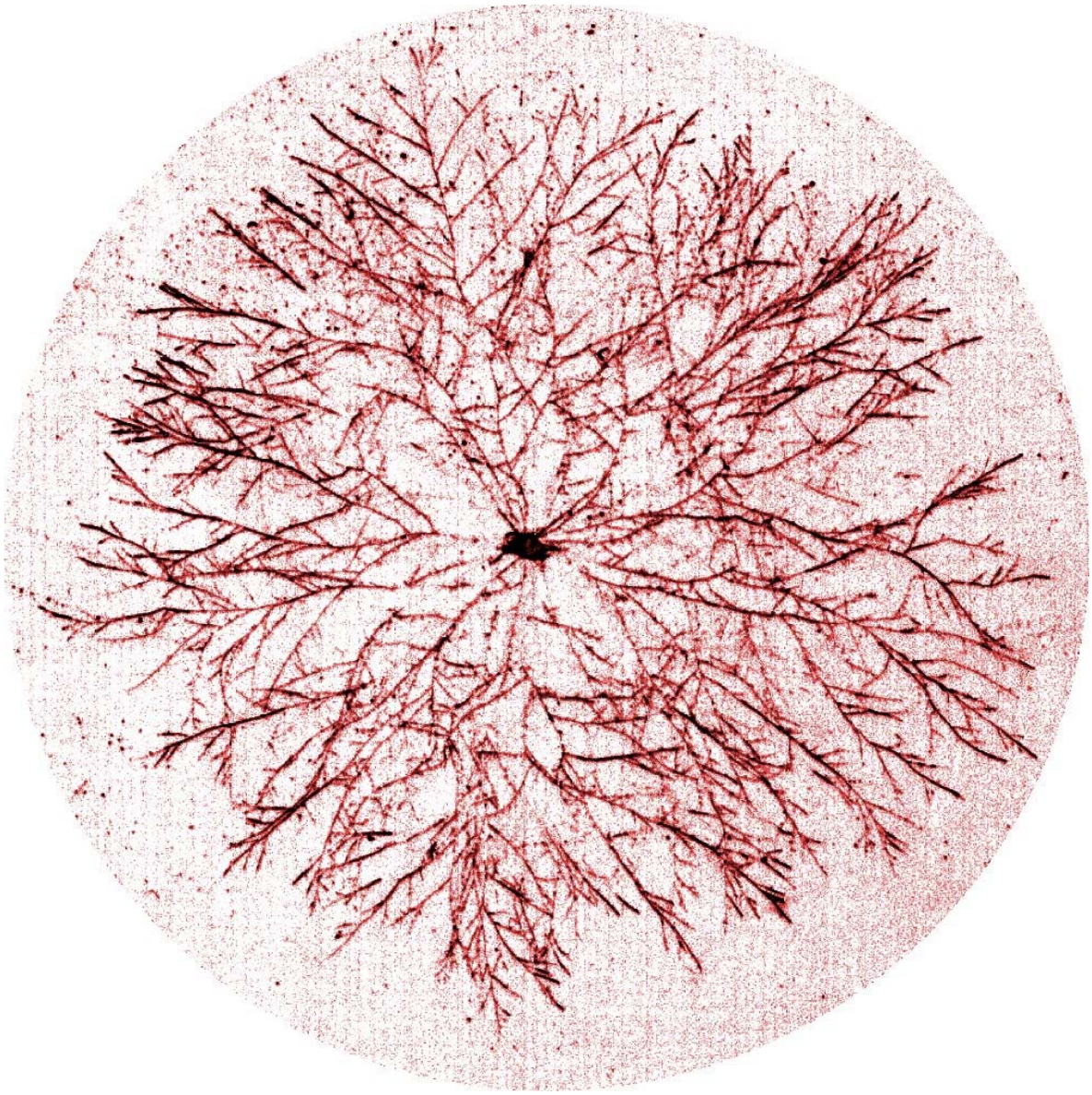


Introduction to fungi

Content

1.The fungal lifestyle

Fungi in everyday life

In every day life we meet fungi and fungal products everywhere. Whenever you go to a shop to buy a food and other things for your household you will come home with a range of products of fungal origin or are results of fungal activities. In Table 1 you find a list of common products:

Table 1.1.

Champignons	Fungal fruit body (biomass)
Bread	Yeast (carbon dioxide, taste)
Wine	Yeast (alcohol, taste, preservation)
Beer	Yeast (alcohol, carbon dioxide, taste)
Cheese	Moulds (ripening by enzymes, taste)
Soft drinks	Moulds (citric acid)
Washing powder	Enzymes
Vegetables (onions)	Mycorrhiza
Wood	Mycorrhiza

Fungi can also be negative to humans and below you see a division of fungal activities into friends and foes (Fig 1.1).

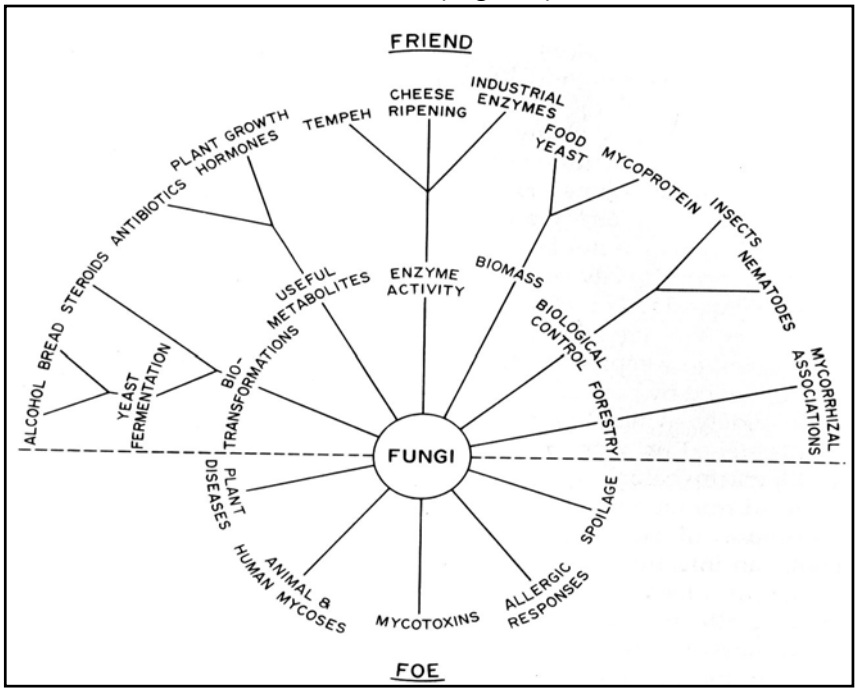


Fig 1.1. Fungal friends and foes

Fungi are not plants

Traditionally fungi have been treated as non-green plants. The reason for grouping fungi with plants was mainly due to the presence of cell walls around cells. This characteristic is not considered to be so important anymore. From biochemical, physiological and genetic analysis fungi are today placed closer to animals and thus we humans are more related to fungi than to plants (Fig 1.2). This is of cause important when trying to find antibiotics aimed towards fungi since it is more difficult to find something that targets this small difference without harming the patient.

Fig 1.2. How fungi are taxonomically related two other main groups of eucaryotes.

True fungi divides into four taxonomic groups, *Chytridiomycota*, *Zygomycota*, *Basidiomycota* and *Ascomycota* (Fig 1.3). These divisions are based on their genetic relatedness and fits with the different modes of producing sexual spores. Some fungi do not produce sexual spores. Most of them seem to be from *Ascomycota* based on genetic similarities. Traditionally, all fungi that produce asexual spores and where no sexual stage is known are place in the order *Deuteromycota*. There is also a group of plants that are seen as fungi from historical reason, the *Oomycota*. The closest relatives to *Oomycota* are Brown algae and their cell wall contains cellulose as in plants. *Oomycota* could be seen as plants with a fungal growth habit that lacks chloroplasts.

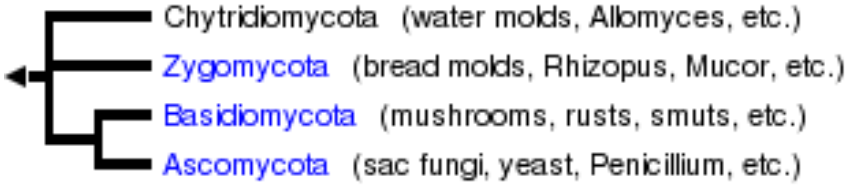


Fig 1.3. Main fungal taxonomic groups

Distinctive features of the true fungi (Kingdom Mycota)

The true fungi share several features that clearly distinguish them from all other organisms, showing that they are a 'natural' (monophyletic) group of organisms. These features are important in practice because they can provide targets for the actions of antifungal agents. In the list below we see that some of the most powerful drugs for treatment of fungal infections of humans, and the fungicides used for plant disease control, are targeted at the unique biochemical or structural features of fungi.

1. **Chitin is a major component of fungal walls** (but also found in insects, etc.). The enzyme that synthesizes chitin (**chitin synthase**) is a target for the polyoxin antibiotics.

2. **Fungi are haploid**, whereas the other major groups of eukaryotes are diploid.

3. **Fungal cell membranes contain ergosterol**, whereas animals have cholesterol and plants have sitosterol and other 'phytosterols'. Several antifungal drugs (e.g. ketoconazole) used in human therapy act by blocking ergosterol synthesis. The antifungal antibiotics (e.g. nystatin, amphotericin B) combine with ergosterol in fungal membranes. And several fungicides used for plant disease control act by disrupting specific steps in the ergosterol synthesis pathway.

4. **Fungi synthesise the amino acid lysine by a unique pathway**, different from that of other organisms.

5. **Fungi have characteristic soluble carbohydrates** (the disaccharide **trehalose** and polyhydric alcohols like **mannitol** and **arabitol**) **and storage compounds** (e.g. **glycogen**), differing from those of most plants and animals.

6. **Fungi have several characteristic ultrastructural features**, such as **plate-like cristae in the mitochondria** (like animals), and **tubular unstacked Golgi cisternae** (unlike animals or plants).

7. **The microtubules of fungi have unique binding affinity for anti-tubulin agents**. In particular, fungal tubulins bind to the antibiotic **griseofulvin** (used to treat some fungal infections of humans) and to the **benzimidazole fungicides** (used widely for control of fungal pathogens of plants).

8. Finally, fungi differ from other organisms in a range of **biochemical and molecular features** such as the regulation of some enzymes, some aspects of mitochondrial codon usage, etc.

What is a fungus

A typical fungus is built up of cellular filaments called **hyphae**. These hyphae grow at the tip and branch. The hyphae are compartmentalized but there is cytoplasmic connection between adjacent compartments (Fig. 1.4).

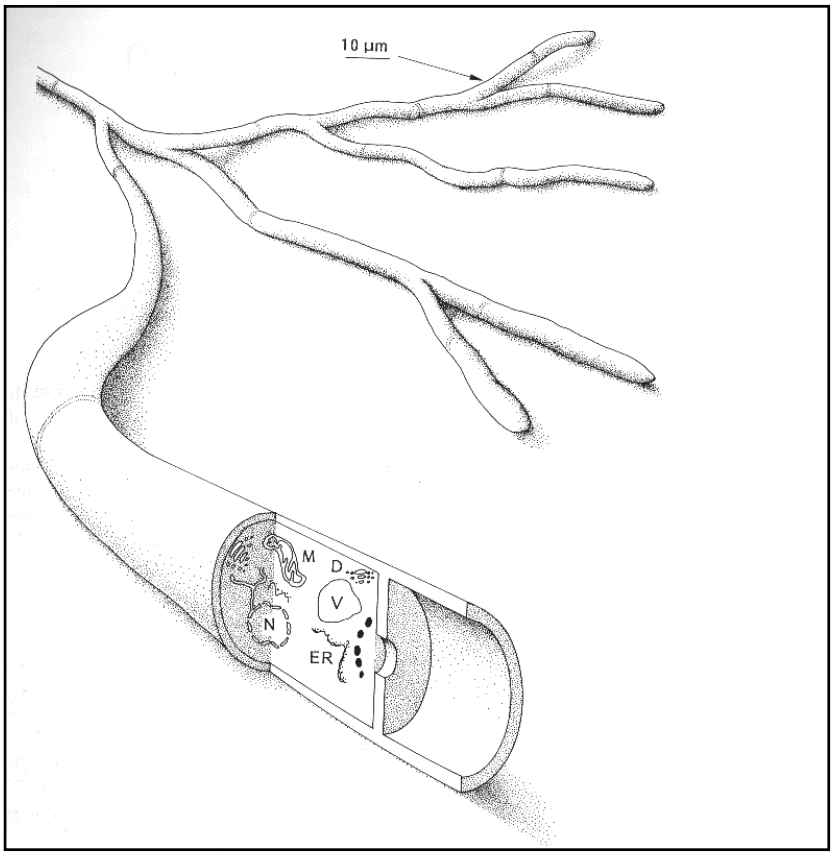


Fig 1.4. Branching fungal hyphae with a tip cut off to show the interior, including a septa (crosswall) with pore.

Hyphal tip growth

Fungal hyphae grow only at the tip and sometimes this tip growth has been seen as the hallmark of the fungal kingdom. In the light microscope a darker body can often be seen when the tip is actively growing. This dark body was named the **spitsenkörper**. We now know that this body is a collection of vesicles (Fig 1.5)

containing enzymes and building material for cell wall synthesis at the tip. These organelles are transported from the older parts of the hyphae along microtubules. The **spitsenkörper** could then be seen as a center for supplying wall-building vesicles to the cell wall at the tip and is also sometimes named the **VSC** (vesicle supply center).

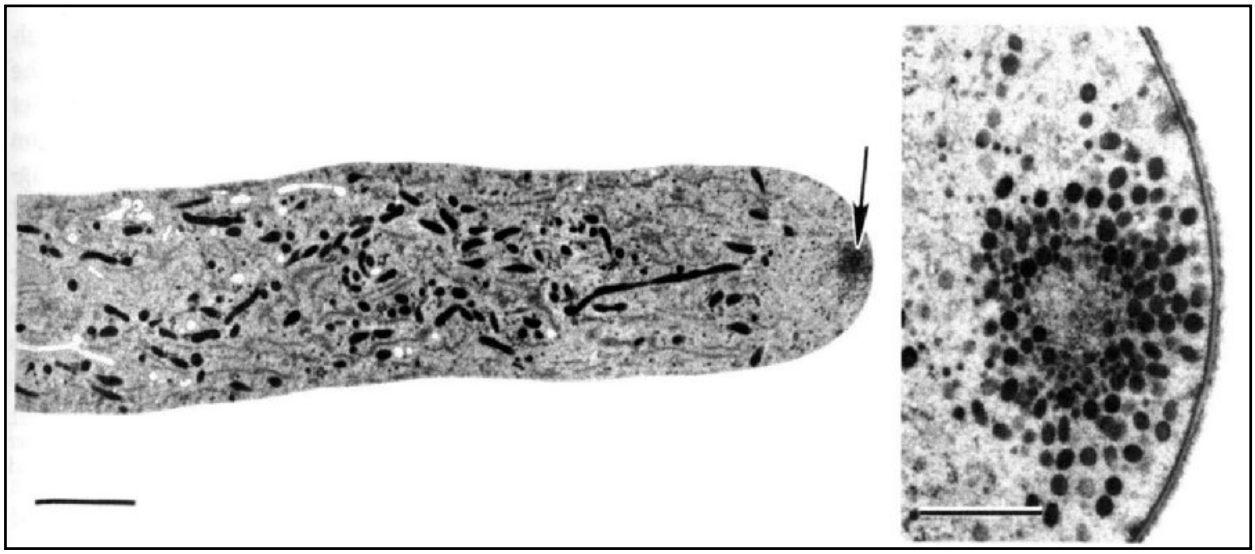


Fig 1.5. Electron micrographs showing a hyphal tip with a dark **spitsenkörper** (left) and an enlargement of the **spitsenkörper** region (right).

Cellular organization

Nuclei

The different classes of mycelial fungi show different distribution patterns of nuclei in the hyphae (Fig 1.6.). Most fungi are equipped with haploid nuclei although there are some diploid exceptions. *Zygomycota* lacks true **septal walls** (a wall with live cytoplasm on both sides) and the nuclei are spread out in the mycelium. The distance between nuclei not random but is highly regulated and nuclei can migrate. In *Ascomycota* there is normally only one haploid nuclei in each compartment. In *Basidiomycota* the vegetative mycelium contains 2 nuclei in each compartment. They are thus **dicaryotic**, either **homocaryotic** with two identical nuclei or **heterocaryotic** with two different nuclei in each compartment.

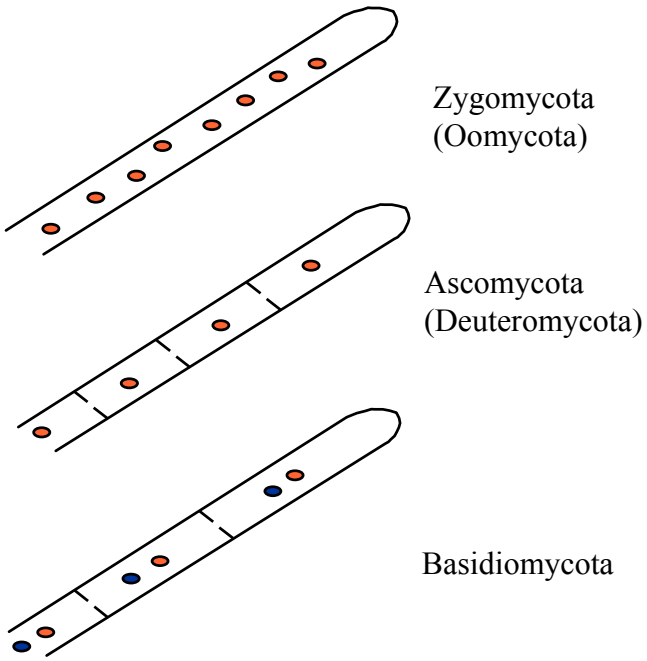


Fig 1.6. Nuclei in fungi.

Septa
Hyphal compartments are divided up by septa in *Ascomycota* and *Basidiomycota*. The septa are secondary walls that grow inwards from the existing walls. The septa are not completely sealed since they have a **septal pore**. This pore has different architectures in *Ascomycota* and *Basidiomycota*. The pore is big enough to make the whole mycelium a cytoplasmic continuum (Fig 1.7). Thus organelles and also nuclei can migrate trough the pore. The pore and surrounding structures (like the **woronin bodies** in *Ascomycota*) functions as emergency doors that can shut if cytoplasm leaks through the door too quickly.

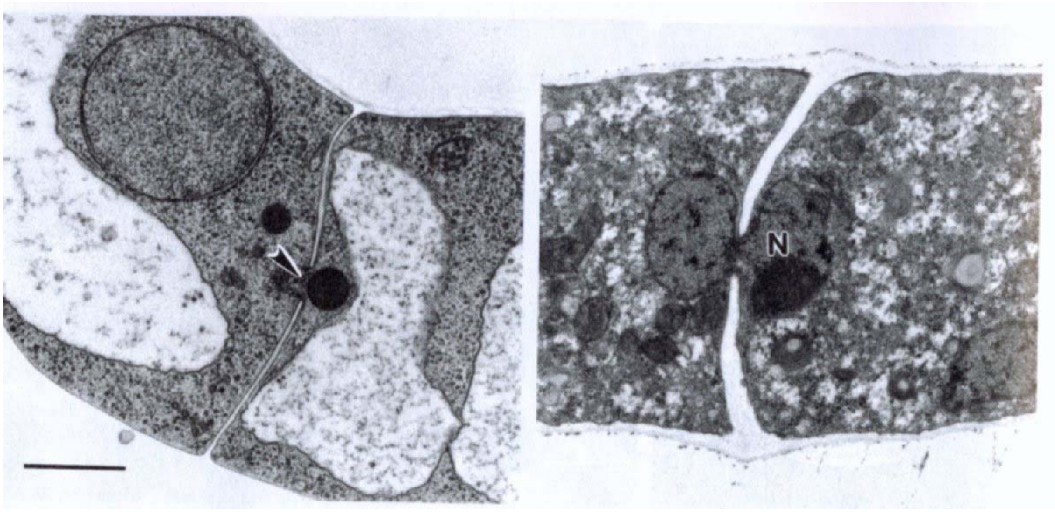


Fig 1.7 Septal pore surrounded by dark woronin bodies (left) and septal pore with a nucleus passing through (right).

Nuclear division
In plant and animal cells the nuclear membrane breaks down when nuclei divide to form two daughter nuclei and two new cells. In fungi the nuclear division takes place inside the nuclear membrane (Fig 1.8).

