerance has not yet been elucidated, perhaps they can enclose them in the cell wall or in vacuoles; they can also regulate uptake of these metals by the plant.

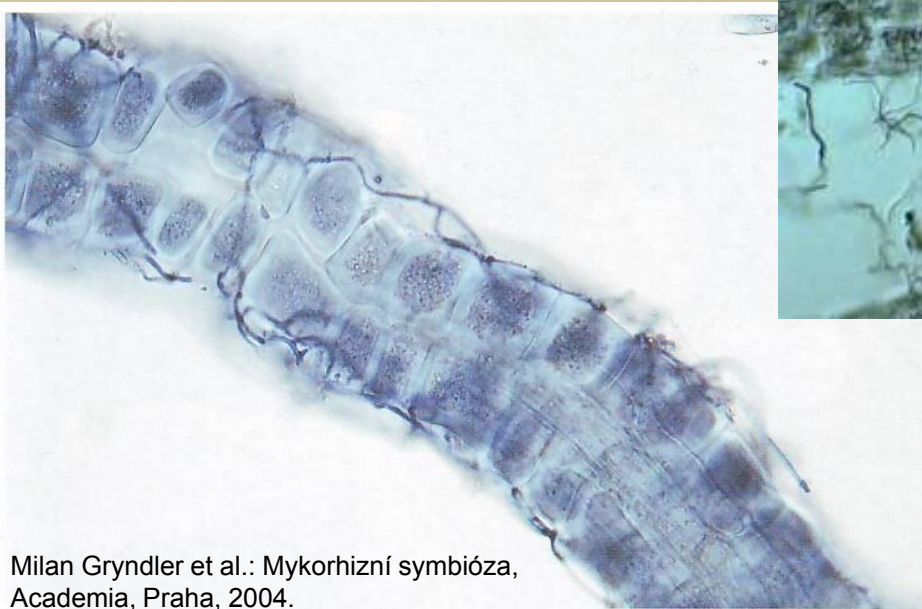
Characteristic anatomical structure of ericoid mycorrhiza are thin ephemeral roots of ericoid plants, the so-called hair roots.

These roots are very primitive, the stele is surrounded by only 1–3 layers of cells without root hairs (unlike arbuscular mycorrhiza).

From the colonised roots, a network of extraradical mycelium penetrates into the surrounding substrate (another difference compared to AM: hyphae of the above-mentioned fungi are septate).

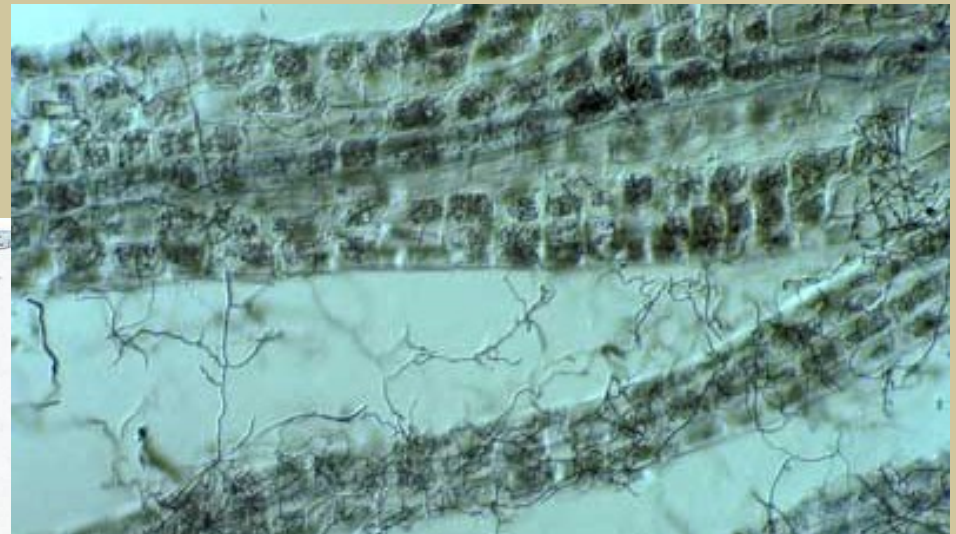
Extraradical mycelium and hyphal coils in hair root cells of *Leucopogon verticillatus*

<http://mycorrhizas.info/ozplants.html#ericoid>



Milan Gryndler et al.: Mykorhizní symbióza, Academia, Praha, 2004.

12 Kořen pěníšníku (*Rhododendron* sp.) kolonizovaný přirozeným společenstvem erikoidních mykorhizních hub. Na povrchu kořenových buněk (které se v levé horní části obrázku od kořene částečně oddělují) jsou vidět mimokořenové distribuční hyfy. Barveno trypanovou modří.



Hyphae penetrate only into the cells of the outer cortical layer of the roots – hypodermis (preferably at the tips, where the plants form hairy roots in the extreme with only one layer of cortical cells).

Detail of mycelium and hyphal coils in hair root cells of *Leucopogon verticillatus* <http://mycorrhizas.info/ozplants.html#ericoid>

During the colonisation (it is important to recognise specific partners) an appressorium is pressed on the plant cell surface => hyphae invaginate into the cell => their loops and coils, where the exchange of substances



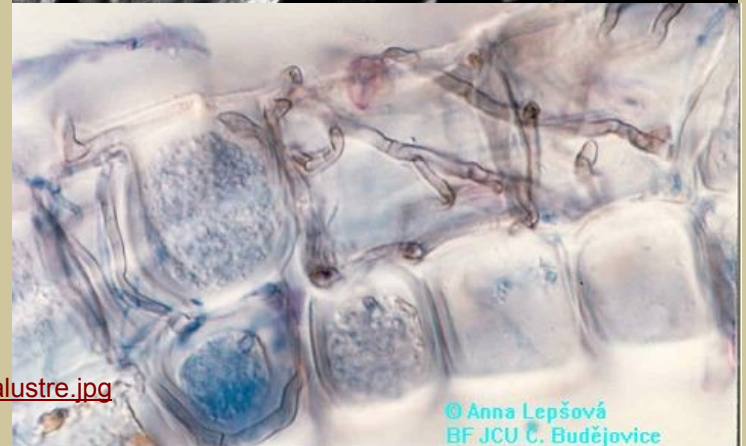
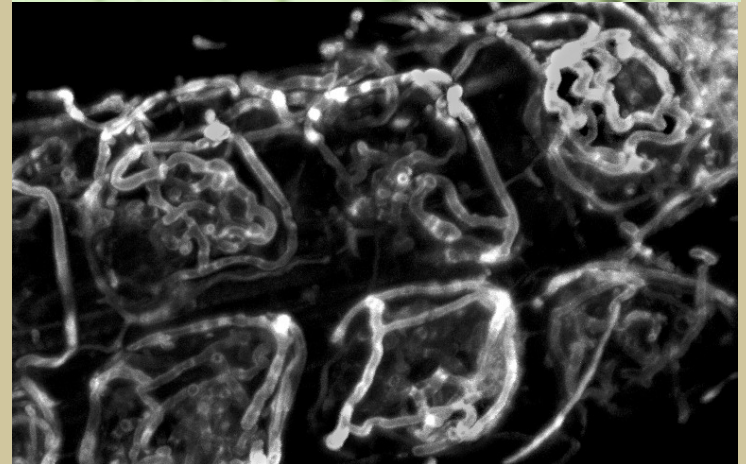
between the fungus and the plant takes place, finally fill almost entire cells.