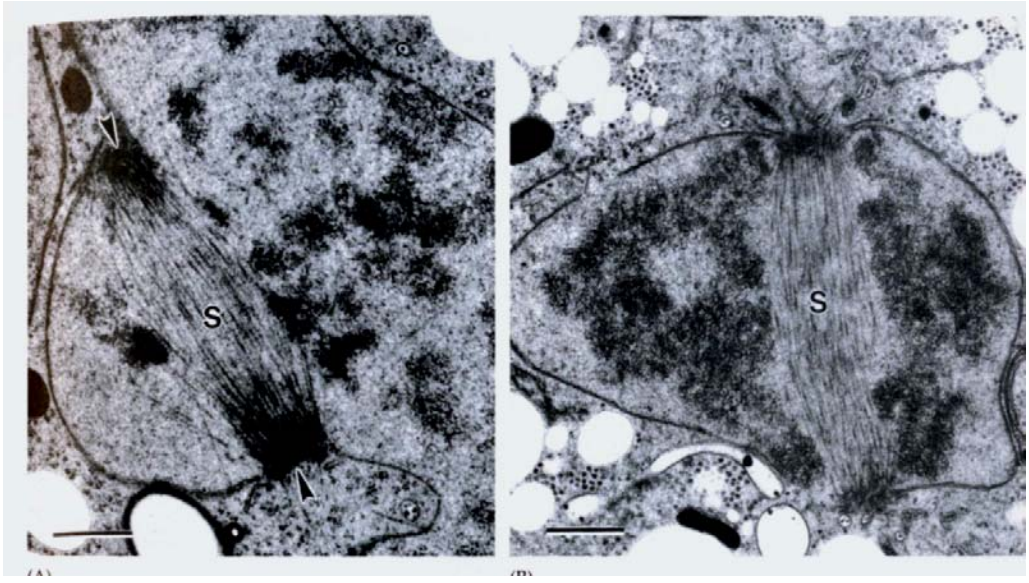


Fig 1.8. Dividing fungal nuclei showing the intact nuclear membrane and the mitotic spindle.

Building a mycelium

A mycelium is built by **branching** of the hyphae (Fig 1.9) and **anastomoses** (Fig 1.9 and 1.10) between adjacent branches. The branching creates a tree-like structures but adding cross-connection by **anastomoses** creates a network of hyphae. It is this hyphal network we call a mycelium.

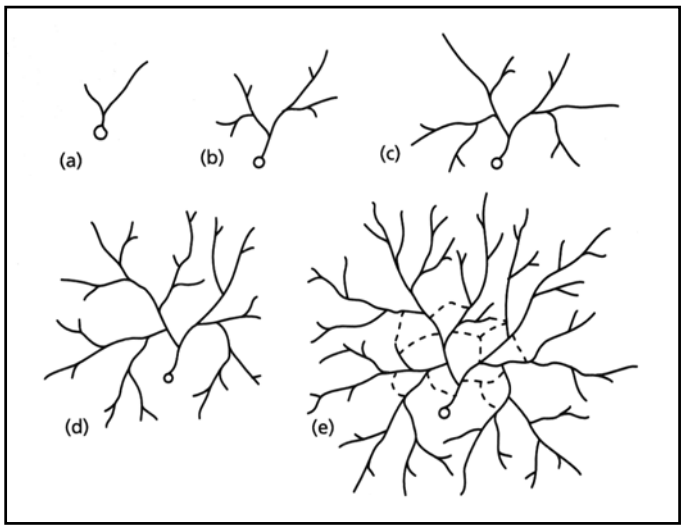


Fig 1.9. A fungal spore germinates (a) the mycelium grows first by branching (a-d) then by anastomoses as well as branching (e).

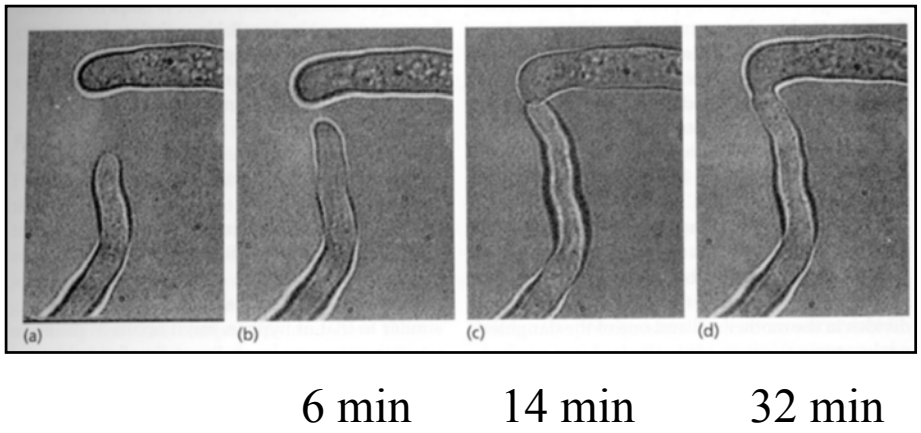


Fig 1.10. Anastomoses between two hyphae of *Rhizoctonia solan*i.

Hyphae can grow parallel and connect to each other forming rope-like structures called **strands** or **rhizomorphs** if more differentiated and elaborate and root-like. Such strands can be seen macroscopically as in Fig 1.11. **Strands** from two different (but genetically similar) mycelia can join to form a large mycelium (Fig 1.11). This can of cause also happen with mycelia not growing as **strands**.

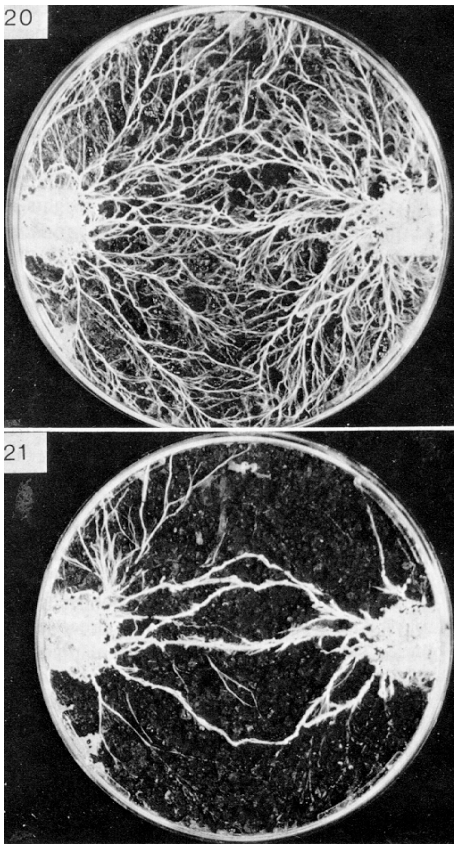


Fig 1.11. Mycelia of a wood decomposing fungi grow out as strands over compacted soil from two wood blocks. They meet and connect by anastomoses (20). After a few weeks only direct connections between the woody resources are kept and strengthened. The rest of the mycelium has died off (been recycled by the mycelium) (20)

Small and large mycelia

The size of a mycelium (a fungal individual) can be both very small and very large. A mycelium just starting to form from a single spore is microscopic in size and just a fraction of a mm (Fig 1.9). On the other hand some fungi can create huge mycelia. The biggest mycelia known are from *Armillaria* species. These fungi are parasites on trees and one can see their effects on the trees as a change in colour and density of the forest from an airplane. Fig 1.12 shows an aerial view of a

mountainside with fully grown spruce trees. The large circular shapes are showing the presence of very big fungal mycelia. In the 1990s researchers took samples from circles like these and could confirm that the same genetically identical individual weighing several hundred tons and probably more than 1000 years old colonized the whole area.

Fig 1.12. Aerial view over a mountainside covered with spruce forest. The circular shapes show the extent of single fungal mycelia of *Armillaria bulbosa* attacking the forest.

Finding mycelial structures in nature

Most people walking in the forest looking for fungi are interested in finding the fruitbodies of the mycelia. It is sometimes possible to see the mycelia themselves. The mycelial rhizomorph network of *Armillaria* species are often seen as net of black threads growing just under the bark of trees (Fig 1.13). Sometimes in forests on stony grounds it is possible to just bend back the vegetation from a stone and

investigate the interface between the soil and the stone (Fig 1.14). The whole underside of the turf is covered with fungal mycelia. These mycelia are mostly of mycorrhizal fungi cooperating with the trees to take up mineral nutrients from the soil. It has also been shown that they are capable of weathering rocks with the help of organic acids. The effect is so strong that they can etch tunnels even through granite.

Fig 1.13. Black network of mycelial *Armillaria* rhizomorphs can often be seen growing just under the bark on attacked trees. In this image the fruitbodies of armillaria are also present.

Fig 1.14. Tuning back the soil from a stone (left) exposes the mycelium of mycorrhizal fungi (right).

Fungal spores

Mycelia are in principle stuck in one place and cannot move to another place very quickly. Thus they have means to escape if the conditions should get unfavorable for growth. There are two ways of escaping, either in time or in space, and fungi form special structures called spores to do this. Spores can be either asexually formed after mitosis or sexually formed where meiosis with recombination of genes is involved in the process of spore formation (Fig 1.15-18). If the asexual spores are formed singly and directly from hyphal branches the spores are called conidia.

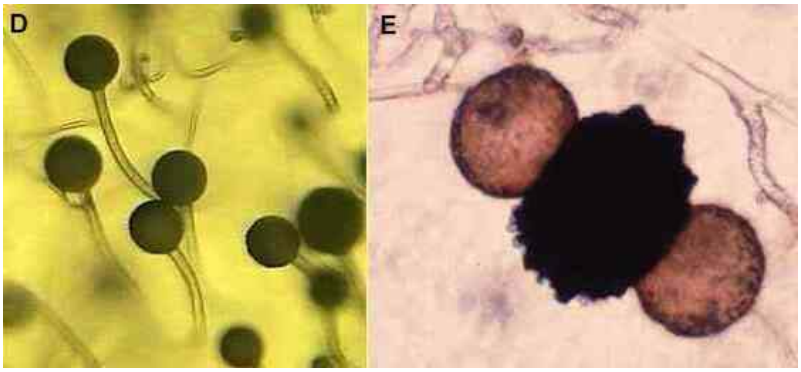


Fig 1.15. *Zygomycota*: **Sporangia** filled with asexually formed **sporangiospores** (left). A large dark sexually formed **Zygospore** (right)

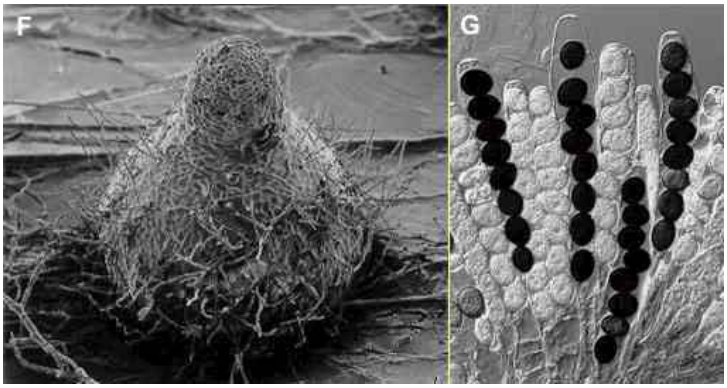


Fig 1.16. *Ascomycota*. **Perithecium** (left) containing **asci** (sacks) with dark and white sexually formed **ascospores**.

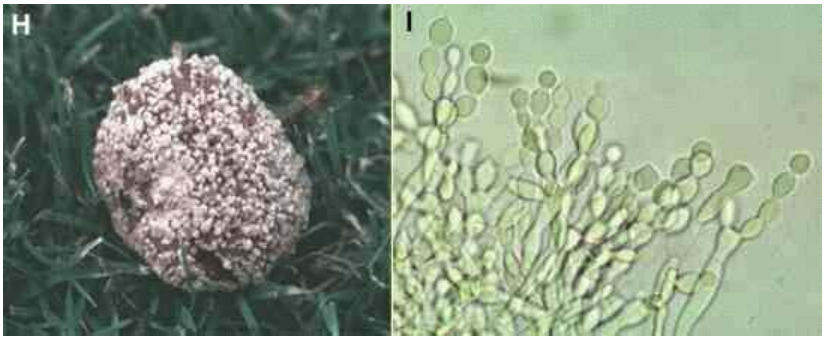


Fig 1.17. *Ascomycota* (*Deuteromycota*). Fungal growth and sporulation on a fruit (left). Magnification showing the asexually formed conidia.

Fig 1.18: *Basidiomycota*. Fruit bodies (J, K;N). Lamelli (L) carrying sexually formed **basidiospores** on **basidia**.

Yeast growth - a special case of spore growth?

Yeast grows by forming a bud from a mother cell (Fig 1.19). The process is very similar to a conidia formation where conidia are formed on hyphae by budding and then from consecutive budding on already formed conidia. The difference is that yeast lacks a mycelium. In the common yeast (*Saccharomyces cereviciae*) each bud leaves a scar on the mother cell. There cannot form another bud from an existing scar and thus there is a maximum number of buds that can be formed from a mother cell. But what are the advantages of growing as yeast compared to growing as a mycelium. In table 1.2 some advantages and disadvantages with each type of growth strategy is listed.

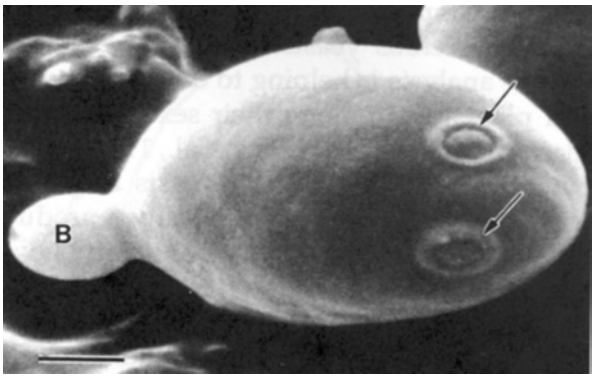


Fig 1.19. A budding yeast cell showing a bud (B) under formation and scars from previously formed and detached buds (arrow).

Table 1.2.

Hyphae
+ Advancing over surfaces
+ Penetrating substrata
+ Simultaneous use of heterogeneously distributed resources
+ Functional differentiation and integration possible
- Expensive in terms of nutrients
- The hyphal apex is vulnerable (drying - wetting)

Yeast
+ Tolerant to osmotic changes (drying - wetting)
+ Economic in terms of nutrients

2.The integrated mycelium

In the chapter about The Fungal Lifestyle a fungal mycelium was treated as a network of hyphae. The aim of this chapter is to demonstrate how integrated a mycelium can be and thus act as a single organism. The concept of a mycelium as the acting organism or that the mycelium (fungal colony) should be seen as a collection of individually acting branches have debated. What has emerged is that both ways of looking at the mycelium can be right but it will depend on the fungus under study. It is also more and more clear that for most fungi we should treat the mycelium as a single organism and not as a collection of organisms.

Colony and mycelium?

The terms fungal colony and fungal mycelium is used in the literature, often for the same things. If one look up these terms in the Dictionary of Fungi they are defined as.

Colony = either the coherent mycelium or a mass of cells of one origin.

Mycelium = network of hyphae

These two terms are really a mixture of concepts one of the origin and one of the structures. None of these terms addresses the function of the mycelium or what could constitute an individual organism. Therefore two other terms have been introduced to better describe the reality.

Genetically identical hyphae occupying a continuous space = The **G**enetic **M**ycelium **U**nit (GMU)

The network of functionally integrated hyphae that forms an individualistic organism = The **F**unctional **M**ycelium **U**nit (FMU).

The GMU is what you see as a fungal colony when growing fungi on an agar medium in a Petri dish. This GMU can be made up of one or many different functional units (individuals). Figure 2.1 illustrates the relations between FMUs and GMUs.

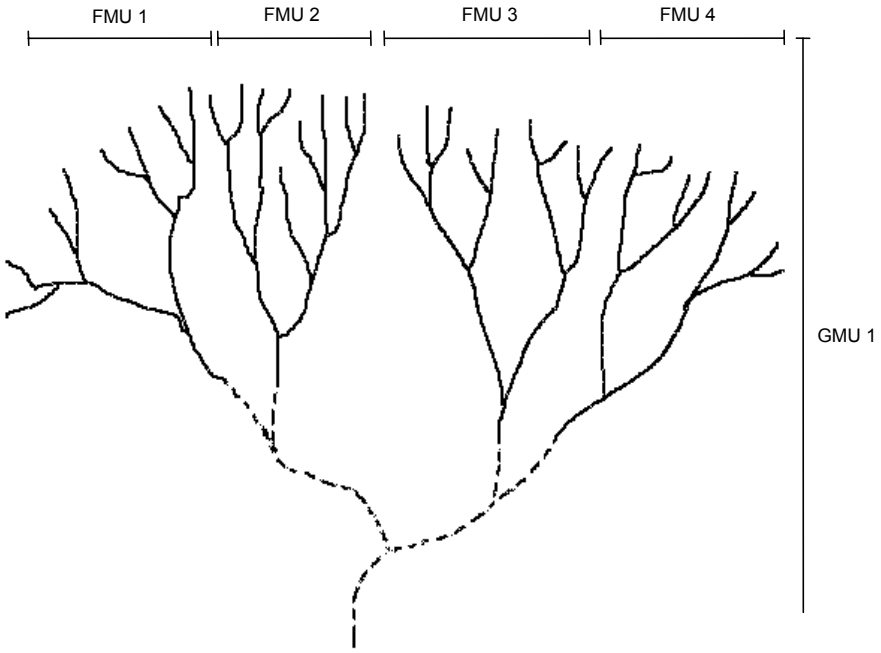


Fig 2.1 Relationship between GMU and FMUs in a branching mycelium where the older parts of the mycelium degenerates and where there is no anastomoses connecting the branches with eachother. If there had been anastomoses connecting the branches or if the older parts of the mycelium had not degenerated completely there would have been just one FMU=GMU. For most fungi keeping some old hyphae alive in combination with anastomoses seems to be the case.

FMU-characteristics

From what was presented about fungal cell physiology it is obvious that the fungal individual (the FMU) should be seen as a unicellular and multinucleate organism rather than a multicellular organism. In other words many nuclei cooperate in a common cytoplasm surrounded by a common plasma membrane. Thus the communication between the different parts of the organism becomes intracellular. The FMU (the fungal individual) have the following characteristics.

1. Many nuclei within the same cytoplasm surrounded by a common plasma membrane.
2. Nutrient translocation between different parts of the FMU is possible. If this takes place the FMU can share nutrients between parts of the FMU that are exposed to local surpluses. This translocation is then by necessity intracellular.
3. If disturbed the whole FMU will act and try to restore intracellular favorable conditions. This is called homeostasis.
4. The cytoplasmic connection makes it possible to signal events and co-ordinate activities within the FMU.

5. Differentiation to form fruit-bodies and spores or other structures is such a concerted effort of the FMU where the whole or large parts of the FMU co-operate.
6. The controlled degeneration of hyphae in parts of the FMU that is under biotic or environmental stress is possible. The FMU “saves” the hyphae in “good” areas and breaks down the hyphae in the “bad” and saves the nutrients by intracellular recycling.

Examples of coordinated activities in FMUs

Nutrient translocation

Translocation of nutrients within an FMU can take place with 4 different mechanisms.

1. Passive diffusion. The FMU takes up the nutrient over the membrane and then the nutrients spread by intracellular diffusion. The uptake shuts down when the local concentration in the FMU is high enough to supply local needs.
2. Passive diffusion aided by active uptake in excess of local needs. If there is no local feedback inhibition of the nutrient uptake there will be a muck greater intracellular concentration gradient of nutrients resulting in a faster passive diffusion (higher nutrient flux) to the other parts of the FMU.
3. Active translocation driven by cytoplasmic activities. This type of translocation involves the activities of motor-proteins acting on the cytoskeleton.
4. Active translocation by pressure driven bulk flow. This type of translocation resembles what we see in plants. A flow of nutrients is canalized through specialized vessel hyphae. The flow is accompanied by water uptake at the source and excretion of water or evaporation at the sink.

All these mechanisms except the first one requires energy for the translocation process although it is only in the last two that energy is directly required for the translocation of nutrients.

All fungi can use the first mechanism and it differs greatly if fungi can use the other mechanisms as well. There is no real rules to which fungi are equipped with efficient active translocation mechanisms. There is however a tendency those fungi known to produce large mycelia are better at translocating than small mycelial fungi. One way of showing this difference between fungi is to expose them to

growth conditions where the mineral nutrients and the carbon/energy has been physically separated into two areas (Fig 2.2).

Glucose side	Mineral side

Fig 2.2. A 20 cm long 2 cm wide dish filled with 2 different agar media. The fungus to test is inoculated in the middle of the whole dish.

If a fungus starts growing between the areas it will grow well at start but become progressivly more dependent on efficient nutrient translocation of all important nutrients as the FMU expands over the two areas. If the fungus just uses mechanism 1 or 2 (above) the fungus will grow less and less biomass further in on both areas (Fig 2.3). On the other hand if the fungus also uses the active mechanisms 2 and/or 3 it will grow well over both areas and there will hardly be any difference compared with growth on a mixed medium (Fig 2.4).

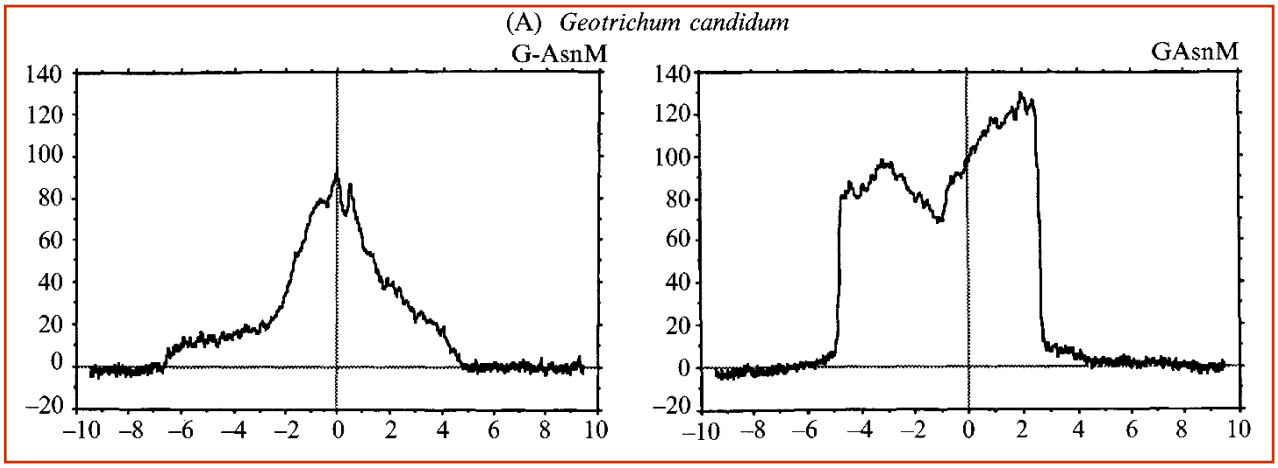


Fig 2.3. Biomass density patterns of *Geotrichum candidum* that does not translocate nutrients actively when grown on a medium with carbon and mineral sources separated (G-AsnM) or when carbon and mineral sources have been mixed (GAsnM). The fungus was inoculated at position “0”.

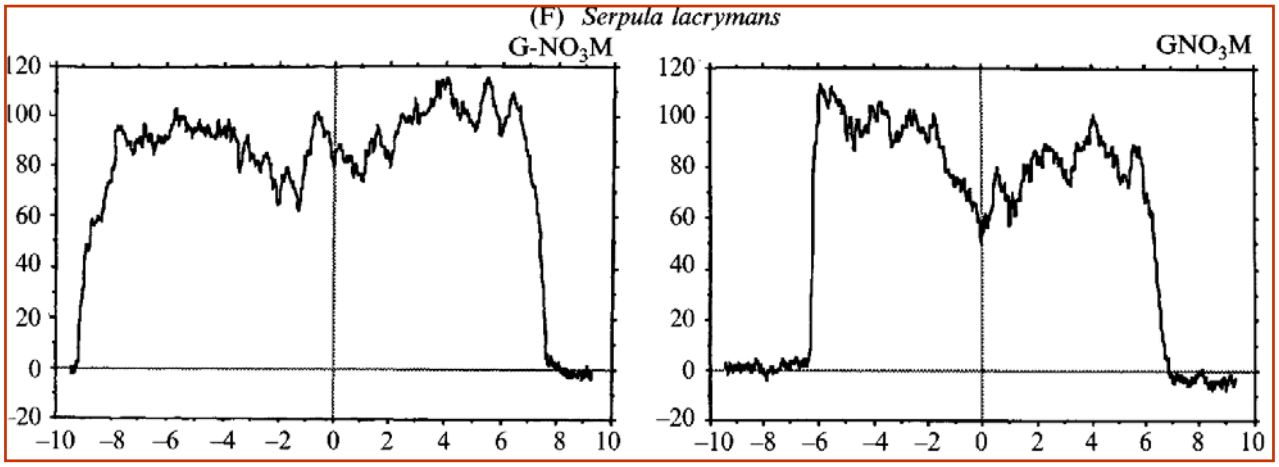


Fig 2.4. Biomass density patterns of *Serpula lacrymans* that translocate nutrients actively when grown on a medium with carbon and mineral sources separated (G-AsnM) or when carbon and mineral sources have been mixed (GAsnM). The fungus was inoculated at position “0”

Visualization of nutrient translocation

The nutrient translocation can be visualized and measured dynamically by repeated recordings of the distribution of a labeled nutrient in a mycelium as shown in Fig 2.5.

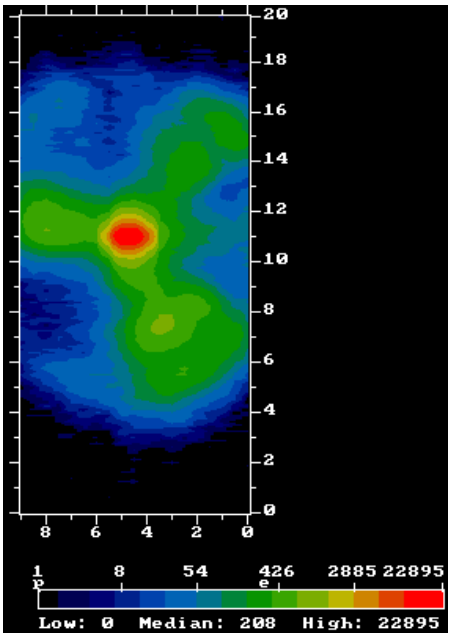


Fig 2.5. Distribution of labeled ^{32}P in a mycelium of *Pleurotus ostreatus* 48 hours after addition in the centre of the colony (red dot). The numbers shows the size of the plate in cm and the colour scale at the bottom the levels of radioactivity. The whole coloured area shows the extent of the circular mycelium. From analyzing the same plate ad various times it was obvious that the phosphorus is translocated towards the colony edge along certain routes in the mycelium.

By analyzing plates like in Fig 2.5 where nutrients had been added either at the centre of the colony or at the edge of the colony it became obvious that the same nutrient (phosphorus) was actively Translocated both from the centre of the colony to the edge and in the reverse direction simultaneously (Fig 2.6). This has been confirmed in many later experiments and makes the nutrient translocation in fungi similar to the circulation of nutrients by the blood flow in animals. In other words the translocation should be seen as a translocation stream circulating in the organism where processes can use nutrients from the stream passing by. This also implies as have been calculated that the internal translocation of nutrients is relatively inexpensive compared to high affinity active nutrient uptake from areas low in nutrient concentrations.

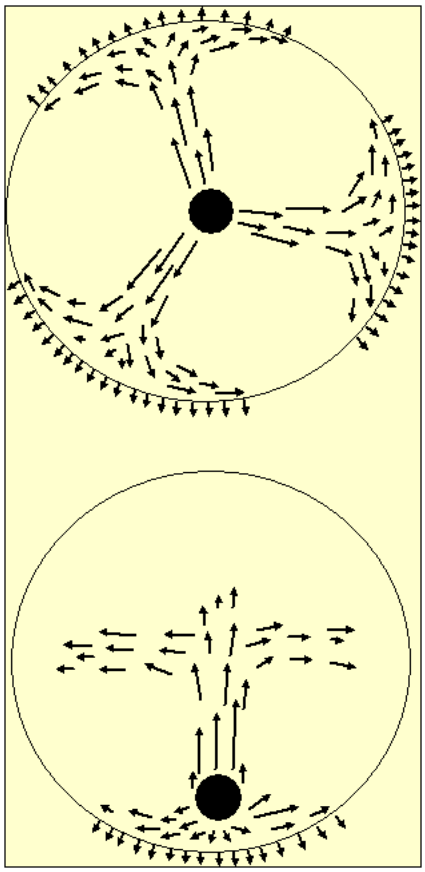


Fig 2.6. Movement of phosphorous in a growing colony of Pleurotus ostreatus (lagre circles) when phosphorus were added either at the centre of the colony (top) or at the edge of the colony (bottom). .

Immobilization and nutrient translocation

From these studies it was also obvious that the nutrients were rapidly taken up and immobilized internally when added to the colony since diffusion through the medium stopped almost instantaneously (Fig 2.7). From the immobilized stores the nutrients were redistributed to the rest of the mycelium.

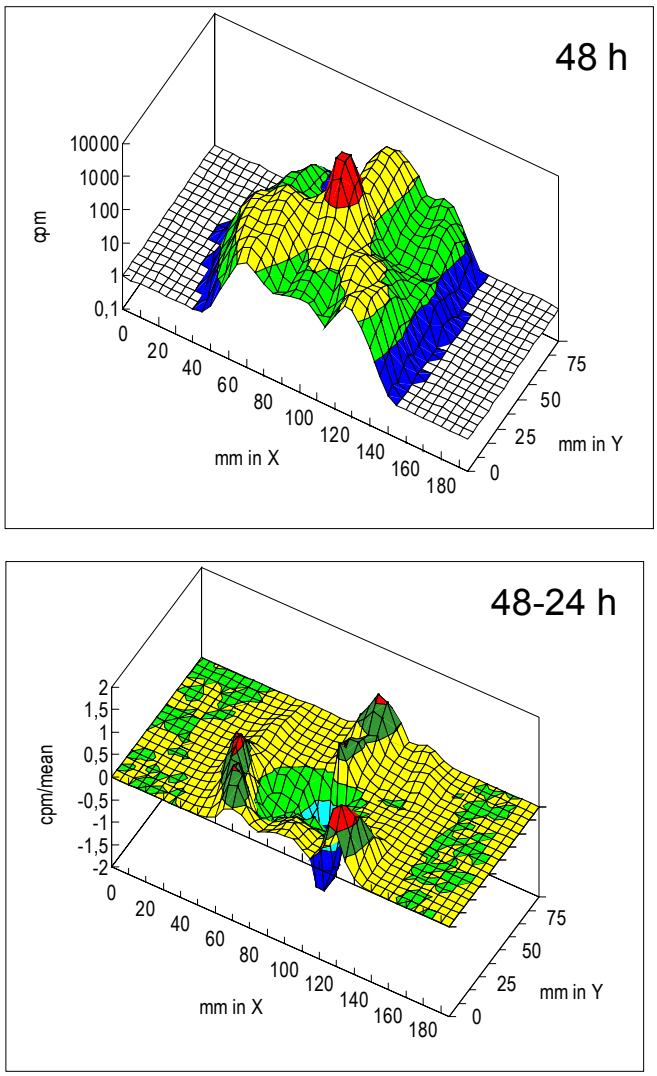


Fig 2.7. Phosphorus distribution in a pleurotus ostreatus colony 48 h after phosphorus addition in the centre of the colony (top). Change in phosphorus distribution the last 24 hours (bottom). From these graphs it is seen that the phosphorus is immobilized in the centre of the colony and it is from this immobilized pool that nutrients are taken for distribution to the other parts of the colony.

Organelle translocation

Organelles including nuclei can travel within the FMU. The roads for these travels are the mirotubules (Fig 2.8). It is known that organelles, including nutrient carrying vesicles, can move with the help of molecular motors (Fig 2.9) along these microtubular tracks through the FMU.

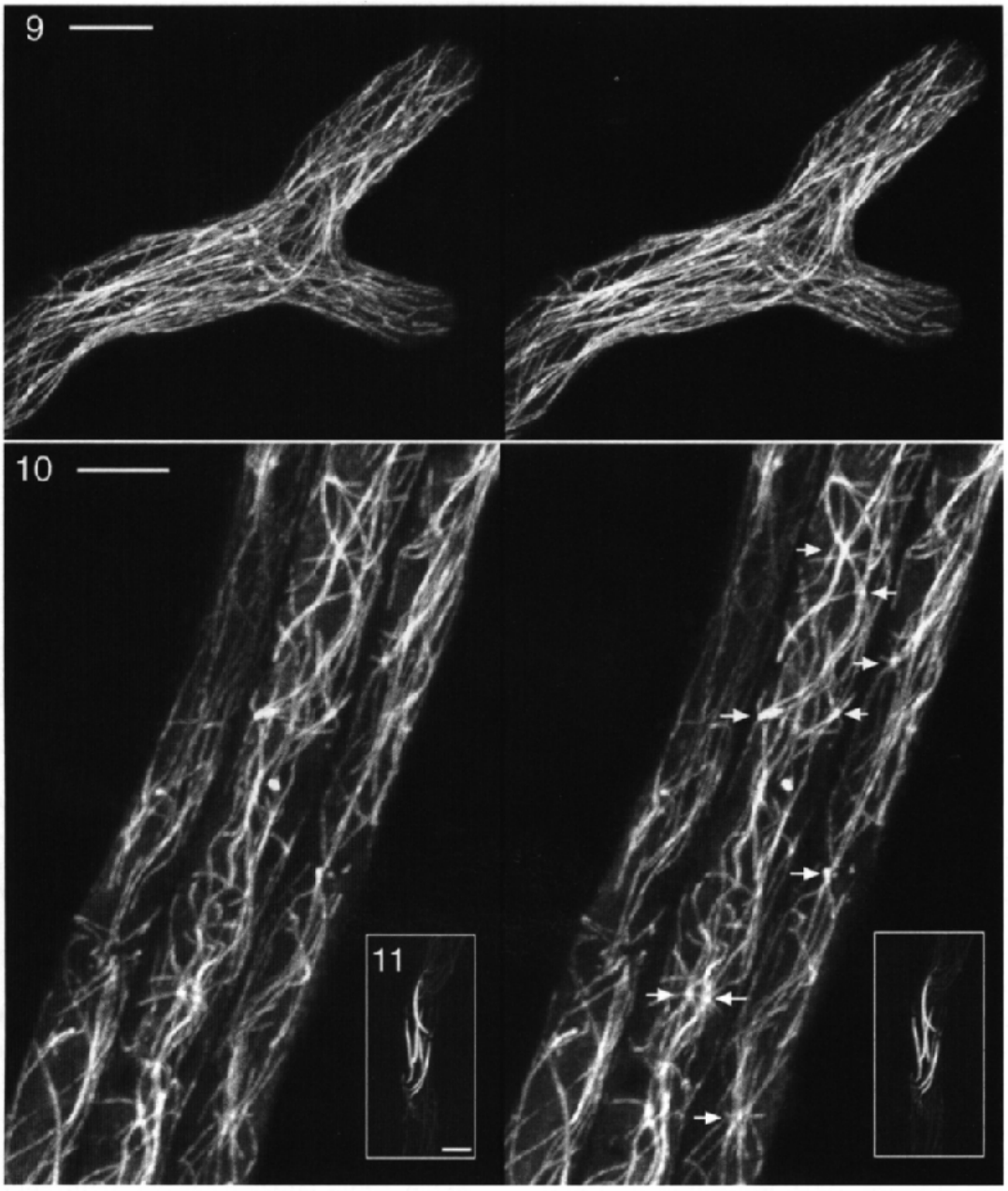


Fig 2.8. Stereo pairs of fluorescent-labeled microtubules in a fungus.

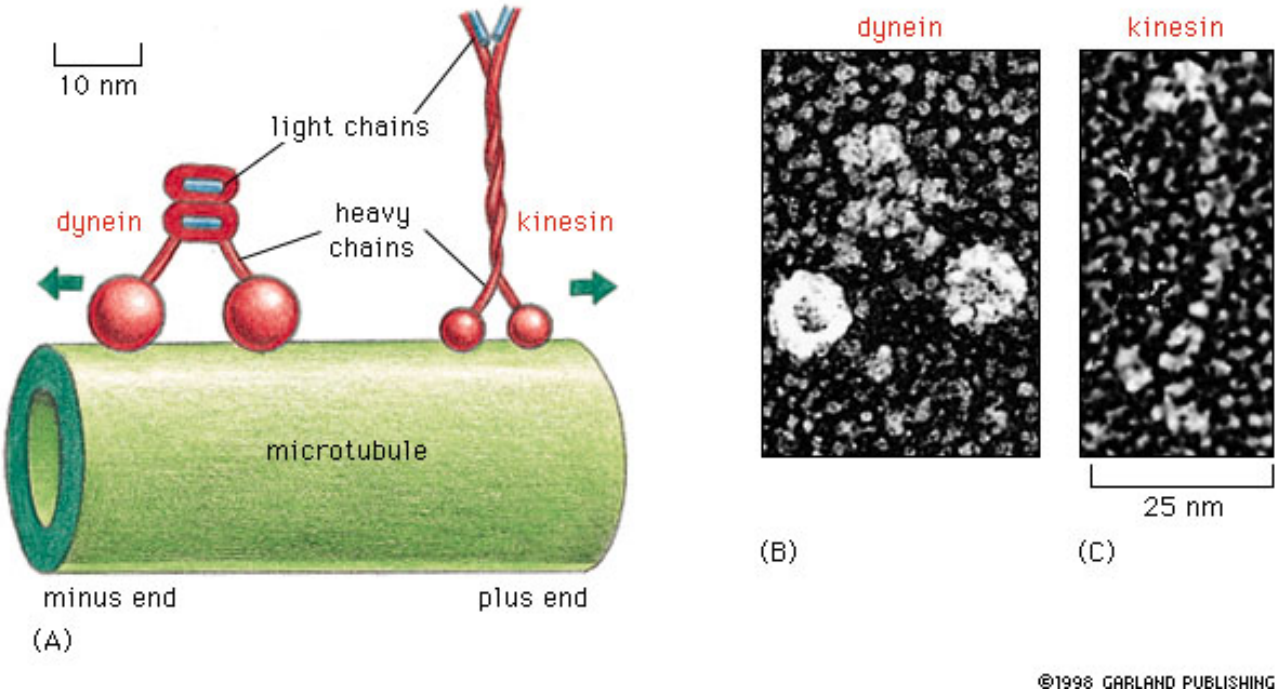


Fig 2.9. The microtubular motors dynein and kinesin can attach to organelles with their light chains and then use energy from ATP to “walk” along the microtubule. Dynein “walks” towards the minus end and kinesin “walks” towards the plus end of the microtubules.

Remodeling the mycelium boundary (or translocation and cell death)

A FMU can move to a different location by cell death in one part combined with recycling of nutrients and translocation and growth in another part of an FMU. This is illustrated in the foraging behavior of wood decomposing fungi (Fig 2.10).

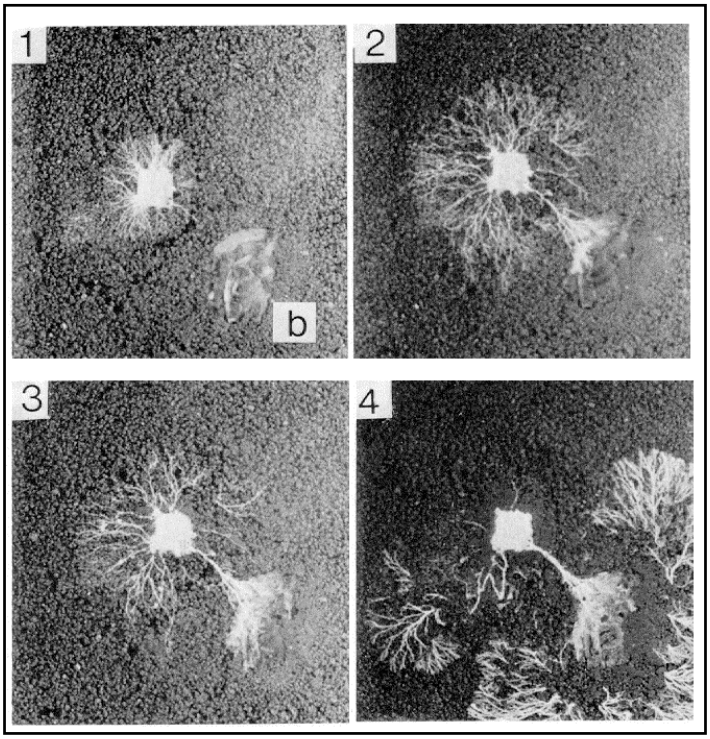
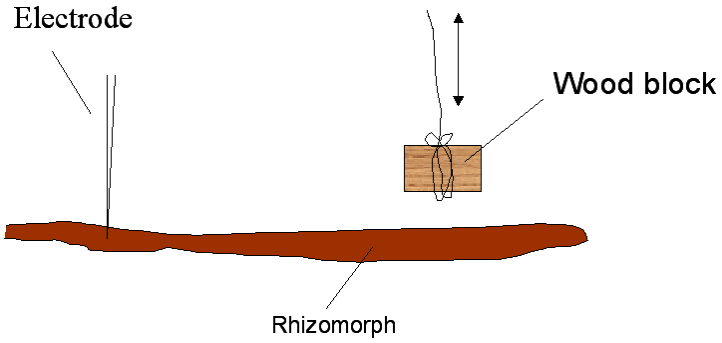


Fig 2.10. A wood decomposing fungus growing out over compacted soil towards a nutrient source in b (1) grows out in all directions. When the fungus finds the nutrients in b (2) the hyphal strands connecting the wood with the new nutrient source is strengthened and the mycelium growing out in the "wrong" directions degenerates (3 & 4). When the nutrients in b is finished the mycelium grows out in a new search for more nutrients (4).