Drawing and photo Hugues Massicotte, <http://web.unbc.ca/forestry/Hugues/Images/>

Over 70% of the cells are colonised; only one species of fungus colonises each individual cell, but different fungal symbionts may be present in neighbouring cells of the same root – however, due to their difficult cultivability, it is difficult to identify them.

Different hyphae colonising root cells of *Ledum palustre*

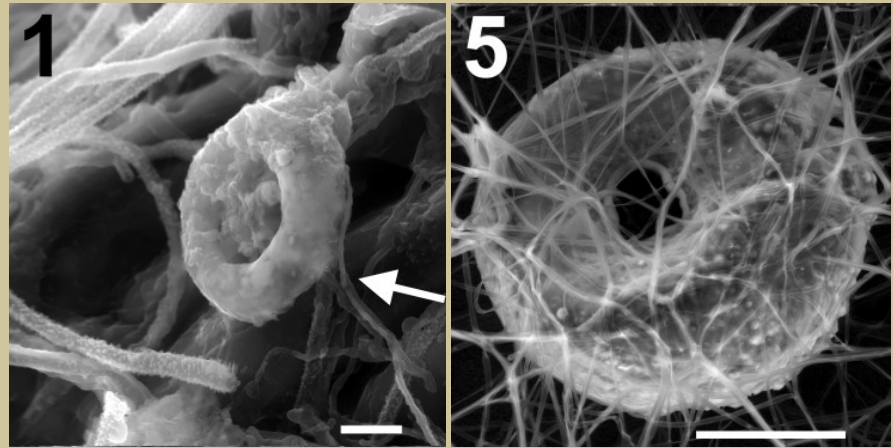
Photo Anna Lepšová, <http://botanika.bf.jcu.cz/mykologie/galerie/mycorhiza/Ledumpalustre.jpg>



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The mycorrhizal connection is physiologically active for a short time (3–4 weeks), then the fungus penetrates into new cells, which is related to formation of new roots.

It was observed that the hyphae of some ericoid mycorrhiza forming fungi contact the tests of testate amoebae.



Pezoloma (Rhizoscyphus) ericae colonising the test.

Source: Vohník et al. 2008; taken from http://botany.natur.cuni.cz/koukol/ekologiehup/EkoHub_5.ppt

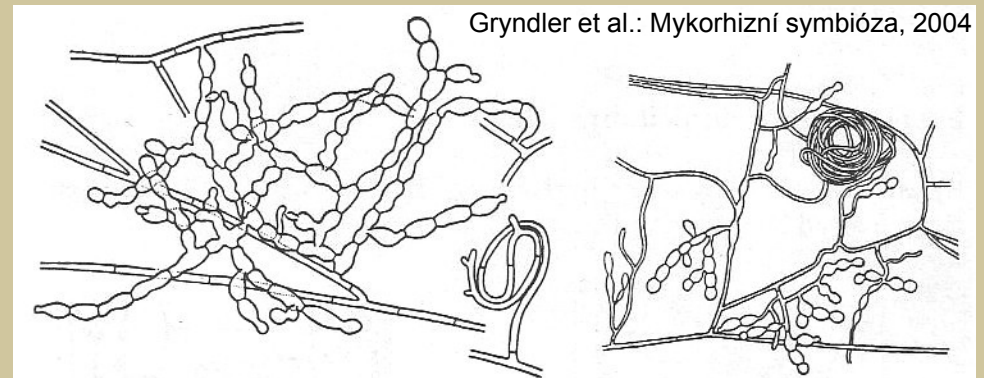
In regions with distinct alternation of dry and rainy periods, plant growth and their mycorrhizal symbiosis has a seasonal character – hair roots, whose cells are colonised by fungi, are formed when the wet period is coming, and die again with the onset of drought. The fungus can survive either in the form of spores in the soil or in specialised plant cells – in the tissues of some species (*Woolsia pungens* etc., Australia) thick-walled cells containing viable hyphae have been found; the hyphae can survive drought in these cells and grow again into newly formed roots, when the wet period is coming.

The fungi forming ericoid mycorrhiza are another group in which the ability to saprotrophic nutrition has been found – *Pezoloma ericae* has this ability even stronger than ectomycorrhizal species. In general, these fungi can survive for a long time without a partner plant, which is obviously related to the above-mentioned enzymatic equipment.

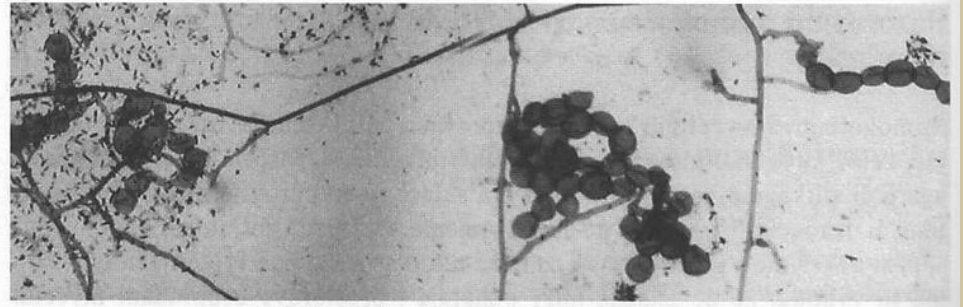
Orchid mycorrhiza is formed by orchids (probably all species of the family *Orchidaceae*) with basidiomycetes of the genera *Sebacina*, *Tulasnella*, *Thanatephorus*, *Ceratobasidium* (species of the anamorphic genus *Rhizoctonia*), observable in the form of anastomosing extraradical mycelium, on which they can form inflated „moniliform“ cells; their clustering creates sclerotia, in which the fungi survive worsened conditions.

Other orchid mycobionts are fungi of the genera *Armillaria*, *Mycena*, *Marasmius*, *Russula*, *Hymenochaete*, *Xerotus*, *Fomes*, *Coriolus*, *Thelephora*, *Tomentella* and even the ascomycete *Tuber*; these fungi represent ectomycorrhizal or phytopathogenic species, which redirect the substances gained from other plants and „feed“ the orchids (it is typical for non-green orchid species).

The species that form OM in nature are referred to as „ecological symbionts“; however, other fungi are also able to support seed germination in laboratory conditions („physiological symbionts“).