Electric signaling in fungi

In the literature there are several examples were there is an apparent very fast signaling within an FMU. Distant parts of the FMU sometimes react to local stimulation much to quickly to be explained with the relatively slow translocation mechanisms talked about above. The typical speed of these translocations is in mm per hours. Speeds of reaction in cm per minutes have been recorded. Thus there might be electric signaling by traveling depolarizations (action potentials) in the cell membrane similar to the signaling in nerve cells. Since the whole FMU is surrounded by the same plasma membrane this would be feasible for fungi. If this way of communication is important for fungal FMUs it would be expected to be most important for wood decomposing fungi creating very large FMUs (Fig 2.11).



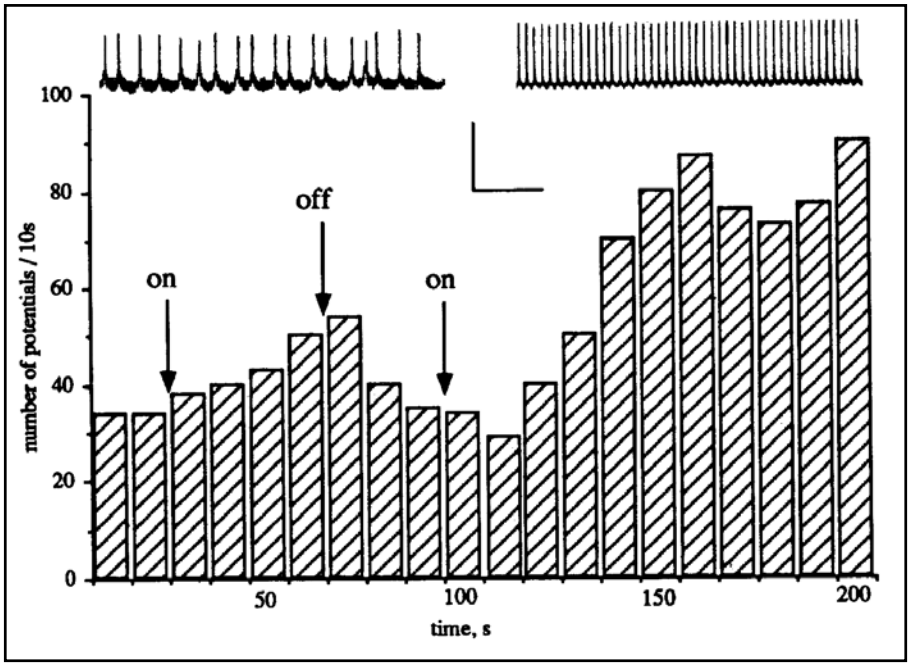


Fig 2.11. An electrode was inserted in a cell in a rhizomorph of *Armillaria bulbosa*. Action potentials could then be measured as depolarization of the membrane potential. The frequency of depolarization events was 3.5 per second. Substrate in the form of a wood block hanging in a string could be added or taken off the rhizomorph. When placed on the rhizomorph the frequency of depolarization increases quickly. When lifted off the frequency decreases and when putting it on again the frequency increases. At the top left of the diagram the depolarisations against time at the start of the experiment is shown. At the top right the depolarizations against time at the end of the experiment is shown.

3.Fungal growth and nutrition

How does a fungus grow?
What are the important nutrients and how do fungi get hold of them?

The fungal hyphae grow at the tip (apex). Cell wall material is transported in vesicles from older parts towards the tip region as described earlier. The newly formed cell wall at the tip is relatively plastic. The tip is stabilised by the actin cytoskeleton. Further back from the tip the cell wall becomes more cross-linked and thus stronger (Fig 3.1)

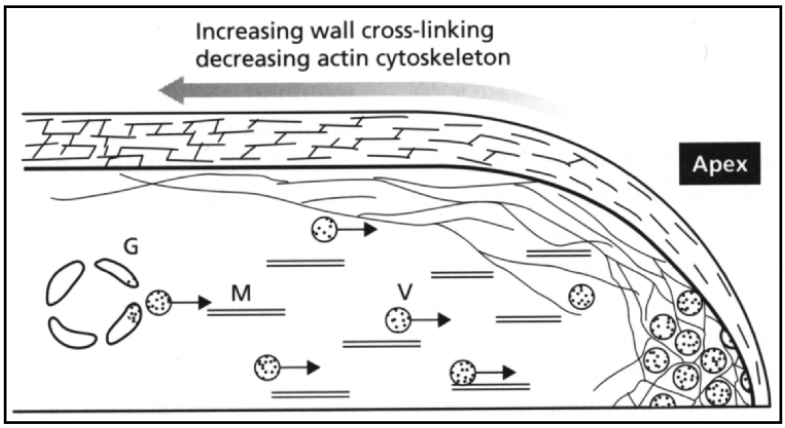


Fig 3.1. Hyphal tip and cell wall grows at the apex. Vesicles (V) containing materials for making the cell walls comes from the golgi complex (G) and are transported to the tip along microtubules (M). The microtubules are much longer than shown in the drawing. At the tip actin filaments stabilizes the tip until the cell wall becomes cross-linked.

Tip growth and branching, the HGU

The hyphal tip grows and when the hyphae has grown for a while it branches. When these branch hyphae have grown for a while more they branch in their turn eventually forming a mycelium. In trying to describe fungal growth this process was studied in detail and it was observed that as the total mycelium length increases the total number of tips also increases. Both these measurements of fungal growth increased exponentially. If you then divided the total hyphal length with the total number of tips in the mycelium a constant was found for each fungus and growth condition. Thus it appeared as if fungal growth could be simply described as an exponential growth of tips with its accompanying length of hyphae, the Hyphal Growth Unit (HGU). Mycelium growth could then be treated as an exponential growth of HGUs like the HGUs had been bacteria. Figure 3.2 illustrates one of the key experiments leading up to the HGU model for describing fungal growth.

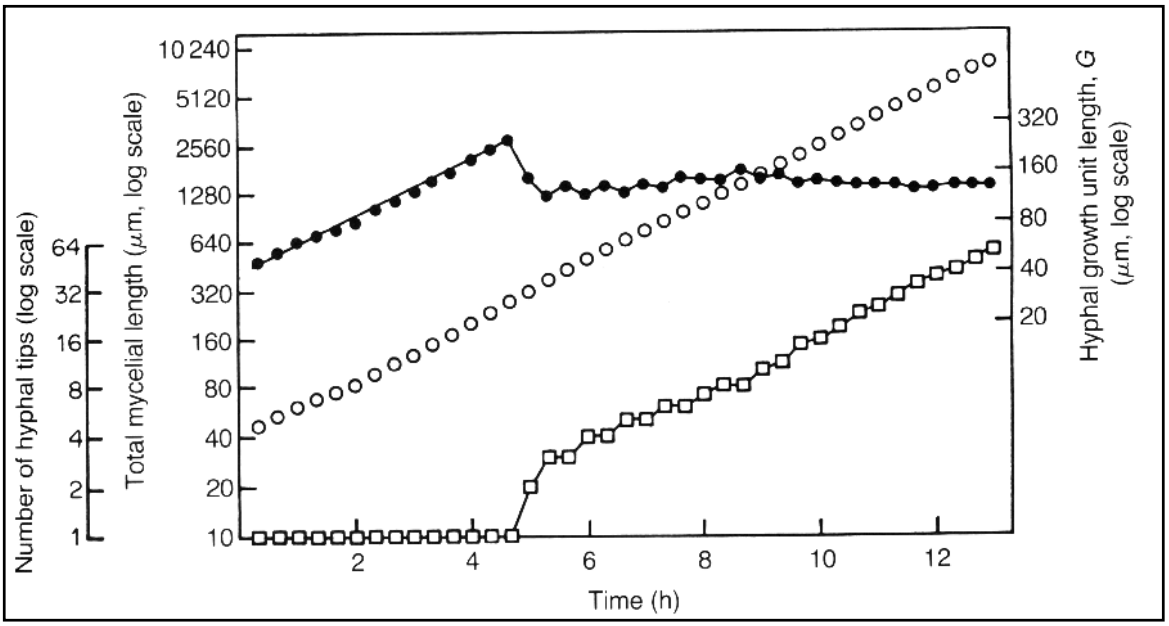


Fig 3.2. Total hyphal length (open circles), total number of hyphal tips (open squares) and hyphal growth unit length (closed circles) against time of incubation of a colony of *Geotrichum candidum*. The hyphal growth unit length was calculated by dividing the total hyphal length with the total number of tips.

The problem with the HGU hypothesis is that it seems to apply only when mycelia are very small and in liquid cultures. Note that in Fig 3.2 the mycelium is microscopic. The total length of the hyphae in the mycelium is less than 10 mm and the number of tips is less than 64. Therefore there has been a lot of work trying to develop models that can describe growth of large, nutrient translocating mycelia growing in heterogenous environments. One rather successful way has been to treat the mycelium as a autocatalytic biomass unit whose biomass growth and spread into the environment is dependent on the nutrients it contacts, uptake of nutrients, translocation of nutrients etc. The mathematics involved is similar to fluid dynamics calculations. Fig 3.3 shows a model for how a fungus grows out in one dimension over soil from a substrate (a piece of wood) using nutrients and energy from the wood for growth.

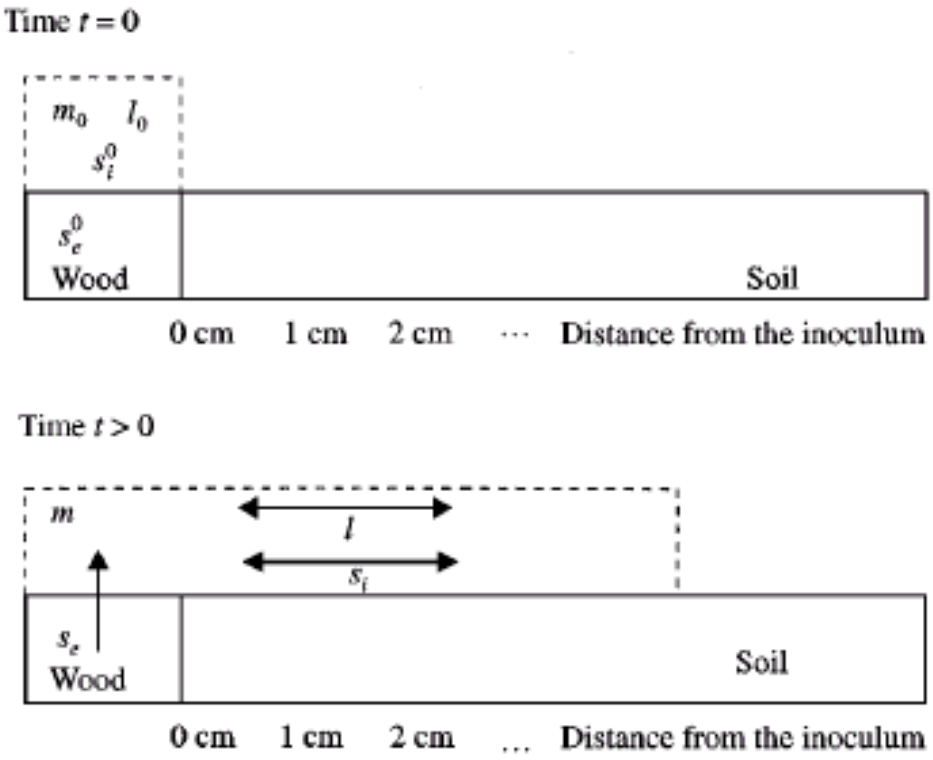


Fig 3.3. The fungal biomass (dotted area) contains basically 3 parameters, m=biomass, l=labelled nutrient, and s_i=intracellular substrate concentration. The biomass starts on top of the wood (top figure) and then grows out over the soil (bottom) that is assumed to contain no available nutrients for growth for this particular fungus. Growth rate in a particular area is assumed to be proportional to the intracellular substrate concentration, S_i. The local S_i is dependent on uptake of substrate, S_e, from the wood, translocation of substrate from the wood, use of the substrate for growth and respiration.

The strength of these new models is that they can be relatively easy tested and the relative contribution of different processes can be assessed. Using the model in Fig 3.3 it was predicted that the label distribution of a labelled substrate would look like in Fig 3.4A if translocation of nutrients inside the mycelium was passive and like in Fig 3.4B if the translocation was active and energy dependent (proportional to the intracellular substrate concentration). These models could then be compared to a real experiment (Fig 3.5) and it can be clearly seen that the label distributions at different times better fit with an active translocation of nutrients.

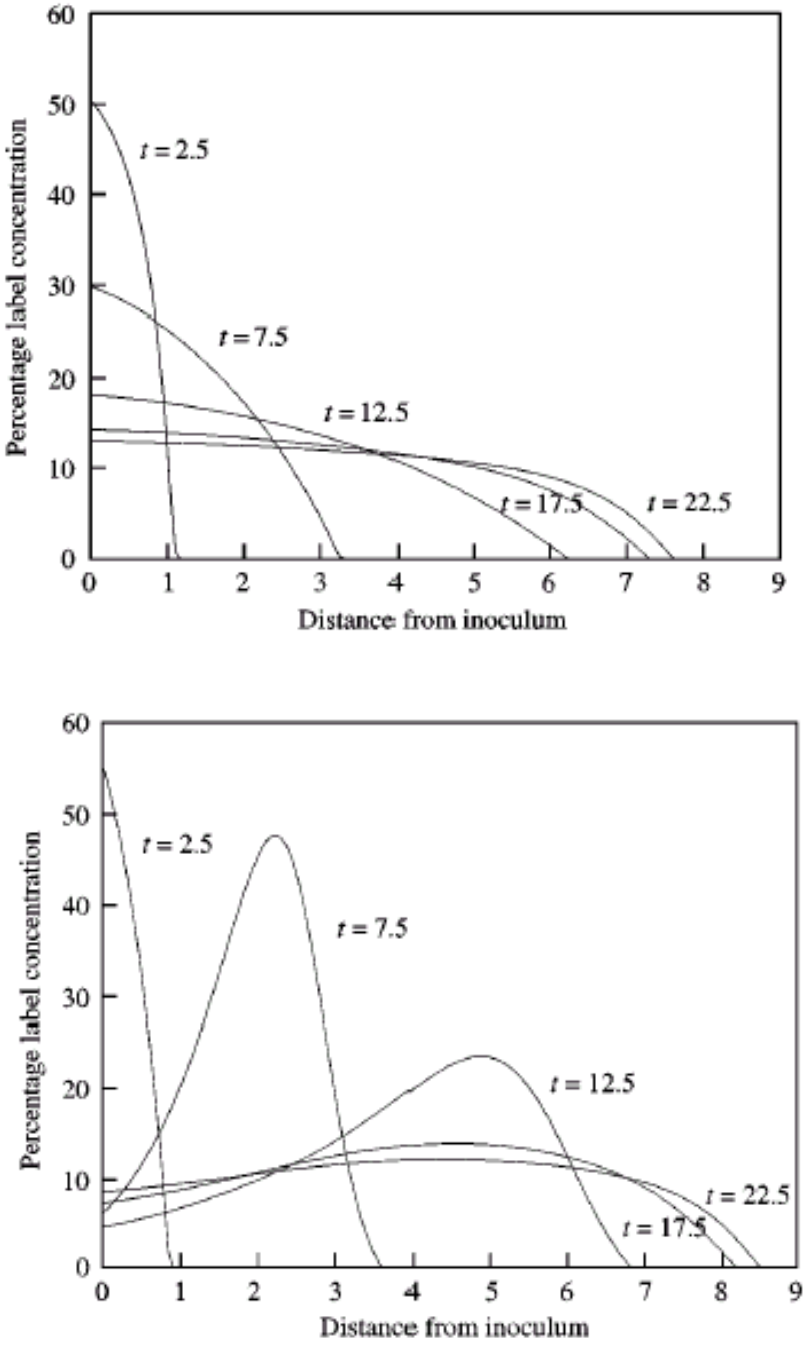


Fig 3.4. Distribution of a labeled nutrient at different times after start of the experiment. The label is added to the inoculum at time 0 and growth is assumed to be out over a non-substrate. In the top diagram (A) the translocation of label is

assumed to be by passive diffusion. The bottom diagram (B) shows the predictions if the translocation of label inside the mycelium is active and dependent on the available energy locally in the mycelium.

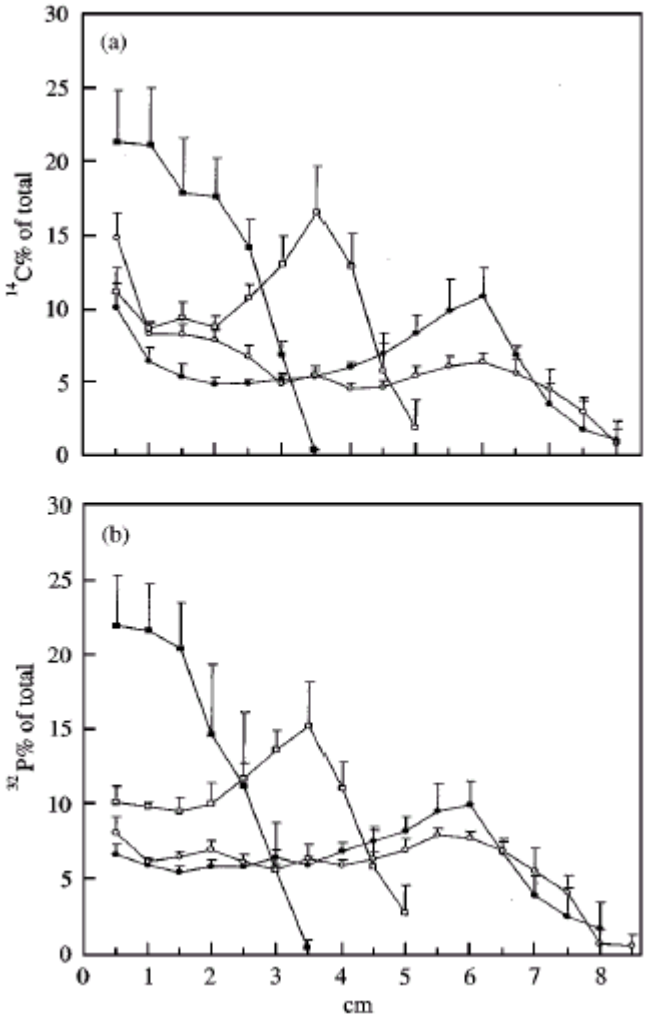


Fig 3.5. Experimentally measured label concentration in a mycelium of *Arthrobotrus superba* growing out from a piece of wood into soil. The top diagram shows the distribution of ^{14}C labelled non-metabolizable glucose analogue added to the wood in trace amounts. The bottom diagram shows the distribution of ^{32}P added to the wood in trace amounts.

Even more interesting it could be seen that there seemed to be a maximum length a mycelium could reach out into the soil from the piece of wood. This could also be modelled (Fig 3.6). In this case a the soil had more bacteria and thus more energy was needed for maintenance compared to what is seen in Fig 3.4 and 3.5

explaining the shorter length the mycelium grows out. It was modelled how far out from the inoculum it could be predicted the mycelium could reach if the size of the wood block was increased. It is obvious that the fungus would be able to reach further out into the soil the larger the food bases size. The advantage of active translocation would be greater the larger the food base size. Experimental results from food base size 1 and 5 fitted the model with active translocation better.