Obr. 24 Bernardovy kresby orchideoidních mykorhizních hub. Jsou patny jak přímé hyfy, tak ztlustlé moniloidní buňky. (Podle Bernarda, 1909.)
diameter of moniloid cells = cca 20 μm



At least in the first phase of ontogenesis, orchids are dependent on the supply of nutrients from the fungus, because their small seeds lack enough nutrients for successful germination – the germination is therefore conditioned by presence of a symbiotic mycobiont. Their „encounter“ can happen in three ways: either there is a successful recognition => mycorrhizal relationship, or they do not „know each other“ => the seed does not start to germinate, or the fungus becomes a parasite => death of the plant. A possible prevention by the plant is the excretion of phytoalexin orchinol – in this case it is important for the plant to survive the initial phase of „infestation“ before it creates enough defensive substances and so-called physiological

compensation occurs (in the next phase mycorrhiza can also protect the plant against other soil fungi).

Germination process (in the case of successful recognition): the seed absorbs water, at the same time the hyphae penetrate its testa and grow into the embryo => invagination of the plasmalemma => formation of hyphal loops in plant cells => septate hyphae in the cells are „digested“ after a certain time.

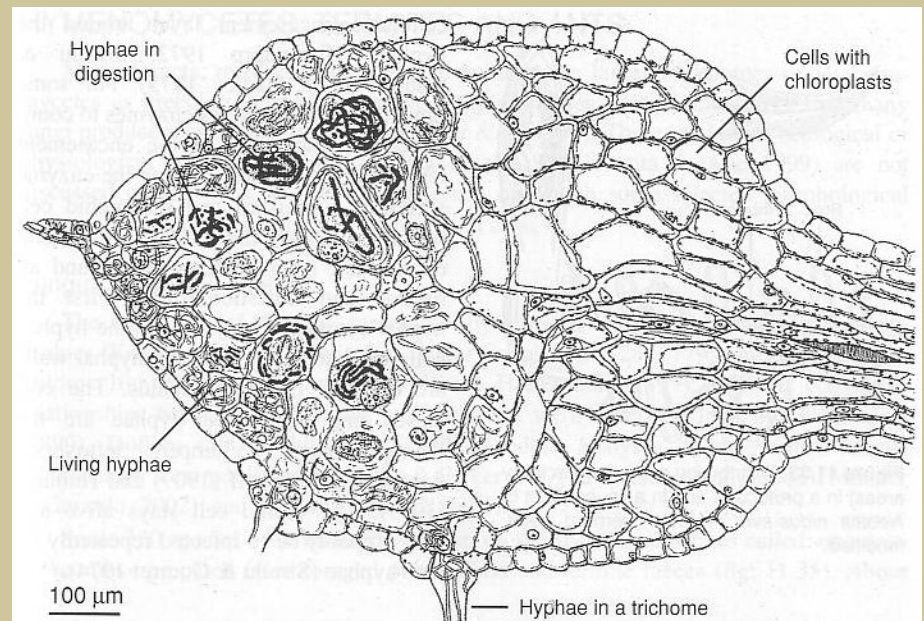


Figure 11.32: Longitudinal section through a young embryo of an *Epidendrum* species with the fungus confined to the lower part (left). Living hyphae are in peripheral cells; the inner cells show hyphae in various stages of digestion. The hyphae in the trichome may be emanating hyphae (as advocated by the author of the drawing) or invading hyphae. – From Burgeff 1909.

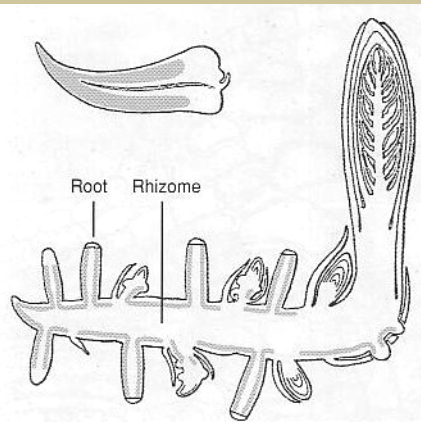
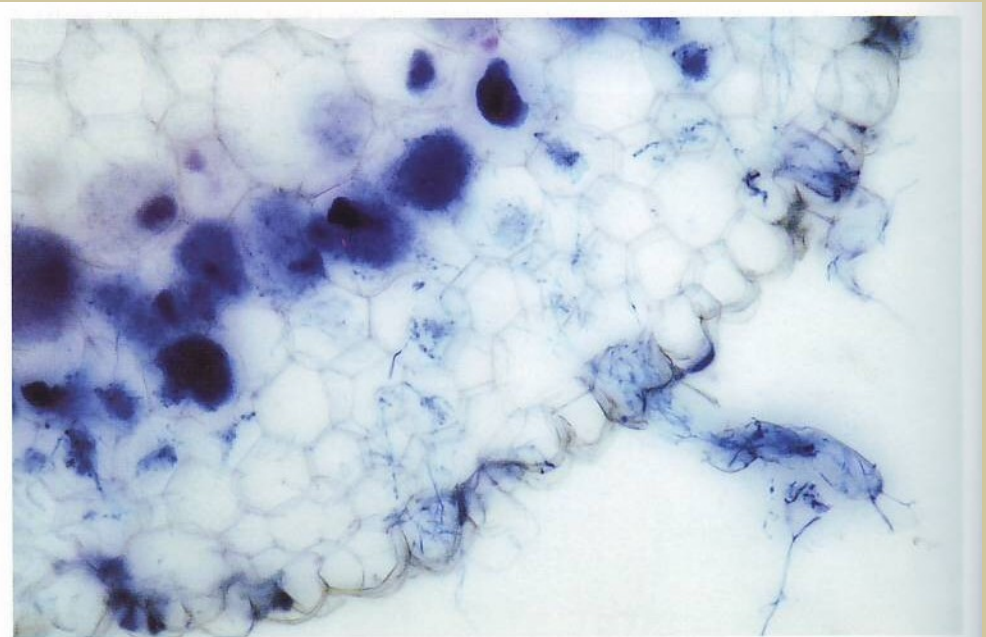


Figure 11.33: Distribution of the fungus (grey areas) in a protocorm and in a young plant of *Neottia nidus-avis*. – From Bernard 1909, modified.

Plectology of the Hymenomycetes. Berlin-Stuttgart, 2004.

Thus, the fungus colonizes the embryo before formation of plant organs => during the plant development, the basal part remains colonised, from which the roots develop, while base of the shoot grows away of the colonisation. While in terrestrial orchids terminal part of the roots is the most colonised, in epiphytic ones it may be different, the fungus is mostly beneficial in places of contact with the substrate.

Heinz Cléménçon:
Cytology and



8 Kolonizace kořenů orchideje *Ophrys lutea* subsp. *galilea* neidentifikovaným druhem houby rodu *Rhizoctonia*. Hyfy pronikají do kořene buď přes kořenové vlásky (vpravo dole), nebo přes kořenovou pokožku (rhizodermis). Zevními vrstvami primární kůry, takzvanými průchozími buňkami, pouze prorůstají, aniž by vytvářely typické hyfové smotky. Ty se vytvářejí až v hlubších vrstvách, kde můžeme nalézt jak plně funkční smotky (na obrázku jsou difuzně modré), tak smotky degenerované (temně modré, ostře ohraničené útvary v některých buňkách).