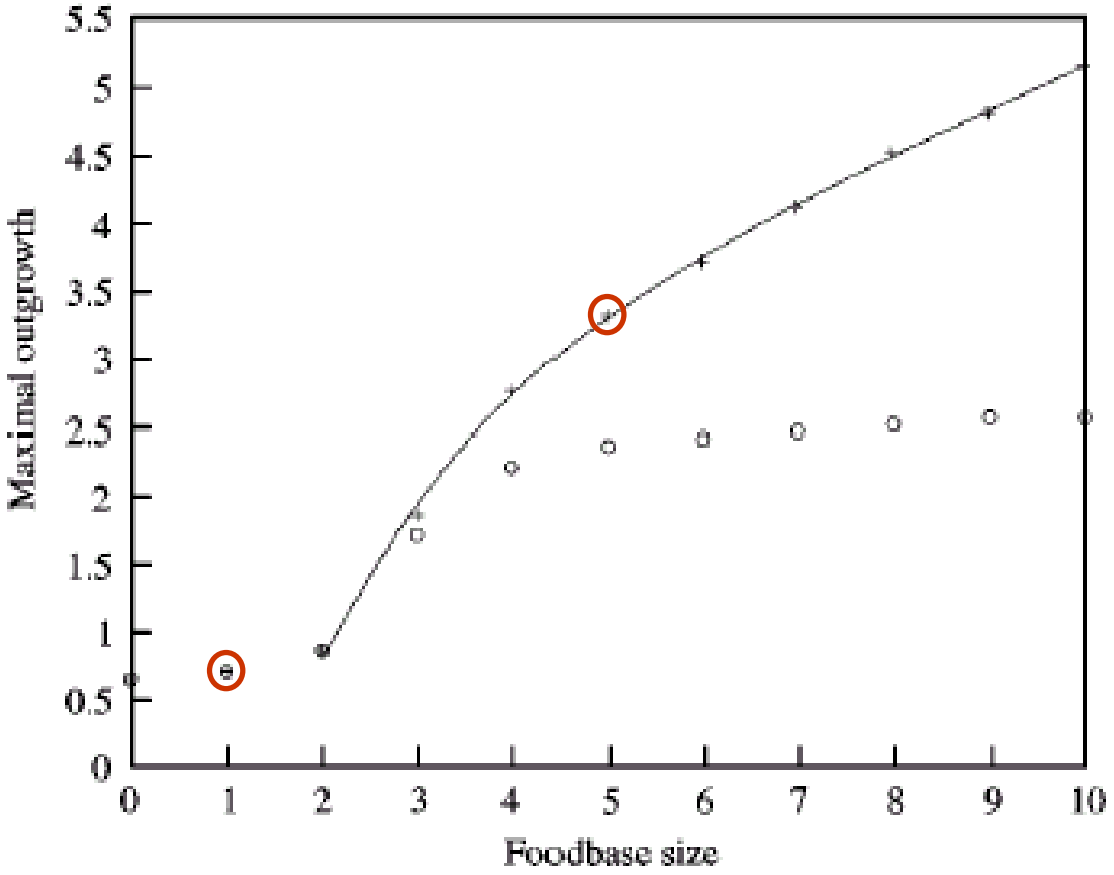


Fig 3.6. Maximal mycelium outgrowth over a non-nutrient area from a food base as a function of food base size. The open circles shows the predictions using a model where nutrient translocation is assumed to be passive. The closed circles and the line shows predictions if translocation is assumed to be active and dependent on intracellular nutrient concentration. For food base size less than 3 there is little difference but for larger sizes the maximal outgrowth is much greater for an actively translocating mycelium. Red circles shows the maximum outgrowth measured experimentally for foodbase size 1 and 5 indicating that the fungus was actively translocating nutrients.

Mycelial growth habit and nutrition

Fungi are not particular good at taking up nutrients compared to bacteria. On the other hand the mycelial growth habit have the following advantages:

- The fungus can often use different types of nutrients present at different locations simultaneously. There could be a large amount of carbon available in one location and a lack of for example nitrogen for good growth at this location. If this nitrogen can be imported from another location good growth can take place.
- The fungal hyphae can penetrate into structures made up of polymers. Fungi have also very high capacities to produce extracellular enzymes to break down and use polymers.
- Fungi can withdraw cytoplasm and resources from an unfriendly area and then “move” to another area. Some fungi can thus “reach” over inert areas including air gaps in cm sizes.
- Fungi can concentrate resources in an area that needs extra resources. Sometimes there is ample supply of nutrients in an area. The problem can be that the resources are inside another organism or in an area already occupied by other organisms. Many fungi can then concentrate their resources in this area producing enzymes and toxic compounds to kill or inhibit the other organisms in order to get hold of the resources.

Carbon

Fungi can use many different carbon sources. There is however differences between different fungal species in how well they can use different carbon sources (Fig 3.7).

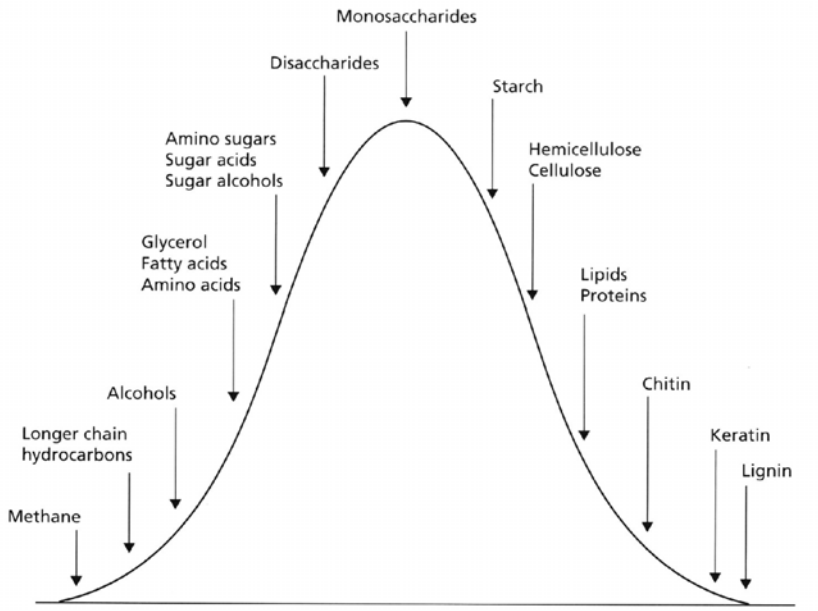


Fig 3.7. Substrates used by fungi. The is an approximation to the relative number of species that can use the different types of carbon sources. Most fungal species can use monosaccharides. Few fungi can use the single carbon compounds like methane and few fungi can break down the very large and recalcitrant polymer lignin.

Wood degradation and fungal succession in wood

One common substrate that is available to fungi and where bacterial degradation plays a very little role is wood. The succession of fungal species in wood is illustrated in Fig 3.8. First wood is colonised by week parasites and pathogens lready on the tree. When a branch or a tree die and fall off next comes pioneer saprotrophs. These first two groups live mainly on easily degradable compounds in the wood. The polymer degrading fungi that attacks the cellulose then follows. After this come the degraders of recalcitrant compounds. These last fungi also attack the lignin in the wood to get to the cellulose. Lignin is a very poor substrate but it protects the cellulose from breakdown. During most of the breakdown process secondary opportunistic fungi are also present. These grow on dead remains of the other fungi, parasitize other fungi or grow commensally with polymer-degraders.

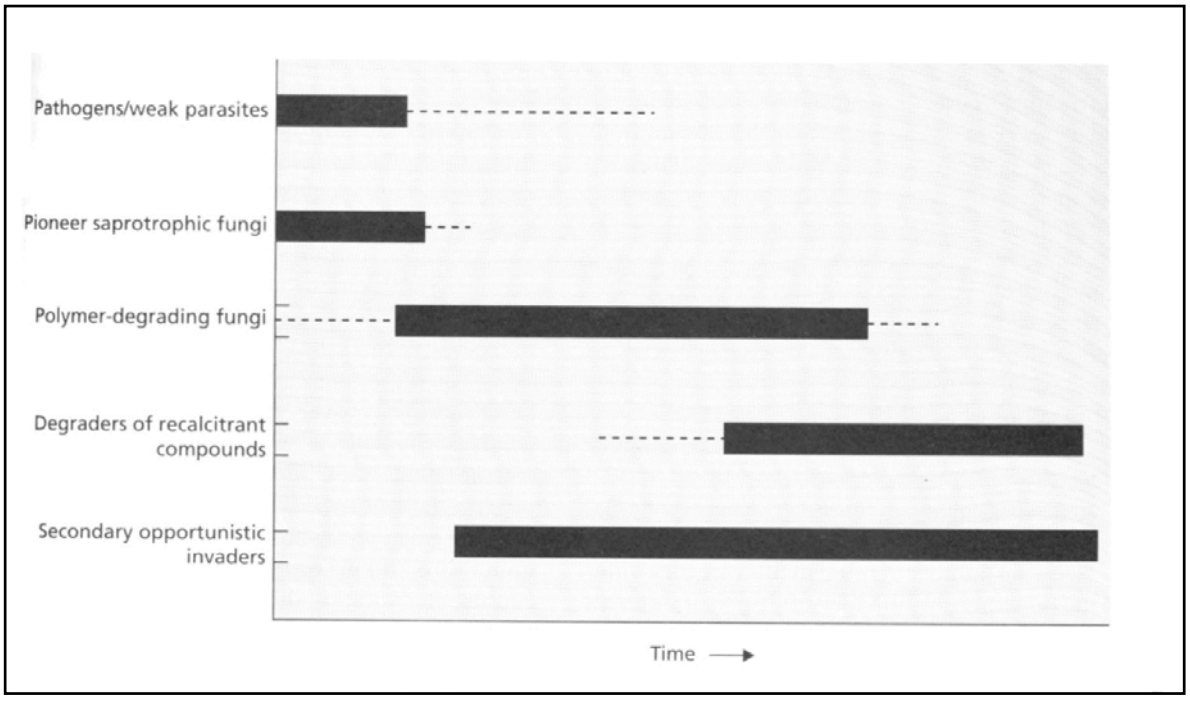


Fig 3.8. Overlapping phases of activity of different types of fungi the decomposition of a woody material.

Many different fungal species can grow on the same piece of wood. They often interact and try to poison each other by excreting phenolic compounds. The defence strategies used by the attacked fungus is to polymerise the phenolics. In wood these areas of “conflict” between adjacent mycelia can be seen as dark polyphenolic demarcation lines (Fig 3.9).

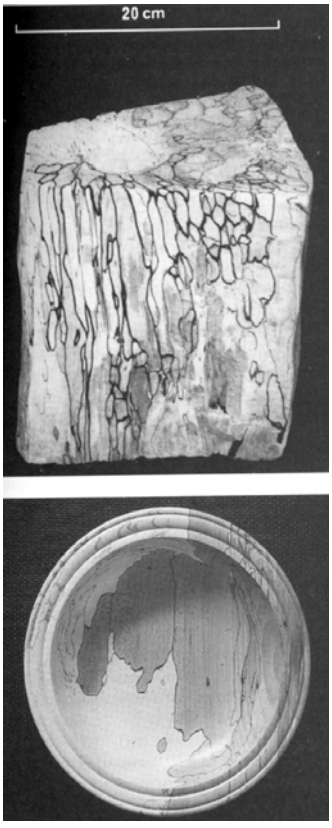


Fig 3.9. Part of a decaying tree stump showing polyphenolic demarcation lines between adjacent mycelia (top). Decorative bowl made from beech wood with dark zone lines

The decomposition of wood

Wood is a composite material made up of cellulose fibres embedded in a relatively hydrophobic lignin matrix (Fig 3.10). For a complete breakdown of wood the lignin matrix has to be removed ore at least made hydrophilic.

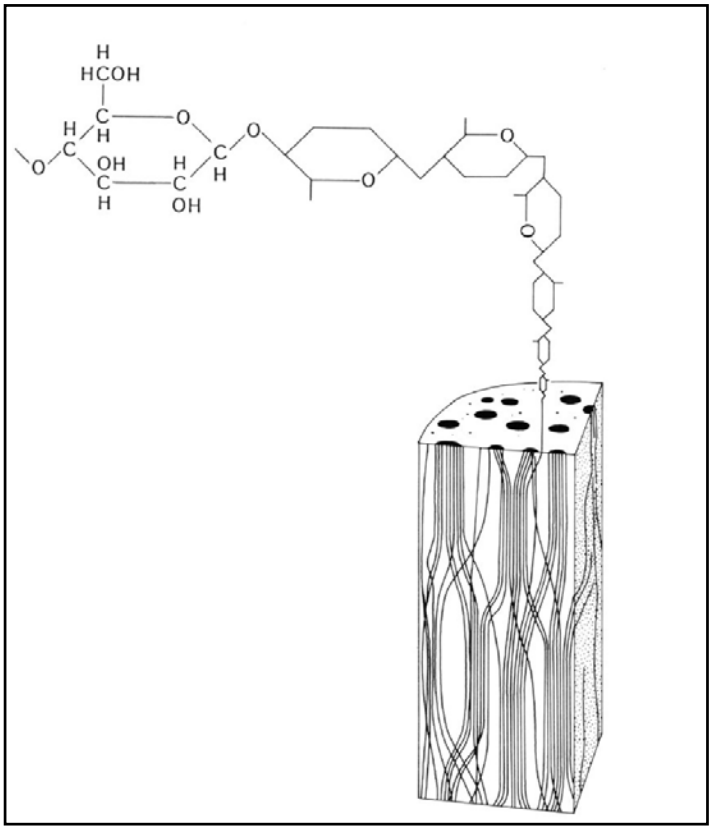


Fig 3.10. Wood is a composite material made up of cellulose fibres in a matrix of lignin.

Cellulose is a polymer that has to be broken down extracellularly. Two different kinds of extracellular enzymes are active, the cellobiohydrolases and the endoglucanases (Fig 3.11). The endoglucanases cuts randomly in the polymer crating many new ends. Then the cellobiohydrolases attaches to the ends of these shorter cellulose fragments and cuts of dimeric cellobiose units from the ends as they move along the polymer. The produced cellobiose is then taken up by active transport trough the membrane. Cellobiose is degraded to glucose intracellularly. There is a constitutive low production of the extracellular enzymes. Cellobiose is sensed and indicates the presence of cellulose. At low cellobiose concentrations cellulolytic enzymes are up-regulated and at high concentrations they are down-regulated to save production on extracellular enzymes.

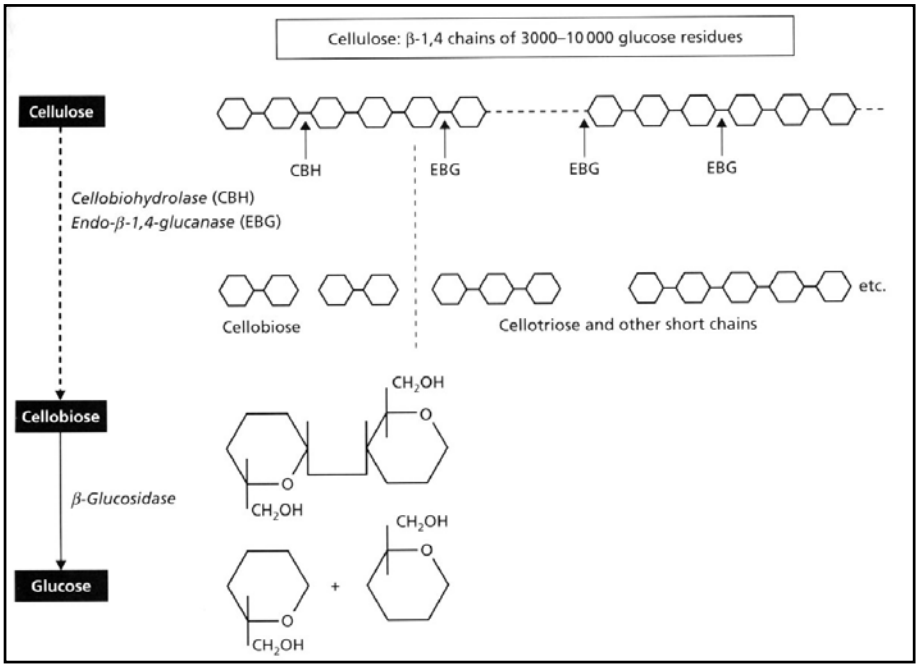


Fig 3.11. Enzymatic processes involved in the degradation of cellulose

A problem for all wood decomposing fungi is to remove or change the lignin so that the cellulose can be attacked. Lignin is a complicated polymer built up of a complex mixture of compounds (a heteropolymer). It is a poor substrate and is not used to generate energy by fungi. On the other hand the fungi have to spend energy to either break it down like the so called white-rotting fungi do or chemically change it like the brown-rotting fungi do. The wood rotting fungi does not attack the lignin by normal enzymatic processes since:1. A normal enzyme can attack only one type of chemical bonds and there are too many different kinds of bonds in lignin (Fig 3.12). Lignin is rather hydrophobic and this limits the access to enzymes. The fungi instead use enzyme systems that generate free radicals that attack the polymer and the breakdown becomes similar to a thermal combustion. These processes have consequently been called enzymatic combustions.

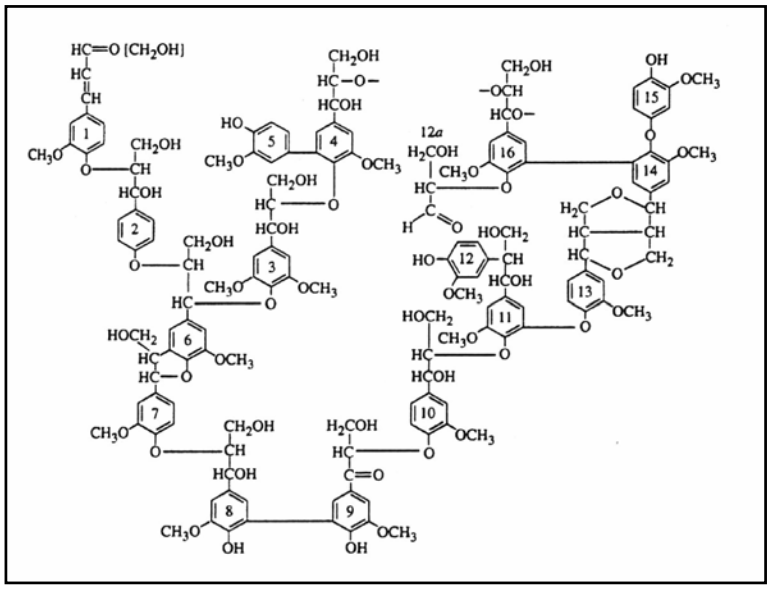


Fig 3.12. Chemical structure of lignin

Nitrogen

Like all organisms fungi needs nitrogen for building proteins and nucleic acids. Fungi also need nitrogen for the amino sugars they use to build the chitin in their cell walls. Polymeric nitrogen sources are broken down by extracellular proteases and nucleases. The resulting peptides, purines, pyrimidines and amino acids are then pumped into the cytoplasm by special transporters in the plasma membrane. From these monomers the fungi can build polymers (Fig 3.13).

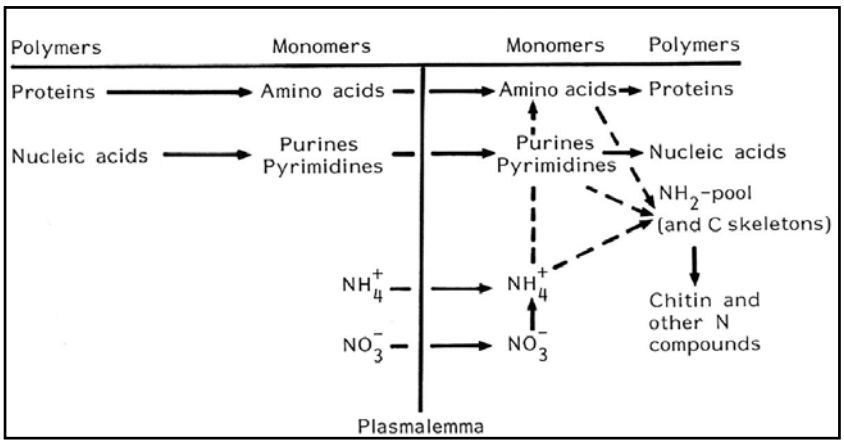


Fig 3.13. Nitrogen metabolism in fungi

Phosphorus

The third most important nutrient phosphorous is taken up from the environment as orthophosphate. Phosphorous are generally strongly bound to compounds in the environment. It can be as phosphate containing organics or as insoluble salts in the soil or present as minerals in rocks. Fungi use extracellular phosphatases to liberate phosphorous from organics. They also produce organic acids to dissolve insoluble salts and rocks to be able to take up phosphorous.

Uptake of carbon and phosphorous from an agar medium

The uptake of these two nutrients can easily be visualized by a simple technique (Fig 3.14). Fungi are grown on top of a cellophane membrane on a defined agar medium containing glucose and minerals (including phosphorous). The fungus cannot penetrate the membrane but can take up small molecular compounds through the membrane. When the fungal colony has grown out the outline of the

colony can be marked on the bottom of the Petri dish and the colony can be removed by lifting off the membrane. The presence of glucose or phosphorus can the be detected in the agar medium by placing a filter paper with a reagent that reacts to produce a coloured product were a glucose or phosphorus is present. Glucose can for example be detected by its ability to reduce copper (Fig 3.15) and phophorus by its reaction to molybdate ions (Fig 3.16).

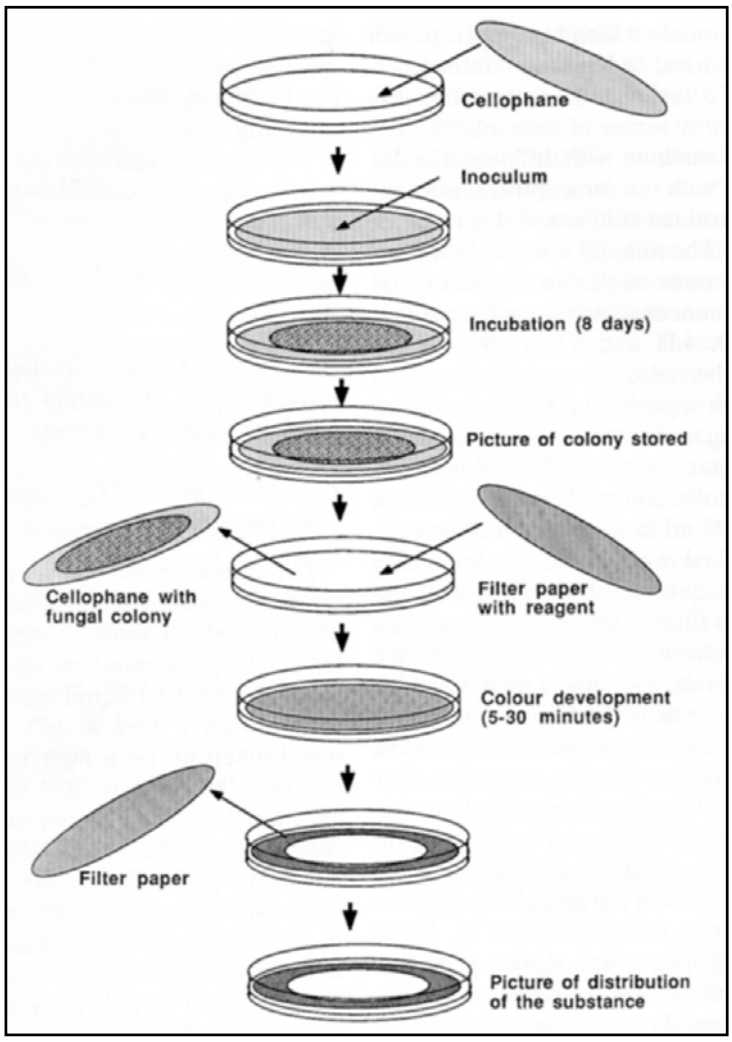


Fig 3.14. Technique for visualizing the uptake of a nutrient from an agar medium.

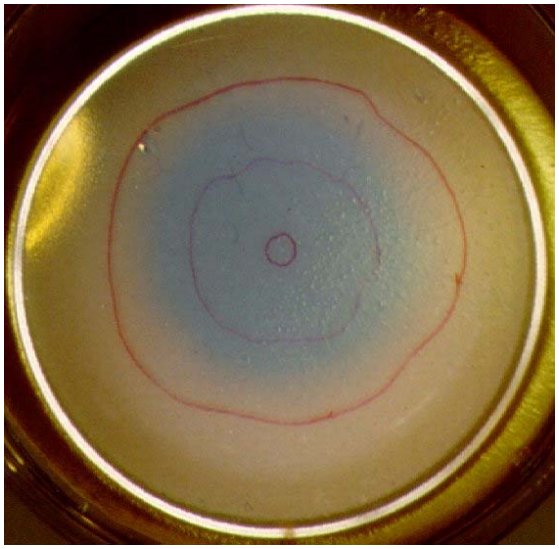


Fig 3.15. Uptake of glucose. Presence of glucose is detected by a precipitation of ´brown Cu_2O when glucose reduce Cu^{2+}(EDTA). The outer red line shows the outline of the fungal colony. Note that the fungus takes up basically all glucose under the colony.

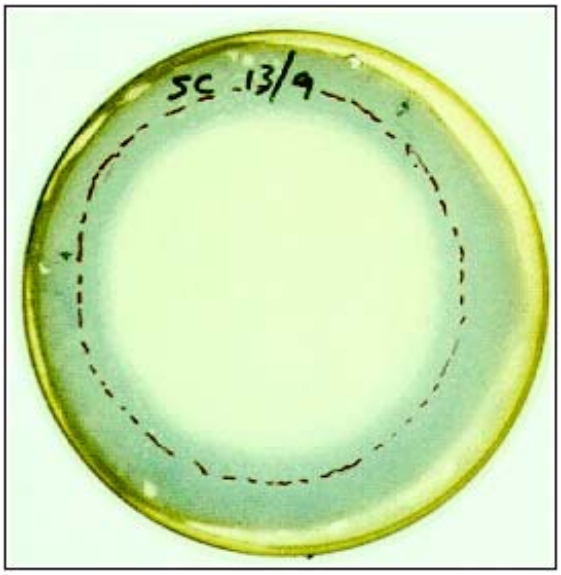


Fig 3.16. Uptake of phosphorus. Presence of glucose is detected by a the reaction with molybdate creating a blue colour. The dashed line shows the outline of the fungal colony. Note that the fungus takes up basically all phosphorus under the colony.

Strategies for nutrient uptake and storage

Often fungi take up all nutrients they get hold of irrespective of their needs. Fungi growing on different media with different mineral nutrient concentrations still show the same depletion of carbon although the need for carbon for growth is greater the higher the mineral nutrient concentration (Fig 3.17 A). Similarly growing on different carbon concentrations the uptake of phosphorous is the same (Fig 3.17B). Thus fungi are able to store both carbon and phosphorus intracellularly and the ratio between elements in a fungus will reflect the ratio of available elements in contact with the mycelium.

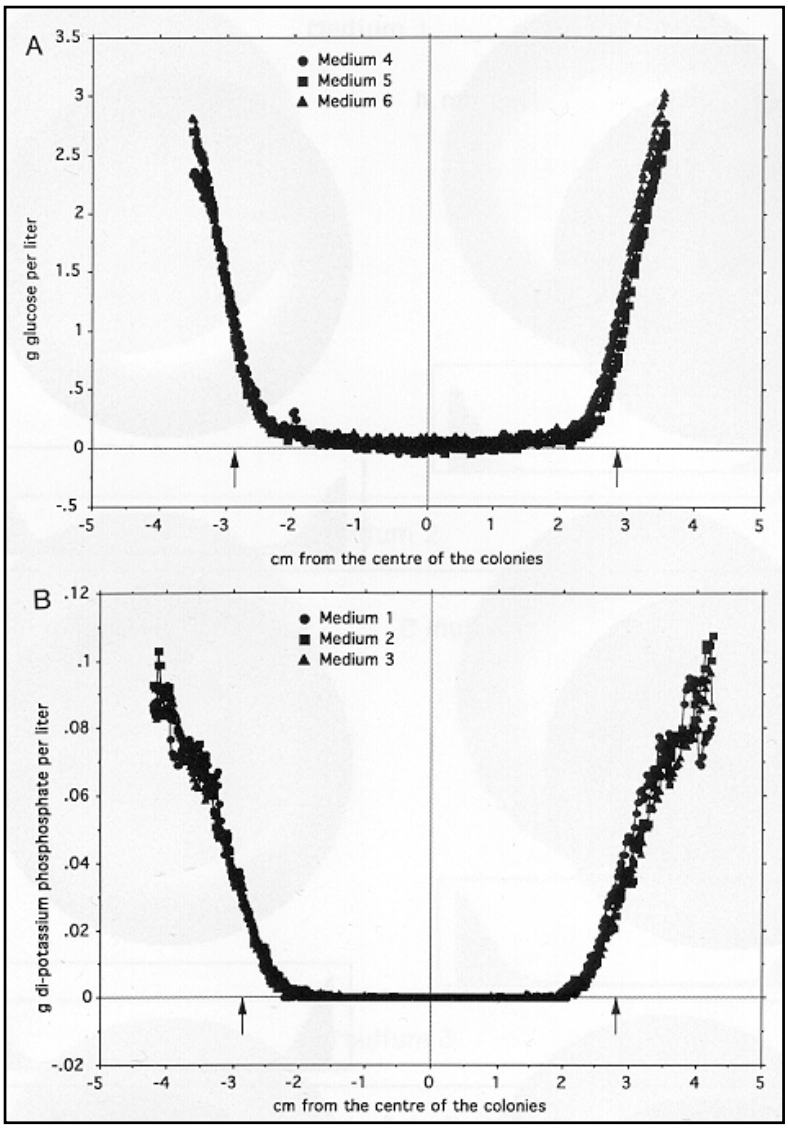


Fig 3.17. Glucose concentration profile under a fungal colonies grown on media with different mineral concentrations (A). Phosphorus concentration under fungal colonies grown on media with different glucose concentrations (B). Medium 3&6 is 2 times more concentrated than 2&5 that is 2 times more concentrated than 1&4.

Storage and translocation

Fungi are thus able to store large amounts of the main nutrients locally either for later use but also as a source for translocation to other parts of the mycelium. Storage of compounds cannot be done as monomers in the cytoplasm. This would create osmotic problems. Thus storage compounds have to be either as polymers or in hydrophobic compounds. Carbon is mostly stored as fats in lipid bodies. Phosphorus is stored as oligophosphates in vacuoles. There is however no known general compound known to be used as storage for proteins. In one case a specific storage protein have been demonstrated (Fig 3.18).

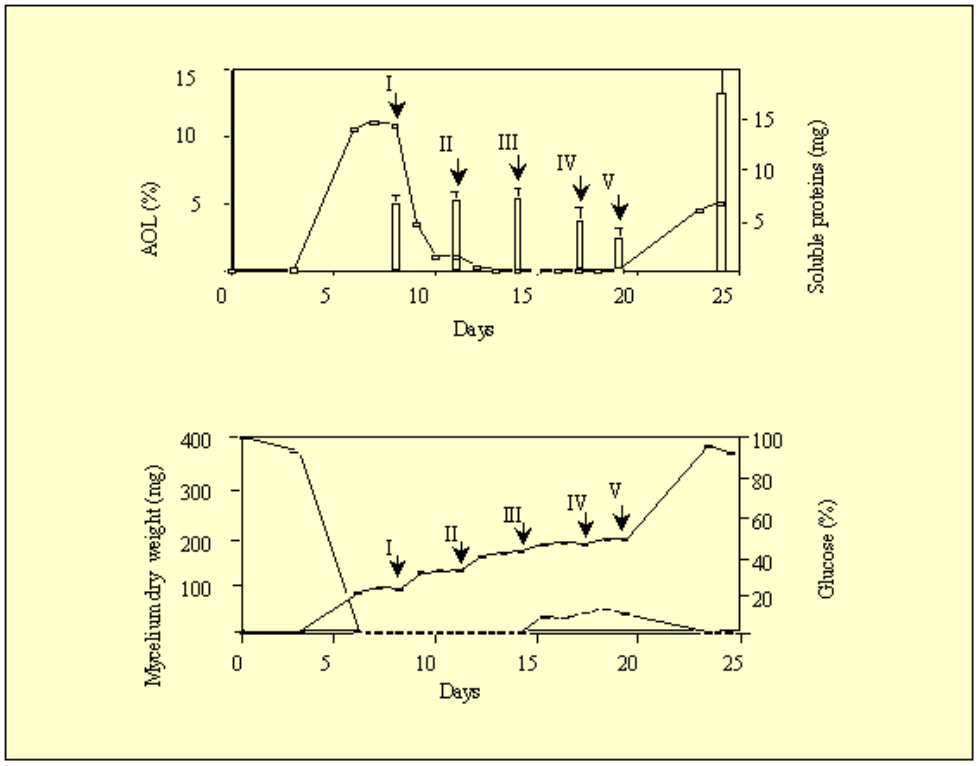


Fig 3.18. Percent AOL (storage protein) of all proteins in a *Arthrobotrus oligospora* and total amount of soluble proteins (top) and mycelium dry weight and medium glucose concentration (bottom) at different times from the start of the experiment. From day 0 until time I the medium had C/N-ratio 5. At time I the medium was exchanged to C/N-ratio 1000. This was repeated at times II-IV. At time V the medium was changed back to C/N-ratio 5 again. Glucose was rapidly finished from the medium after e few days. When glucose was becoming limited there was nitrogen in excess and the content of AOL increased. When C were added at time I AOL started to be broken down. The fungus was able to continue growth by using the stored nitrogen in AOL and completely remove the glucose until the medium change at time III. Then there was a small surplus glucose present in the medium since the fungus was not able to take it up. When shifted to the high nitrogen content medium again at time V the accumulation of AOL started again and glucose was depleted. .

Secondary metabolism

When fungi run out of a limiting nitrogen source (or other mineral source) they start producing secondary metabolites (Fig 3.19). These are small molecular compounds excreted into the environment. They are called secondary because they are not produced during unlimited growth (primary growth).

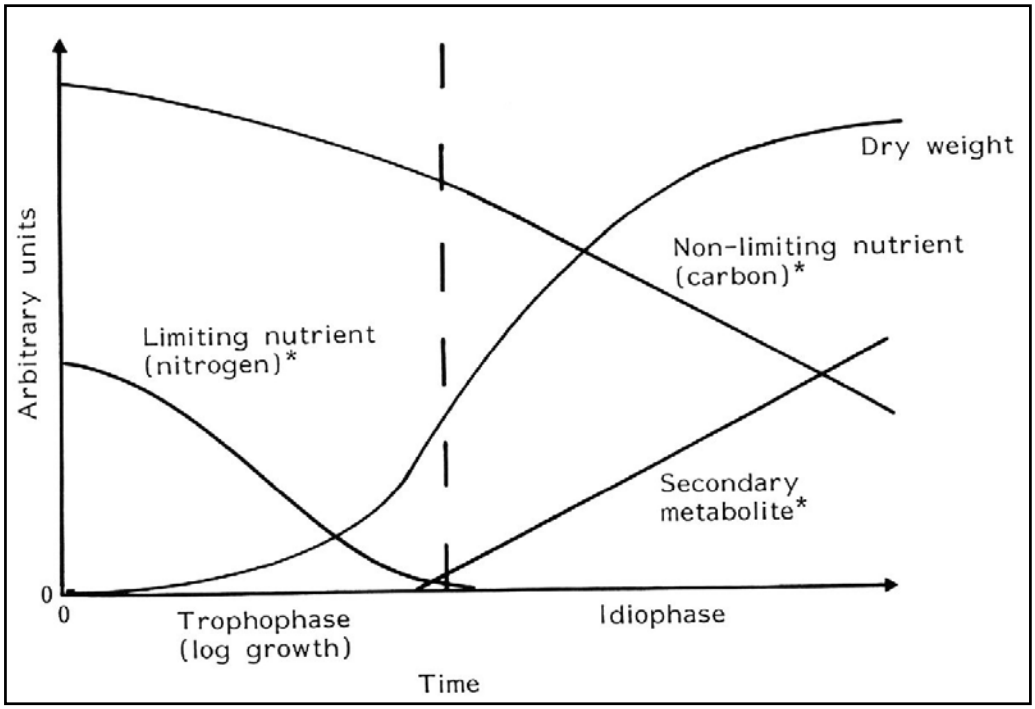


Fig 3.19. Batch growth of a fungus in a liquid medium with nitrogen as a limiting nutrient. When the limiting nutrient is finished (dashed line) the secondary metabolites appear. Dry weight continues to increase but the exponential growth stops and the fungus slowly enters stationary phase.

The compounds produced are of many kinds and characteristic for the fungal species and strains. Many of the compounds are bioactive and have become important products for industry (Fig 3.20).

Table 1.4 Fungal secondary metabolites produced commercially for pharmaceutical, agricultural and research uses.

Usage	Product	Fungal source	Application
Medicine	Penicillins	*Penicillium chrysogenum*	Antibacterial
	Cephalosporins	*Cephalosporium acremonium*	Antibacterial
	Griseofulvin	*P. griseofulvum*	Antifungal
	Fusidin	*Fusidium coccineum*	Antibacterial
	Cyclosporin	*Trichoderma polysporum*	Immunosuppressant
	Ergot alkaloids	*Claviceps purpurea*	Induces labour; migraine treatment
Agriculture	Zearalenone	*Gibberella zeae*	Growth promoter for cattle
	Gibberellins	*G. fujikuroi*	Plant hormones
Research	Gliotoxin	*Trichoderma virens*	Immunosuppressant
	Cytochalasins	*Helminthosporium dermatoideum*, etc.	Anti-actin agents
	Fusicoccin	*Fusicoccum amygdali*	Stomatal opening
	Phalloidin	*Amanita phalloides*	Actin binding
	α-Amanitin	*A. phalloides*	RNA polymerase II inhibitor

Fig 3.20. Examples of secondary metabolites.

The different classes of secondary metabolites are made from compounds originating in the primary metabolism (Fig 3.21). To make a secondary metabolite a special metabolic pathway for its synthesis is needed. Often all the enzymes needed plus regulatory proteins and export proteins are clustered at the same location of the genome. The secondary metabolites where once regarded as some kind of surplus metabolism not needed in nature but rather a consequence of growing in rich laboratory media. Today they are considered as important compounds used in the interactions with other organisms or the environment and not secondary to fungal survival in nature at all.

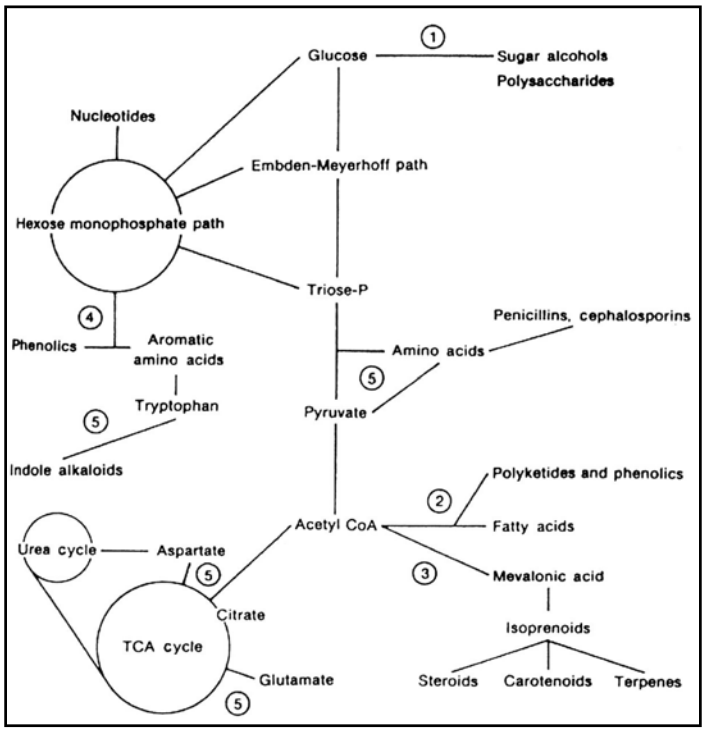


Fig 3.21. Numbers illustrates the starting compounds for different classes of secondary metabolites.

4.The physical environment and biodegradation

Physical environment

Fungal growth in an environment is not just dependent on available nutrients. Other environmental factors like the availability of oxygen, water and the temperature are important determinants of fungal growth.

Oxygen

Nearly all fungi are strictly aerobic. They need oxygen for respiration to use organic substrates completely. They also need oxygen to aid in the biosynthesis of certain compounds like phenolics and some secondary metabolites. Oxygen is also directly involved in oxidation of phenolics by some fungi. Fungi often change morphology and the formation of the cell wall can be affected by the availability of oxygen (Fig 4.1).