Hyphae penetrate into the roots through „passage cells“ in the exodermis (or mesodermis) => further, the fungus can spread through neighbouring cells, it does not penetrate between the cells (similar to Paris-type endomycorrhiza or ectendomycorrhiza).

Milan Gryndler et al.: Mykorrhizní symbióza,
Academia, Praha, 2004.

The exchange of nutrients takes place mainly in trophocytes („host cells“) in the primary cortex (neither endodermis nor tissues of the middle cylinder are colonised). Transfer of compounds is started after hyphal invagination into the plant cell, where clusters of branched hyphae (pelotons) are formed; subsequently, the hyphae can grow into other cells.

After a certain time, the plant cell enters the phase of phagocyte („digestive cell“) – enzymatic digestion of the hyphal content

occurs => coils of dead hyphae can then be observed in the cells, or the cell „digests“ the hyphal walls; over time it can be colonised again by new hyphae. (However, the „digestion“ of mycelia that has penetrated the cells is not a specialty of orchid mycorrhiza; a similar process takes place, for example, in lycophytes, ferns or *Pyrolaceae*.)



Obr. 26 Mladý hyfový smotek – peloton – v buňce kořenové kůry orchideje *Platanthera bifolia*; barveno trypanovou modří.

Milan Gryndler et al.: Mykorhizní symbióza, Academia, Praha, 2004.

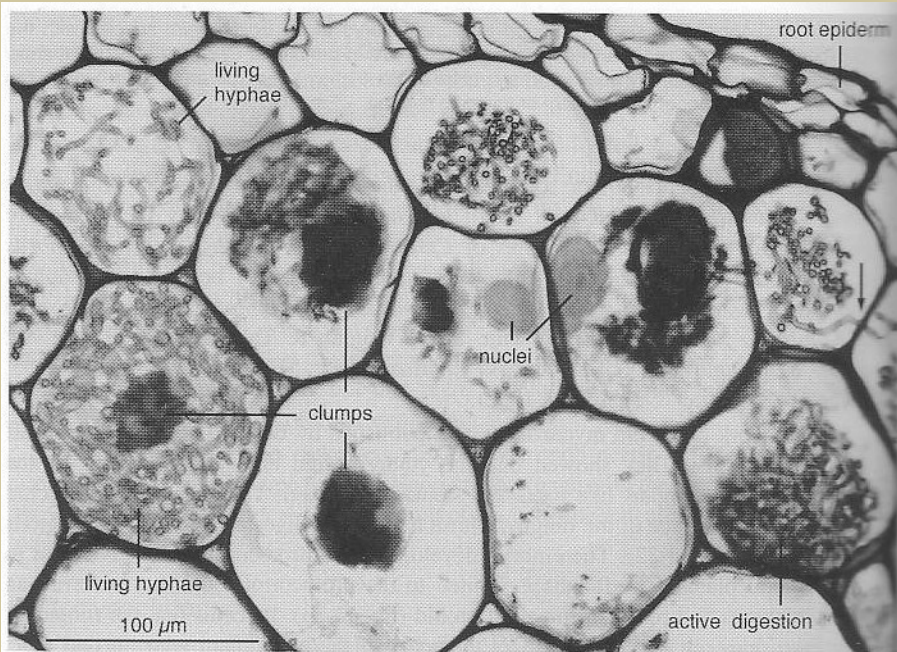
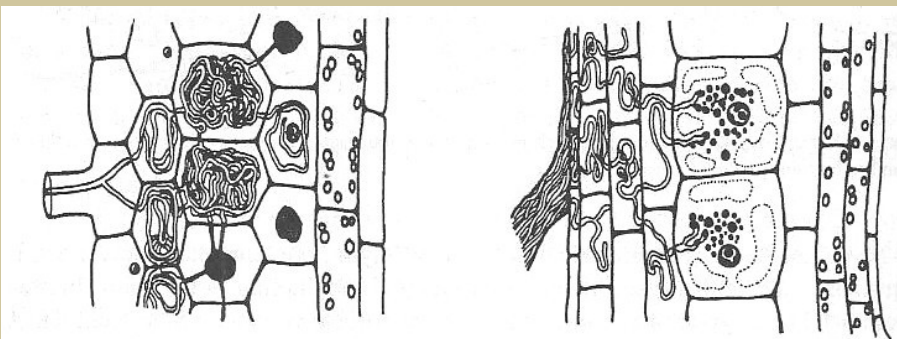


Figure 11.34: Orchid endomycorrhiza. Cross section through a root of *Cypripedium pubescens*. The hyphae of the *Rhizoctonia*-type fungus are seen in various stages, from fully alive to almost fully digested, forming compact clumps of hyphal wall remnants. Some root cells contain clumps and living hyphae simultaneously. In two root cells the nuclei are visible. **Arrow:** passage of a hypha from one cell to the other. – Microtome section stained with iron haematoxylin. Original photograph.

Although the plant also obtains substances from the fungal body in this way, the elimination of hyphae in plant cells is rather considered to be a defense mechanism against excessive development of the symbiont, which could potentially become a pathogen (however, a simple autolysis of aging hyphae is also possible).

Heinz Cléménçon: Cytology and Plectology of the Hymenomycetes. Bibliotheca Mycologica 199. J. Cramer, Berlin-Stuttgart, 2004.

A common tolypophagous form of orchid mycorrhiza is described above. In several non-green tropical species, the ptyophagous form is known – in this case hyphal coils are not formed in the cells, but individual hyphae grow into the cells (from a rhizomorph outside the root), which are lysed here and their content is poured into vesicles (ptyosomes).



Tolyfágní forma

Ptyofágní forma

Milan Gryndler et al.: Mykorhizní symbióza, Academia, Praha, 2004.

Obr. 25 Formy orchideoidní mykorhizní symbiózy. /Podle Bernarda (1909) a Burgeffa (1936)./

Orchid mycorrhiza is the only symbiotic relationship with the participation of fungi, in which the flow direction of carbon compounds is from the fungus to its partner. During the initial development of a non-green young plant, it is a fungus that produces cellulases, which contribute to the degradation of cellulose (in epiphytic species it is also important in adulthood) => the plant then takes the obtained oligosaccharides from the fungus (some plants also draw other nutrients from the fungi – they serve as a source of nitrogen and phosphorus, vitamins, amino acids or growth hormones) – in this phase of plant ontogenesis it is basically parasitism until the plant fully begins photosynthesis (later the metabolism is probably mycorrhizal, but carbon transfer from plant into the fungus still remains unconfirmed, although some experiments suggest it).

Permanently non-green orchids thus remain parasites on „their“ fungus for the whole life (the fungi can also form ectomycorrhizal connections with other plants in the vicinity and then it can actually be phytoparasitism through the fungus, see above).

Some orchids are perennial, others survive in underground organs (tubers) – at this stage the fungus is excluded outside, it can be isolated from the root or tuber surface, but does not penetrate into the tissues.

Fungi entering orchid mycorrhiza also have saprotrophic abilities, in addition to the above-mentioned cellulases they also possess pectinases or polyphenol oxidases.

In anamorphic fungi of the genus ***Rhizoctonia*** (mentioned in orchids) with teleomorphs in the orders *Ceratobasidiales*, *Platyglloeales*, *Exidiales*, *Tulasnellales* and *Sebacinales*, we find a wide range of relationships with various plants.

Teleomorphic genus ***Sebacina*** (including anamorphs in the genus *Rhizoctonia*) is a genus with the largest known number of mycorrhizal associations – it forms orchid mycorrhiza (*Neottia nidus-avis*), ericoid mycorrhiza (*Gaultheria shallon*), endomycorrhiza in liverworts, ectomycorrhiza (*Dryas octopetala*), eventually both ectendomycorrhiza and ectomycorrhiza (*Pinus*, *Salix*, *Tilia* or *Eucalyptus*).



Hyphae of the genus *Rhizoctonia* are characterized by more or less rectangular branching.

<http://www.forestpests.org/nursery/rhizoctoniablight.html>

Sebacina incrustans

http://users.skynet.be/bs133881/champis/sebacina_incrustans_%28yd%29_2.htm

As already mentioned, mycorrhiza is not limited to vascular plants – some ascomycetes and basidiomycetes (again mainly *Sebacinaceae*) enter a symbiosis with frondose liverworts and form **jungermanniid mycorrhiza**. Hyphae of fungi grow into rhizoids of liverworts; these hyphae can also form ectomycorrhiza with surrounding plants. Purely mycotrophic is the non-green liverwort *Aneura mirabilis* (*Cryptothallus mirabilis*, right photo), nourished by a fungus (*Tulasnella* sp.), in which a connection to birch or pine has been found.

Heinz Clémenton: Cytology and Plectology of the Hymenomycetes. Bibliotheca Mycologica 199. J. Cramer, Berlin-Stuttgart, 2004.

<http://rbg-web2.rbge.org.uk/bbs/Resources/gallery/cryptothallus%20mirabilis%203.jpg>

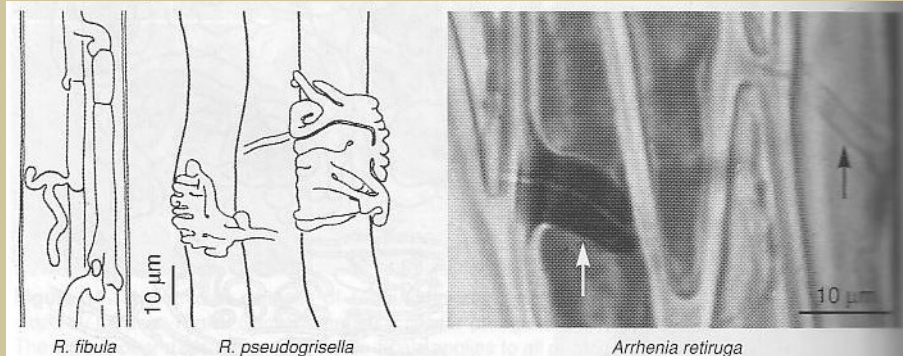
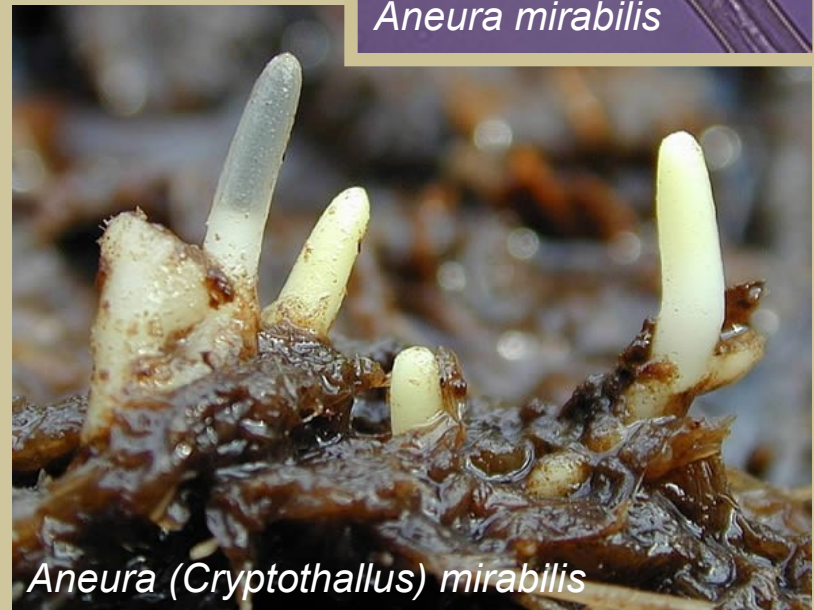


Figure 11.20: Drawings: Hyphae of *Rickenella fibula* in a caulonema of a moss; and palmate-like appressoria of *Rickenella pseudogrisella* on the surface of rhizoids of the liverwort *Blasia pusilla*. – From Kost 1988. – **Photograph:** Infection canal (white arrow) of *Arrhenia retiruga* surrounded by a massive, dark deposit in a leaf cell of *Brachythecium rutabulum*, and a hypha in a cell (black arrow). – From Hassel & Kost 1998, modified.

Not every connection with the fungus is mycorrhizal, in the right photo it is obviously a parasitic penetration.





Epipactis microphylla is nourished by truffles (so far the only known ascomycetes).



Galeola septentrionalis draws nutrients from *Armillaria*, parasitising on woody plants.

Besides other mycorrhizal relationships, it is worth mentioning the **mycoheterotrophic plants** (the term epiparasitic might be more accurate, because in principle they parasitise on fungi and thus through them on other plants). So far accepted view that they are saprotrophic perennial herbs is clearly wrong – these plants are not able to decompose organic matter. Some plant species are non-green for the whole life, others only initially; in some species we find even photosynthetic and non-photosynthetic individuals.

http://www.aho-nrw.de/Arten/Arten_Ep-micr.htm <http://www.parasiticplants.siu.edu/Mycotrophs/images/Orchids/GaleolaSeptentrionalis1.jpg>

These are not only non-green orchids or species using monotropoid mycorrhiza – mycoheterotrophy has already been found in at least 400 species of plants belonging to various groups (liverworts, ferns, *Orchidaceae*, *Ericaceae*, *Pyrolaceae*, *Monotropaceae*, *Gentianaceae* or *Fabales*); their symbionts are mostly *Basidiomycota* and they are quite strictly bound to the species of fungal host.