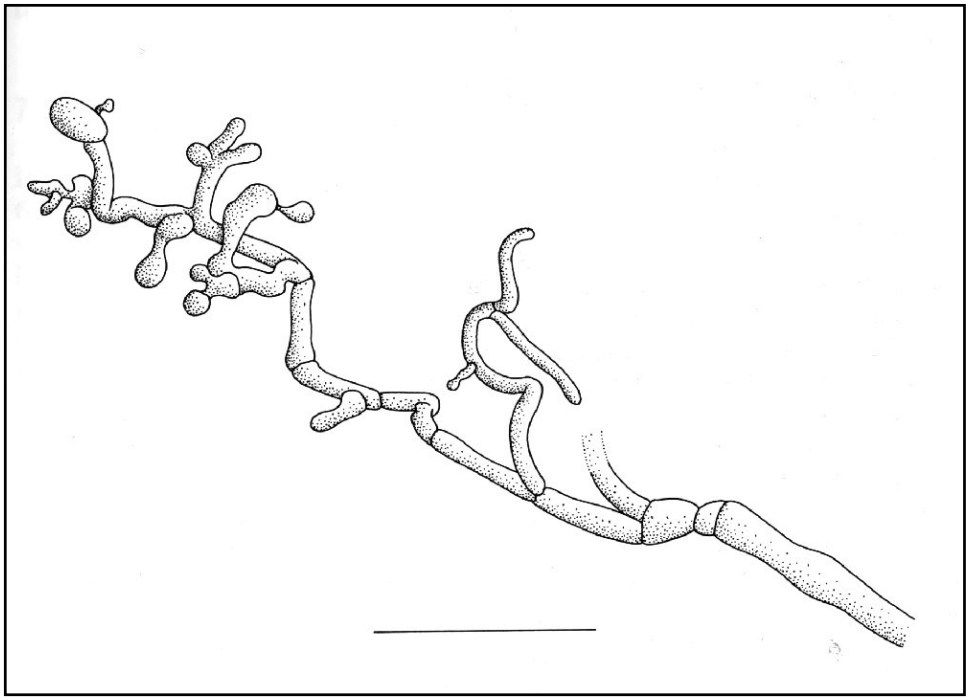


Fig 4.1. Fungus growing into area with lower and lower oxygen tension (towards the left). Note the more and more yeast like structure of the mycelium.Desiccation stress

Water is of cause necessary for growth but drying (desiccation) creates a mixture of osmotic stress and oxygen stress (too much oxygen). Short time desiccation leads to an accumulation to increased osmolarity in the vacuole by accumulation of polyols, as mannitol, in the vacuoles to counteract the loss of water. Prolonged desiccation case oxygen stress by oxidation of vital cellular compounds. This oxidation is caused by:

- Slower oxygen removal due to lower respiration rate
- Decreased repair activities due to less energy available
- Oxidation of polyunsaturated fatty acids in the membrane. These lipid peroxidation products are poisonous.

Thus it is difficult to separate water-stress from oxygen stress during desiccation. Although vulnerable fungal cells are very well adapted to withstand desiccation and the lichens are the real specialists. Lichens can withstand years of desiccation and being exposed to sunlight that increases oxygen stress.

Temperature

Fungal growth can be found at temperatures from below zero to above 60 C. There is no fungi that can grow close to the boiling point as some bacteria can (Fig 4.2).

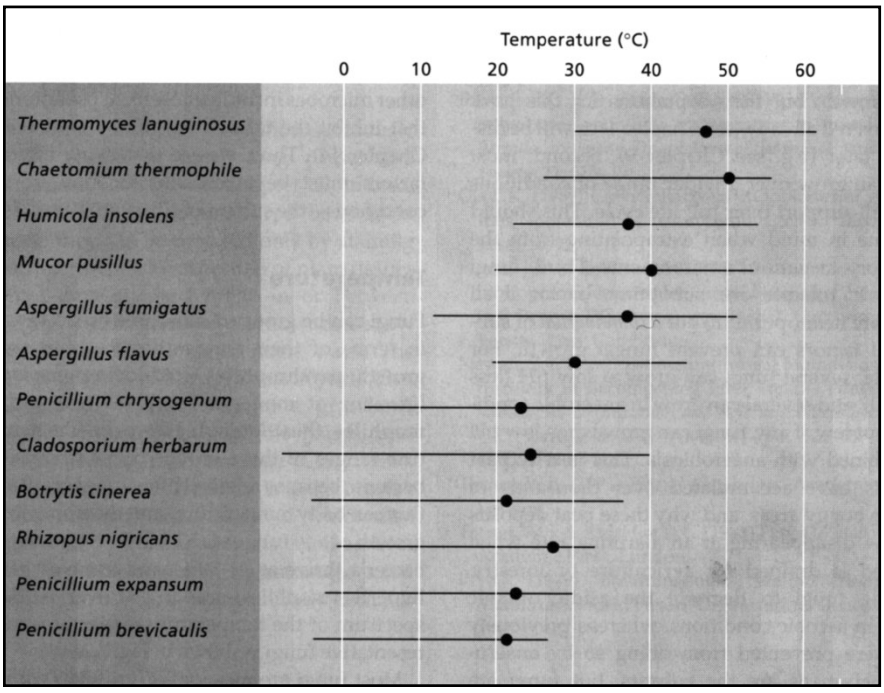


Fig 4.2. Temperature ranges for a selection of different fungi. Black dot indicates optimum temperature for growth.

Each fungal species have a temperature range and a water potential range allowing its growth. If one want to keep a material from being degraded by fungi it

is possible to do this by keeping the material outside these ranges for the fungi capable of degrading the material (Fig 4.3).

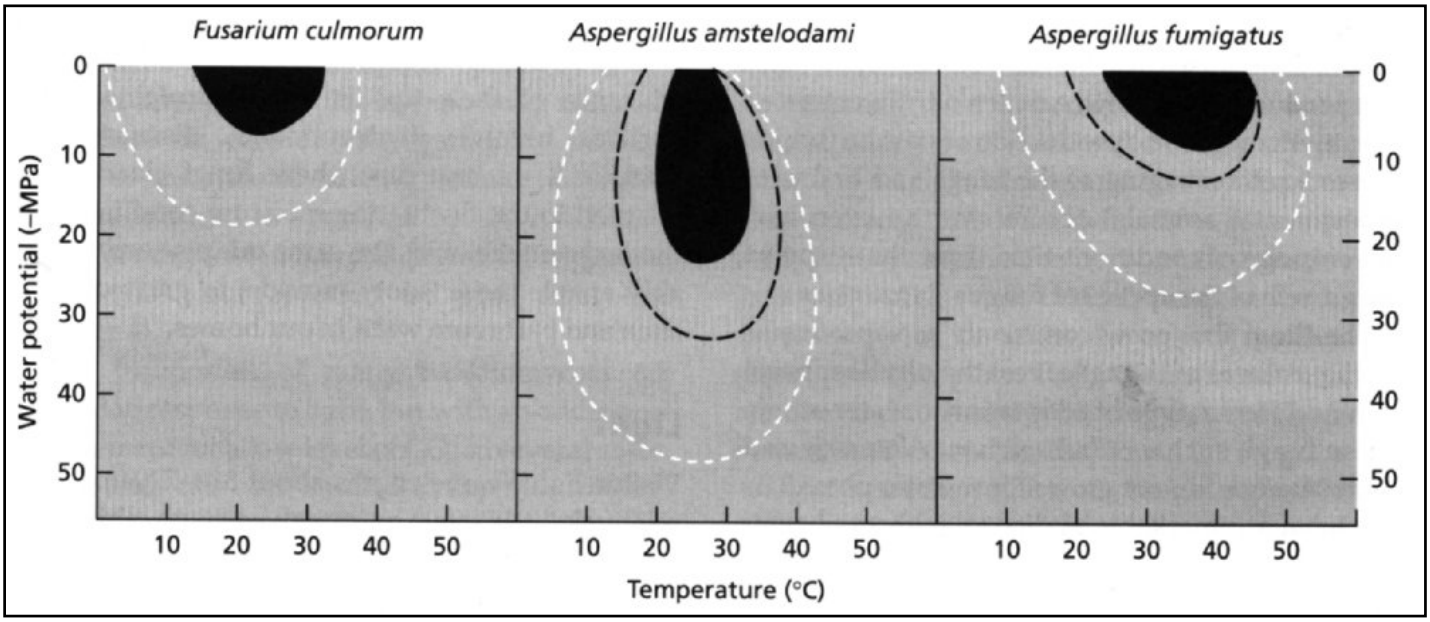


Fig 4.3. Growth rate isopleths (the combination of temperature and water potential at which different growth rates are seen) for three fungi that cause spoilage of cereal grains. Broken white line = 0.1 mm/day, broken black line = 2.0 mm/day and black area = faster than 4.0 mm/day.

Biodegradation of organic pollutants

Humans have produced many organic compounds that are totally artificial and foreign to life, xenobiotics. These xenobiotic compounds can be very recalcitrant (long lasting in the environment and thus difficult to break down). Thus the recalcitrant compounds may accumulate to toxic levels in the food chain. One of the most well known examples is DDT. The reason why these compounds are recalcitrant can be that they are aromatic or even polyaromatic. Some compounds are toxic to most organisms. Many have low water solubility so they become inaccessible to ordinary enzymatic breakdown. Even though the compound can be present in dangerous concentrations the concentration might be too low to be used as a carbon source for growth of a microorganism, metabolic breakdown. The compound can however be broken down by enzymes excreted into the environment by an organism using another carbon source, co-metabolic breakdown..

Examples of compounds:

- Polycyclic aromatic hydrocarbons (PAHs) in coal tar or creosote.
- Explosives (TNT, etc)
- Dyes (like aniline)
- Chlorinated compounds
    - Poly Chlorinated Biphenyls (PCBs)
    - Dioxin

Wood decomposing fungi as bioremediators

Wood decomposing fungi are potentially very good for bioremediation (biological cleanup) of xenobiotic compounds. The main reason is that all types of organic compounds can be attacked by the enzymatic combustion characteristic for the lignin attacking ability of these fungi. Thus the degradation is both extracellular and non-specific. The other advantage is that many wood decomposing fungi are good soil colonizers and can grow into an unsterile environment like soil full of indigenous microorganisms. Common enzymes involved in the degradation of xenobiotics are the lignin attacking laccases, phenol oxidases and cellobiose dehydrogenases. All these enzymes generate free radicals that can attack most compounds in a process similar to combustion.

5.Fungal interactions

This chapter is about fungi interacting with bacteria, other fungi and nematodes. Plant and animal fungal pathogens are not treated. Mycorrhizal symbionts will be treated in the next chapter.

Bacterial fungal interactions
The effect of antibiotics of fungal origins on bacterial growth is probably the best known interaction between bacteria and fungi. There are several other antibacterial compounds in use for inhibiting bacterial growth. For the control of fungi antifungal compounds from bacteria could be used. There is however very few antifungal compounds known for use against fungal infections on humans. The reason is mainly that humans and fungi are both eucaryotic and the have the same targets of the antibiotics. On the other hand there are promising bacteria producing antifungal compounds and that could be used against plant pathogenic fungi. One such interaction is shown in Fig 5.1.

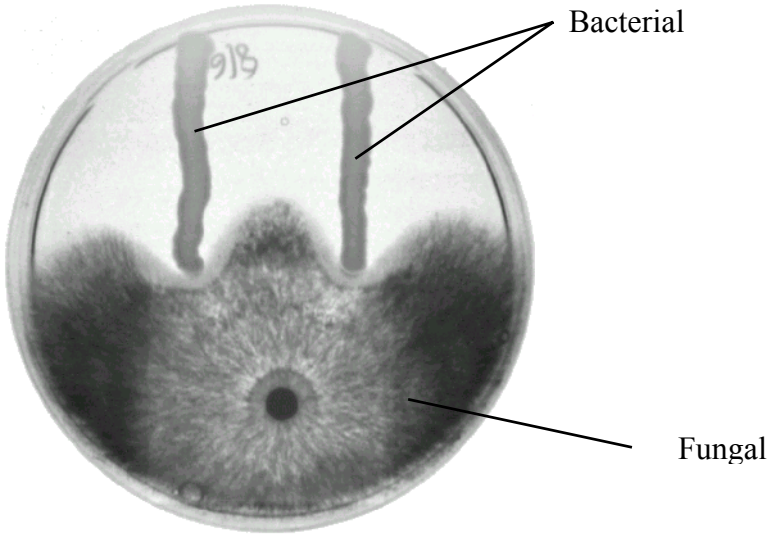


Fig 5.1. Pseudomonas fluorescence isolate producing an antifungal compound inhibiting the growth of *Rhizoctonia solani*. Not that there is inhibition zones surrounding the bacterial colonies.

Case: Biological control of fungi using a bacteria producing an antifungal compound
A lot of work has been done to try to use the bacteria in Fig 5.1 for biological control purposes. Often one or several excreted compounds are responsible for the effect on the fungi. It is important both to identify the compounds and describe their

effects on target fungi. The identification of viscosinamide produced by certain *Pseudomonas fluorescence* isolates and its effect on fungi is taken as an example.

Isolation of the antifungal substance

The bacteria apparently produce some kind of diffusible substance. Extracts from bacterial cultures were fractionated and a compound highly effective in inhibiting fungal growth was isolated. The pure compound was active on its own and it was shown to be a new substance, viscosinamide. The compound is a cyclic peptide Fig 5.2 with a lipid tail and has strong surfactant properties (amphiphatic molecule). The compound is similar to other compounds that have been shown to create holes in membranes.
. When added to a glass fibre filter paper and put on a fungal colony large inhibition zones was visible (Fig 5.3).

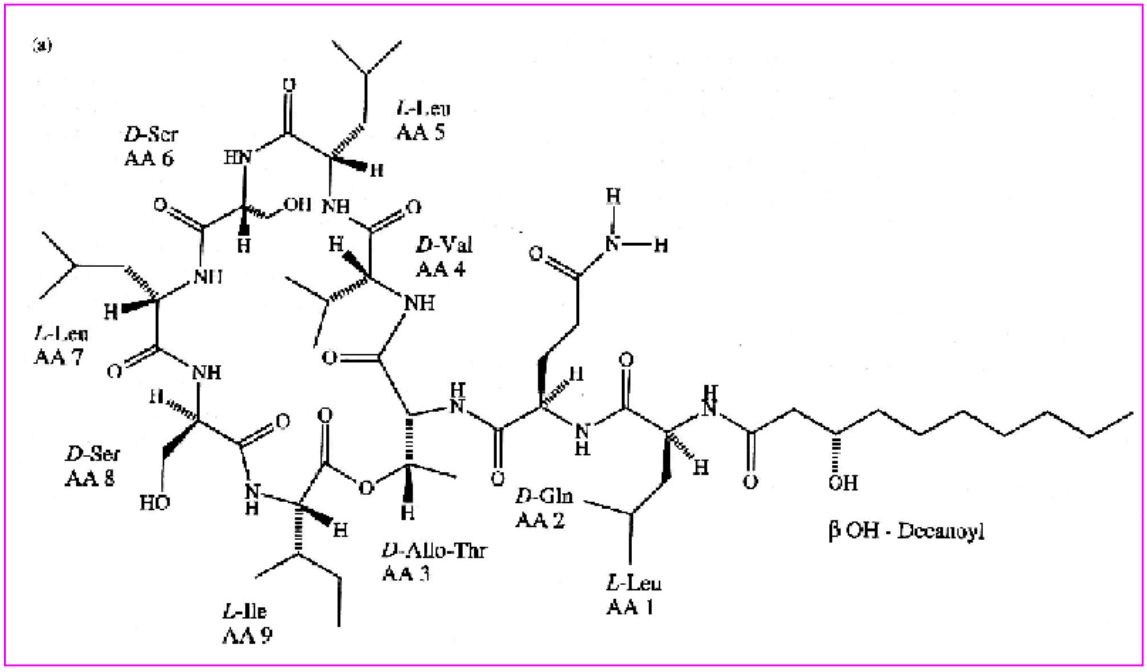


Fig 5.2. Molecular structure of viscosinamide. To the left is the cyclic peptide and to the right the lipid tail.

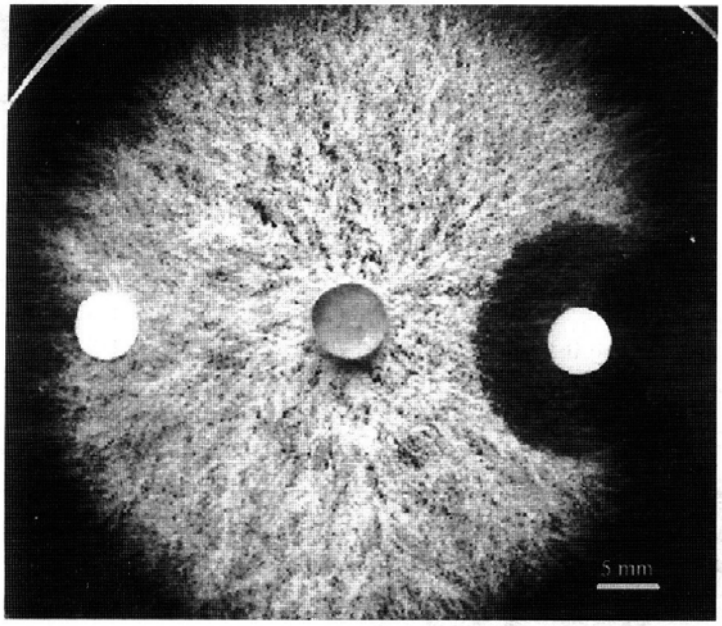


Fig 5.3.

Fig. 3 *In vitro* test of *Rhizoctonia solani* growth on Potato Dextrose agar plate containing filters with 100 μg viscosinamide (right of fungal mycelium) and methanol solvent control (left of fungal mycelium)

Effect as biological control agent

The way one could use the bacteria for controlling fungi could be for inhibiting fungi attacking germinating seeds. In a pot experiment to test the effect of the bacterial treatment it was found that the bacteria was at least as effective as the standard fungicide treatment (Fig 5.4).

TABLE 3. Effects of seed inoculation with *P. fluorescens* DR54 isolate or Thiram[a] fungicide on appearance of healthy seedlings in pot experiments with *Pythium ultimum*-infested soil

Treatment	% Healthy seedlings[b]	Odds ratio[c]
DR54	36 ± 29	7.6*
Thiram	26 ± 13	4.8*
None (untreated)	7 ± 8	1.0

Fig 5.4.

Effect of viscosinamide on fungi

When pure viscosinamide was used to challenge fungi it could be observed that the fungi reacted by decreased hyphal growth, increased branching and swelling hyphal tips (Fig 5.5). This type of reaction is common in fungi if membranes have been affected so that calcium from the environment can enter the cytoplasm.

Intracellular calcium is kept very low by all organisms. An increase in the intracellular calcium content is used in intracellular signalling and prolonged increases can trigger stress reactions. Thus it was believed that viscosinamide could make the membrane more permeable to calcium.

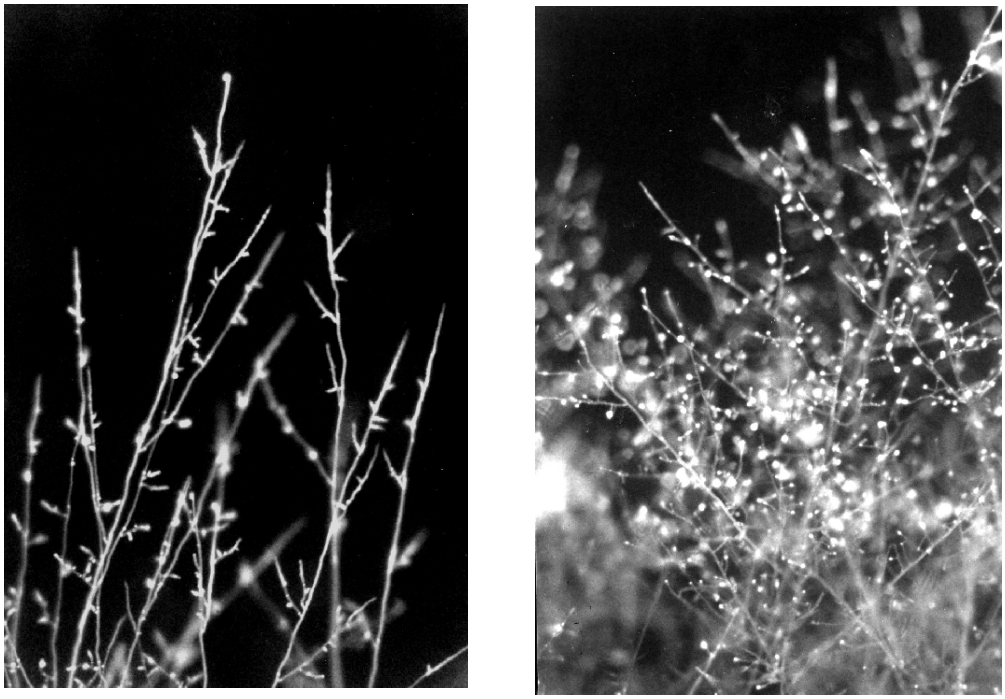


Fig 5.5. Extending hyphae of *Rhizoctonia solani* growing out on control agar medium (left) and in the presence of viscocinamide (right).

To test the calcium hypothesis a fungal assay using a transformed isolate (a reporter strain) of the fungus *Aspergillus awamori* was used. The fungus have been transformed to constitutively produce a protein from a jelly fish. This special jellyfish protein sends out light dependent on intracellular calcium concentration. More light is produced the higher the calcium concentration. When treating the reporter fungus with different concentrations of amphotericin B (antifungal antibiotic used in humans) a compound known to make holes in the membrane a dose response can be observed (Fig 5.6).