ENDOPHYTIC AND EPIPHYTIC FUNGI

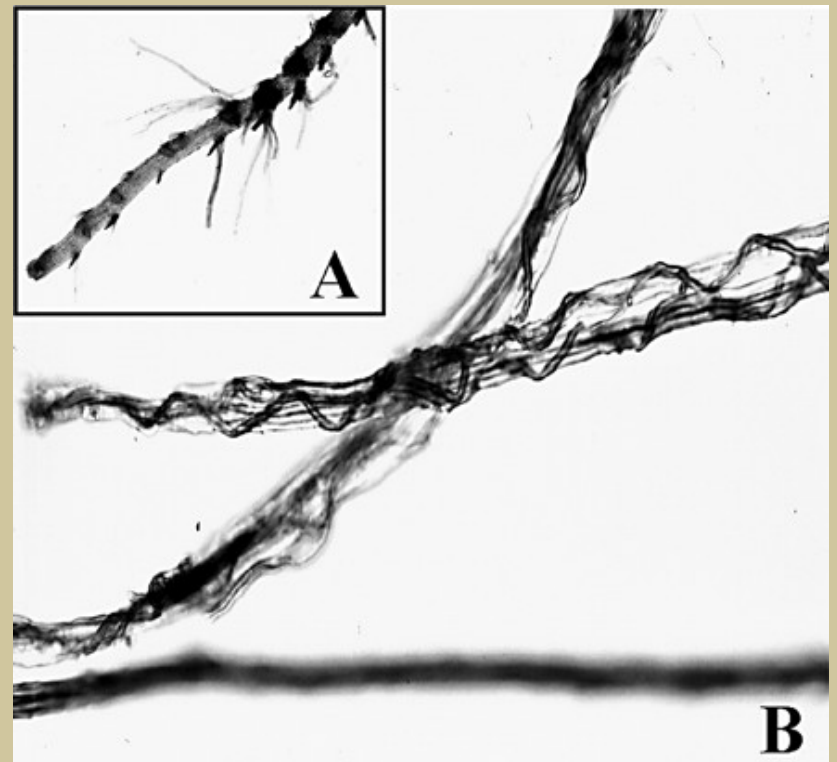
A special case is **fungi living endophytically** in the intercellular spaces of plant tissues, which are neither parasites nor symbionts directly connected to plant cells; usually there are no apparent external manifestations of colonisation – we are talking about asymptomatic colonisers. Plants probably provide them with a suitable niche for growth, as they represent a stable environment and source of organic carbon; on the contrary, fungal metabolites can protect plants against herbivorous insects, pathogenic fungi, bacteria and other organisms (antibiotics, alkaloid production in *Clavicipitaceae*, etc.).

In almost all plants recently tested for the presence of endophytes, the result was positive, so this is probably a widespread phenomenon; endophytic fungi are recruited from practically all divisions of fungi (mostly ascomycete anamorphs, also some *Xylariales*, endophyticism was also observed in *Coprinus* group!) ...

Hyphae of *Xylaria* growing through and braiding rhizoids of the *Bazzania* liverwort (orig. 1000x).

Inset: stolon with fascicles of rhizoids (orig. 40x).

Source: Davis et al. 2003, <http://www.amjbot.org/cgi/content/full/90/11/1661>



and have been recorded in tissues (especially in leaves) of many species of terrestrial and aquatic plants, as well as red and brown algae). Hundreds to thousands of strains belonging to dozens of species are commonly found in various parts of the plant. Colonisation can vary in size from single cells (*Rhabdocline*) to ingrowth of the entire shoot, stems and leaves (*Chaetomium*), as well as root endophytes (*Phialocephala*); likewise, among endophytes there are species widely distributed ecologically and geographically ...

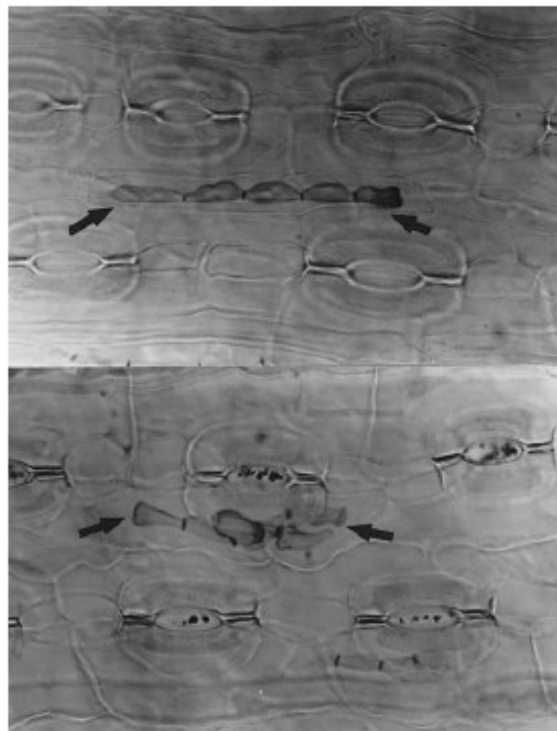


FIGURE 12.1 Intracellular *Rhabdocline parkeri* hyphae (arrows) in Douglas fir (*Pseudotsuga taxifolia*) needles ($\times 500$).

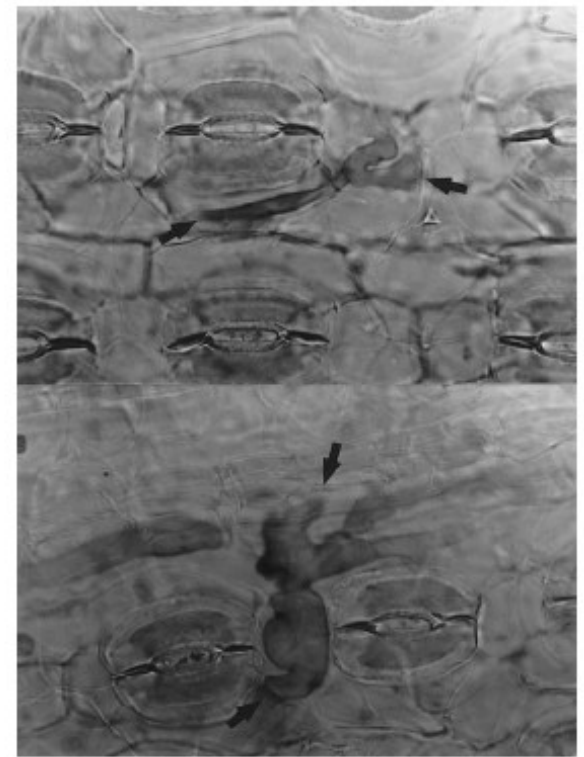


FIGURE 12.2 Intracellular *Phyllosticta abietis* hyphae (arrows) in Giant fir (*Abies grandis*) needles ($\times 500$).

Source: Müller et al. 2004; taken from http://botany.natur.cuni.cz/koukol/ekologiehub/EkoHub_8.ppt

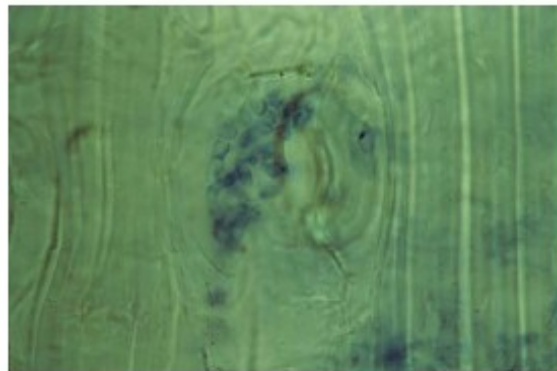


FIGURE 12.3 Hypha of an unidentified endophyte in epidermal cells of *Picea pungens*. Needles were cleared in 10% KOH and stained with 0.05% trypan blue in lactoglycerol.

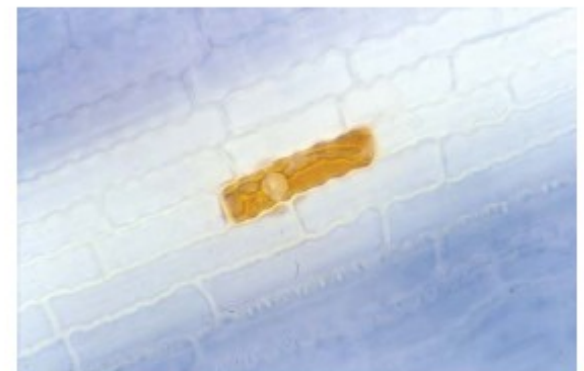


FIGURE 12.4 Hypha of *Stagonospora innumerosa* in an epidermal cell of *Juncus effusus* var. *pacificus*. The epidermis was excised with a razor blade, cleared by boiling in lactophenol-ethanol (1:2 v/v), and stained in acid fuchsin-malachite green (Cabral et al. 1993).

... and, conversely, species with a narrow host specificity and a rare and limited occurrence. Spread of fungal diaspores takes place in air, but also by insects (even a few entomopathogenic fungi have been found to be endophytes).

It is assumed that this is a mutually beneficial relationship – plants provide the fungi with a stable environment and source of nutrients; on the other hand, in order for endophytic fungi not to grow uncontrollably, plants must have developed mechanisms by which they can limit their growth. These mechanisms are usually physical and chemical (production of phenolic compounds etc.) and their application can also increase the resistance (stimulate the response) of plants against other, pathogenic fungi.

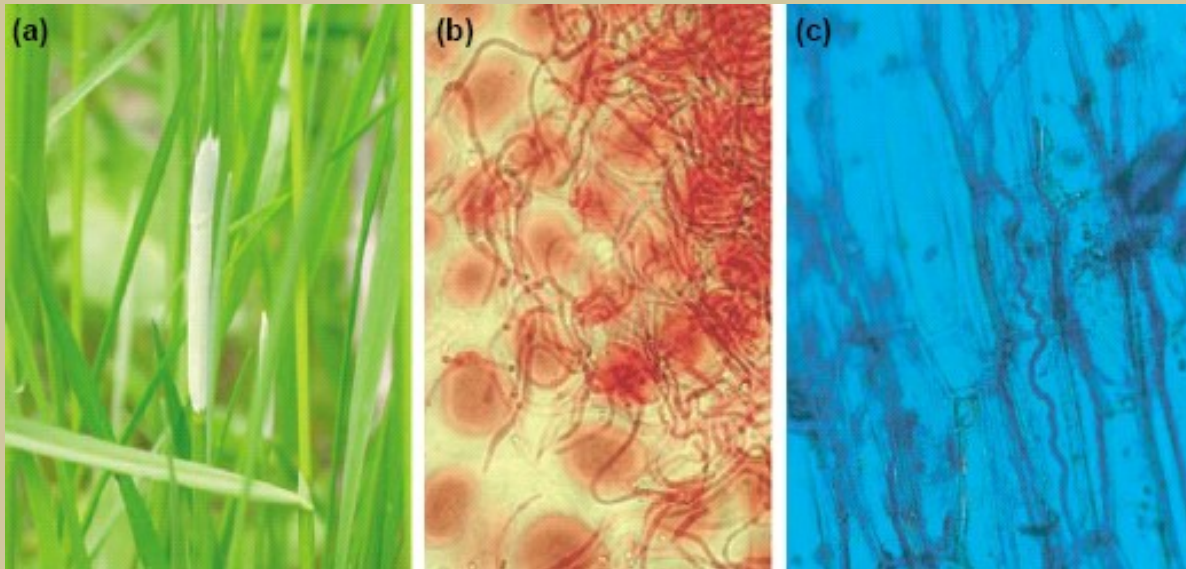
Plants also benefit from endophytic symbiosis: fungal metabolites are a source of nutrients, the already mentioned stimulation of defense against pathogens (e.g. *Chaetomium*), protection against herbivores (*Phomopsis*, *Lecanicillium*; animal toxic substances are distributed throughout the plant body, even if the fungus colonises only part of the tissue) and increase in tolerance to drying was noted (perhaps by changing the osmotic conditions in the tissue).

Source: Redman et al. 2002

Thermotolerance of *Dichantheium lanuginosum* – right plants colonised by an endophytic fungus of the genus *Curvularia*, left without endophytes.



Plants may also have developed mechanisms that facilitate fungal colonisation in the next generation – for example, the genus *Neotyphodium* on grasses, where there is a „vertical transfer“ (in the seeds to the next generation). *Neotyphodium* (teleomorph: parasitic genus *Epichloë*) belongs to the specific grass endophytes of the order *Hypocreales*; in this genus, it was well observed that the proportion of individuals with endophytes increased over time compared to non-colonised ones, which were grazed by herbivores, while colonised plants did not. The negative effect on herbivores, as well as invertebrate parasites of plants, is due to the formation of alkaloids that repel these animals and can be toxic to them – clavine alkaloids repel mammals (tested on rabbits, but can also bother cattle), while ergovalines and lysergic acid amides have negative effect to insects.



(In addition to this effect, the osmoregulatory function of alkaloids is also considered, but results of different studies are contradictory.)

Left: teleomorph stroma of *Epichloë typhina*, centre: *Neotyphodium* sp. hyphae in seed, right: in leaf.

Source: Mueller & Krauss 2005;
Taken from http://botany.natur.cuni.cz/koukol/ekologiehub/EkoHub_8.ppt

Epiphytic fungi differ somewhat from endophytic fungi; these fungi rather only use leaf exudates and do not penetrate into the tissues. They are usually melanised (UV resistant) and some are able to break down fats and thus utilise a wax layer on the leaf surface.

Endophytes (or also epiphytes) do not damage the healthy tissue of their „hosts“, but they can also be **latent pathogens** – either physiological changes in the host tissue (they can be caused by fungal activity, or even natural tissue aging) can lead to the change of symbiont into pathogen, or changes in the environment that increase the stress on the plant (e.g. *Alternaria* becomes a pathogen in potassium deficiency).

Latent infection (can last for days or even years) can then change into a disease that manifests itself externally. The direct cause of its outbreak is the action of fungal metabolites or depletion of nutrients available for the host; it must also not be forgotten that there are a number of fungi in plant tissues, so in many cases the action of one weakens the plant and the other fungus kills it.

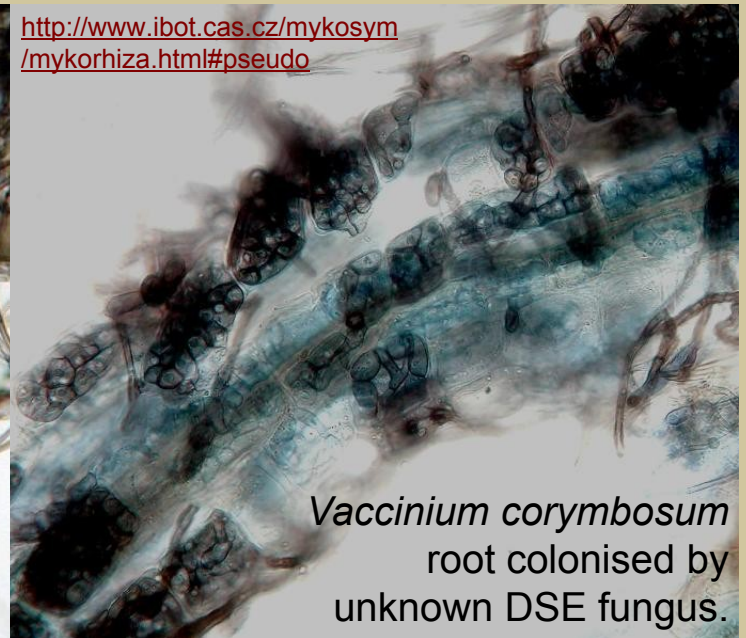
After fall of the colonised part of the plant, the endophytes and epiphytes are the first colonisers to start the decomposition process.

Besides typical endophytes, we can mention „pseudomycorrhizal“ fungi forming **DSE-associations** (dark septate endophytes), manifested by asymptomatic colonisation of conifer roots, herbs (grasses) or ericoid plants. These are anamorphs of saprotrophic ascomycetes with melanised hyphae (e.g. *Phialocephala fortinii*, *Meliniomyces variabilis*, *Cadophora finlandica*). In their case, slight „harmless“ parasitism of the fungus on the plant was previously considered, but it appears that the connection could be neutral or mutually beneficial (it seems that the relationship may vary depending on environmental conditions).

Thick melanized septate hyphae form a dense network around the host root. They often form appressoria, penetrate the root tissues and grow along the stele.



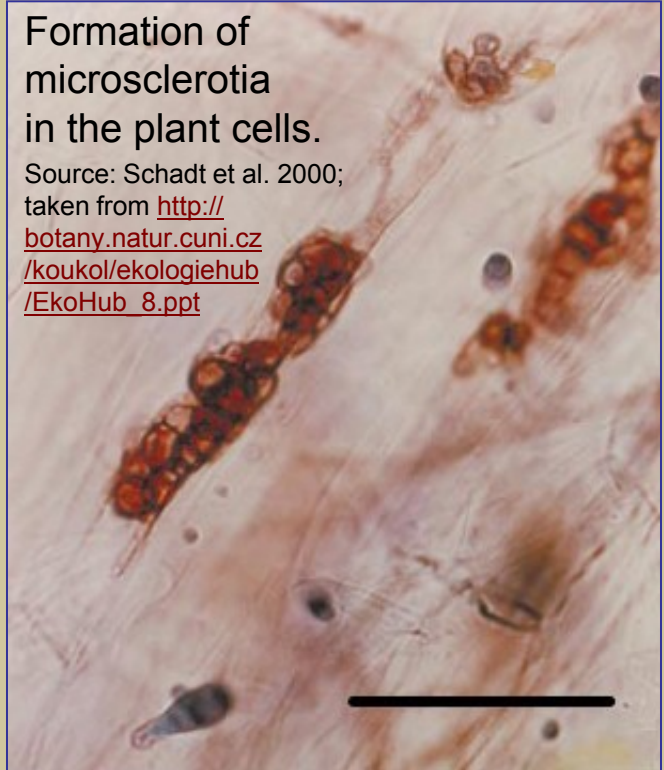
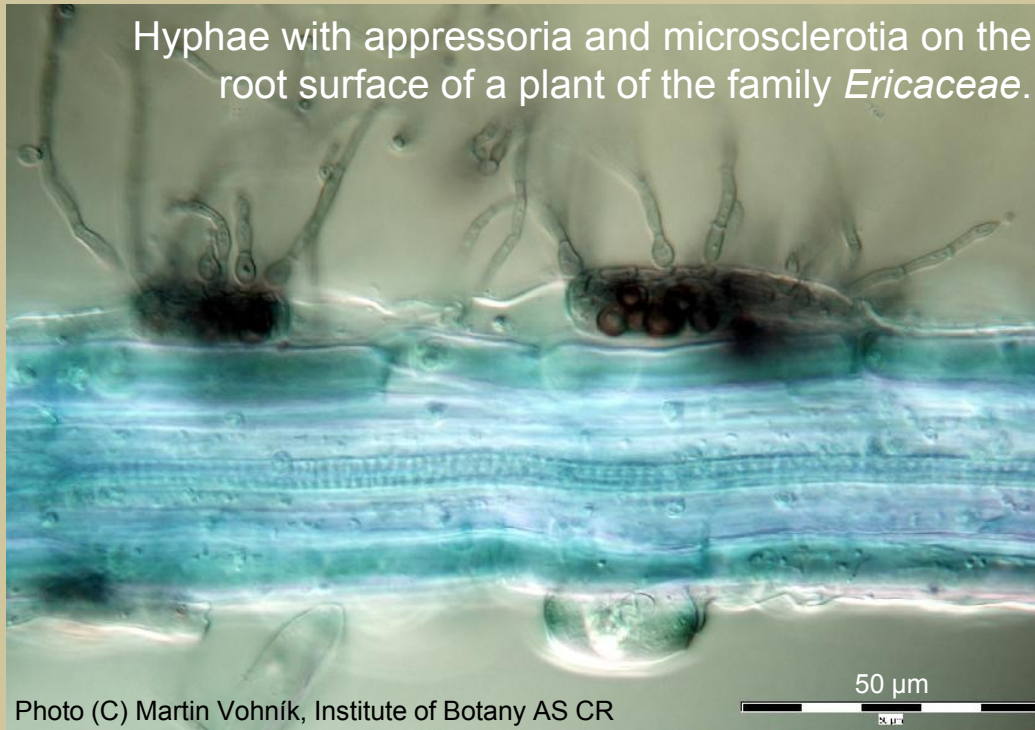
Photo (C) Martin Vohník, Institute of Botany AS CR



<http://www.ibot.cas.cz/mykosym/mykorrhiza.html#pseudo>

Vaccinium corymbosum
root colonised by
unknown DSE fungus.

Inside individual (most often rhizodermal) cells, these fungi form typical structures, so-called mikrosclerotia.



This type of symbiosis predominates in cold nutrient-poor areas (where it dominates over arbuscular fungi), but also in semi-arid steppes; the occurrence of pseudomycorrhizal fungi is still probably underestimated.

It is limited by extreme environmental conditions, DSE fungi do not occur in aquatic or waterlogged ecosystems, but have been found, for example, in soil samples collected on the Antarctic Peninsula.