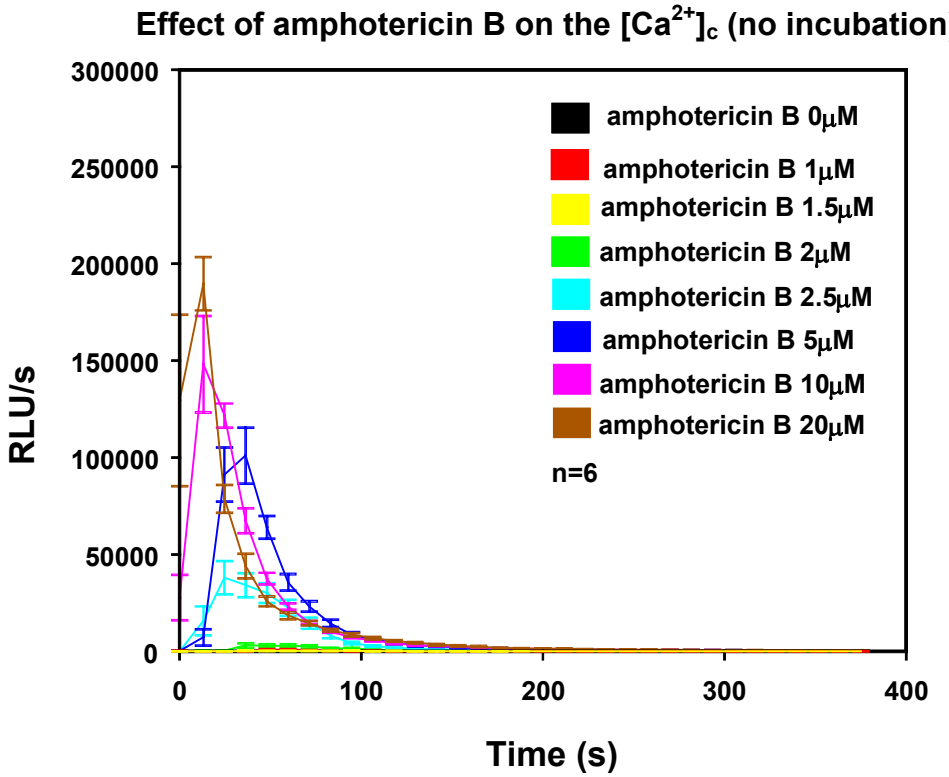


Fig 5.6. Effect of amphotericin B additions on the intracellular calcium levels as indicated by the relative luminescence (RLU) from cultures of a transgenic *Asperillus awamori* calcium reporter strain.

If bacteria producing viscosinamide were added to colonies of the reporter strain the bioluminescence of the fungus increased greatly compared to controls (Fig 5.7) indicating that the effect of viscosinamide is to make holes in the fungal membrane.

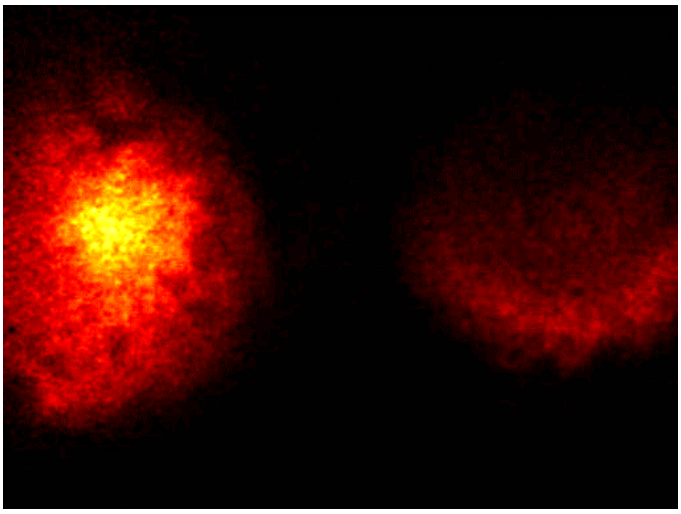


Fig 5.7. Light emitted from a transgenic *Asperillus awamori* calcium reporter strain when challenged with a viscosinamide producing strain of Pseudomonas (left) compared to the light output when challenged with a strain not producing viscosinamide (right).

The fungus tries to counteract the stress caused by viscosinamide

When en organism is chemically attacked it can defend itself to make itself less vulnerable to the compound. There are several was of doing this.

1. It can produce an enzyme that breaks down or modify the compound
2. It can polymerise the compound
3. The cell wall can be made more impermeable to the compound
4. The cell membrane can be made more impermeable to the compound
5. The compound could be more effectively pumped out of the cytoplasm or removed from the membrane.
6. The target of the compound in the fungus could be changed to make the fungus less sensitive.

In many fungi one of the way they seem to defend themselves against attack from other organisms is to make their cell walls less permeable, An enzyme that can be used for this in laccase. Laccase can polymerise phenolics in the cell wall making it more hydrophobic. *Rhizoctonia solani* reacts by producing laccase when challenged by viscosinamide producing bacteria (Fig 5.8). And the cell walls also becomes more autofluorescent (more polyphenolic) close to challenging bacteria (Fig 5.9).

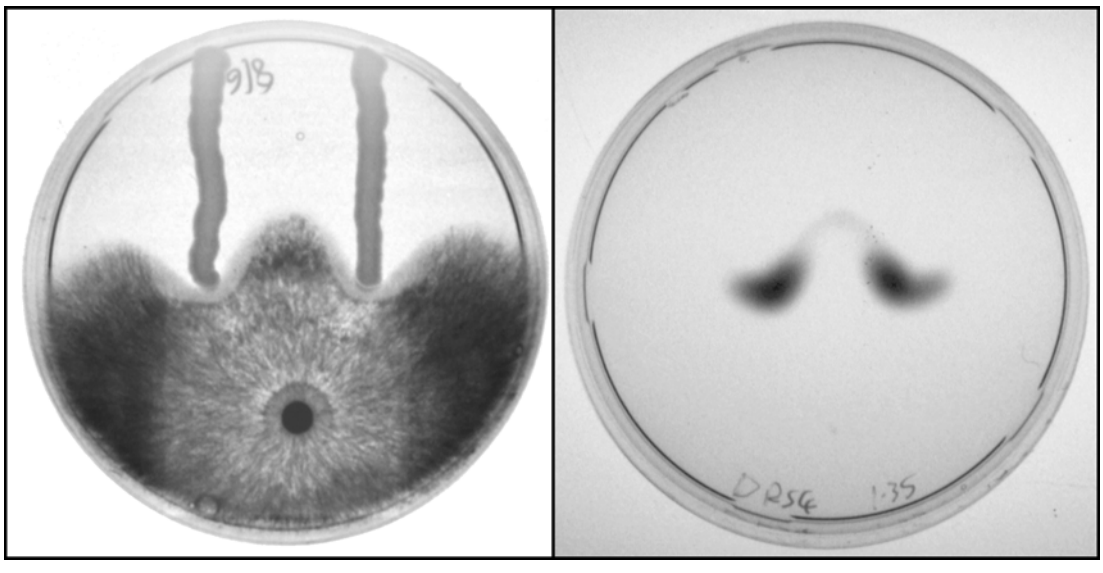


Fig 5.8. *Rhizoctonia solani* challenged by viscosinamide producing bacteria (left). An agar overlay containing the laccase substrate ABTS revealed high laccase activity in the zone of interaction between the fungus and the bacteria (right).

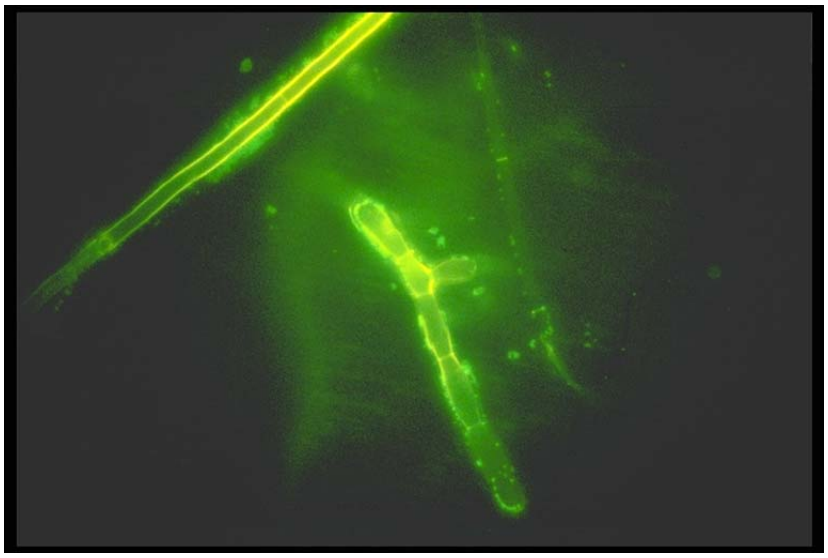


Fig 5.9. Increased auto fluorescence indicating polyphenolics present in the cell walls could be seen were bacteria were close to hyphae of *Rhizoctonia solani*.

Fungal-fungal interactions

Some fungi are very aggressive and attacks fungi of many other species. A fungi commonly used as a biological control agent against other fungi is *Trichoderma harzianum* (Fig 5.10). When it attacks a fungus *T harzianum* grows around the hyphae and produces cell wall degrading enzymes at the contact points. This interaction is used commercially to control fungal pathogens in greenhouse soils..

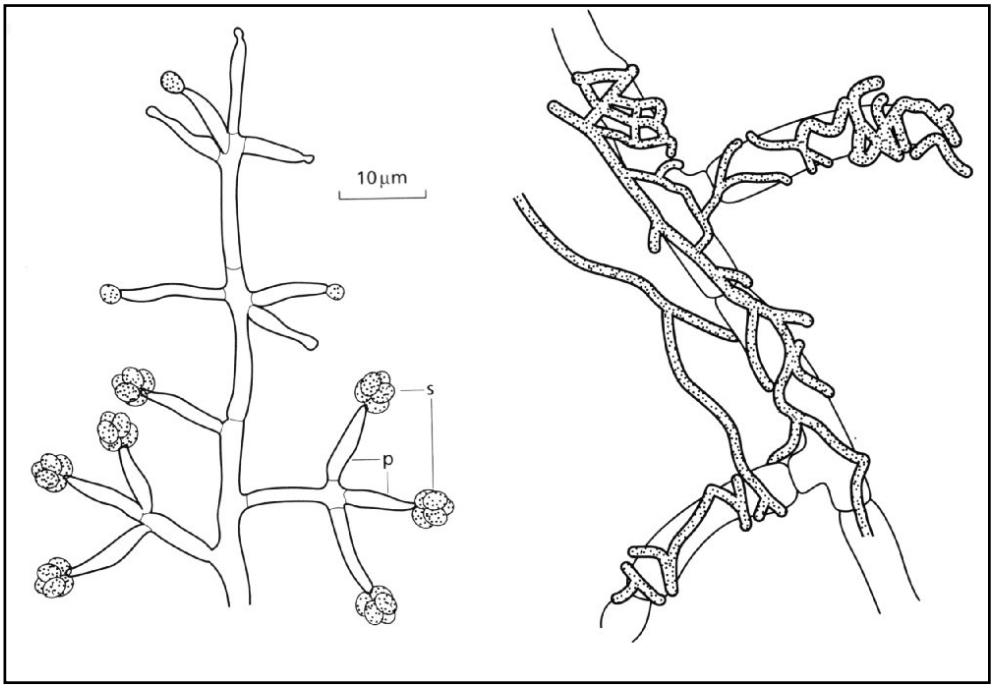


Fig 5.10. Trichoderma harzianum. Sporophore with spores in slimy heds (left). Hyphae of *T. harzianum* growing around hyphae of *R. solani* and attacking it.

Another interesting fungal fungal interaction is the one used to stop tree infections of *Heterobasidion annosum*. *H. annosum* attacs by spores landing on the stump of a newly felled tree (Fig 5.11) It then spreads down the stump then infects adjacent trees by the root-root contacts. The pathogen can be controlled by applying spores of a saprothroph with high competitive ability to the stump at tree felling.

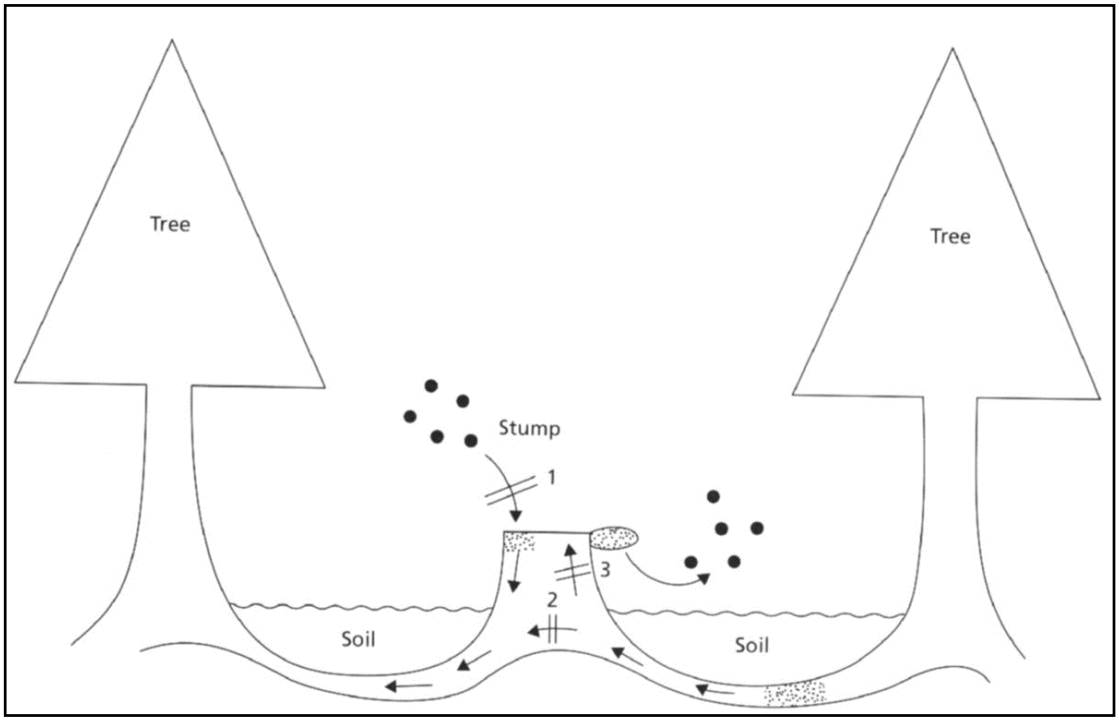


Fig 5.11. Mode of action of *Phlebiopsis gigantea* in controlling *Heterobasidion annosum* (stippled) in recently felled pine stumps. 1, *P. gigantea* is established on the stump surface and prevents colonization from airborne spores of *H. annosum*; 2, when *P. gigentea* has colonized the dying stump and root tissues it prevents spread of *H. annosum* from existing foci of infection in the root zone so it cannot spread to healthy tree roots; 3, *P. gigantea* also prevents *H. annosum* from growing up to the stump surface to sporulate.

Nematophageous fungi

There are around 150 species of nematophageous (nematode eating) fungi described. These fungi are common in most soils and belong to many taxonomic groups and nematode feeding by fungi is an example of convergent evolution. Many ways of catching and destroying the nematodes have developed. Some fungi make adhesive three dimensional nets (Fig 5.12). The three dimensional net is covered with an adhesive. When a nematode come in contact with the adhesive

the nematode sticks irreversibly. The fungus then produce a penetration hyphae that enters into the living nematode. The nematode is soon after killed and consumed by the fungus. There are other structures made to catch nematodes like adhesive knobs (Fig 5,13A&B). The adhesive knobs sit on thin stalks (Fig 5.13A). When a nematode come in contact the knob sticks irreversible to the nematode surface. It sticks so that if the mnematode wiggles it may tear the knob off the stalk and swim away. This does not however help the doomed nematode. The stuck knob has enough resources so that it can penetrate the nematode and infect it. Thus the nematode can by swimming away help to spread the fungus. Some nematophagous fungi have specialised so much in growing in nematodes that they cannot grow very well in the environment. They infect by their spores and only form vegetative mycelia inside nematodes (they are often referred to as endoparasitic) (Fig 5.14).

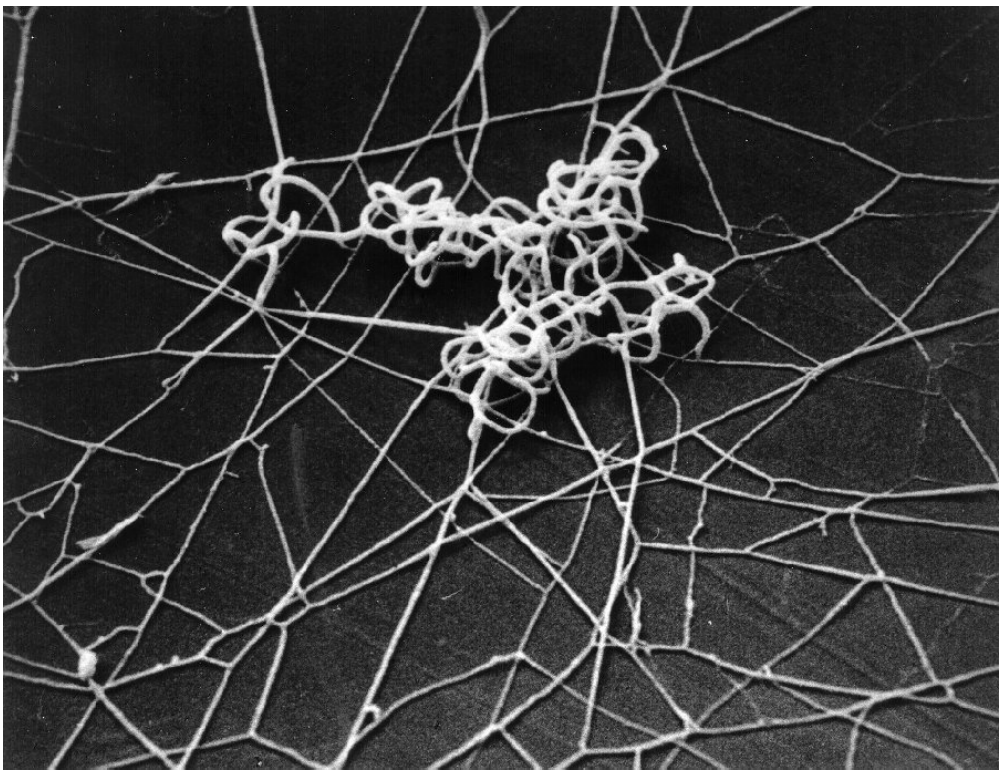


Fig 5.12 Adhesive three-dimensional traps produced by the nematophagous fungus *Arthrobotrus oligospora*.

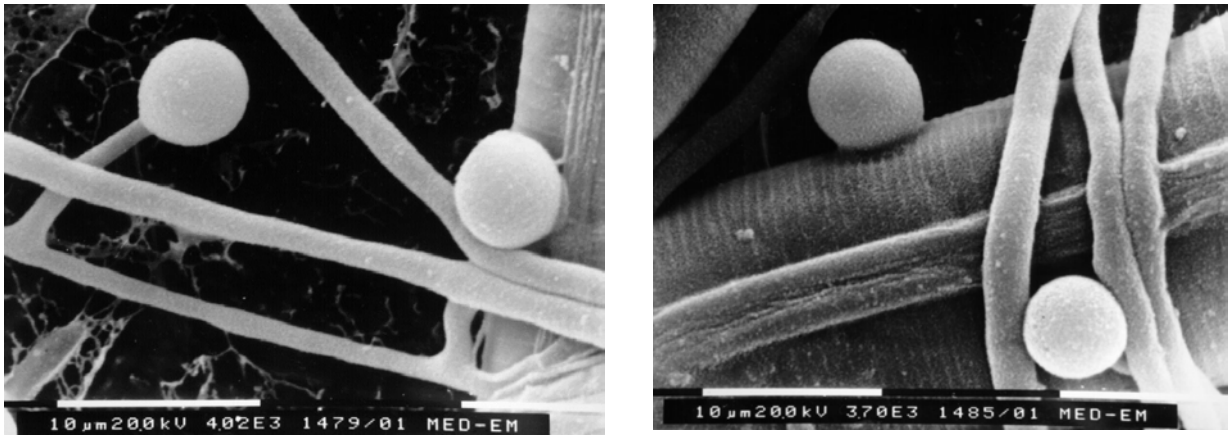


Fig 5.13. Nematophagous fungus producing traps as adhesive knobs on thin stalks (left). The knob might be detached from the stalk but will still adhere to the nematode and be infective (right). The large “stripy” structure in the image to the right is a nematode.

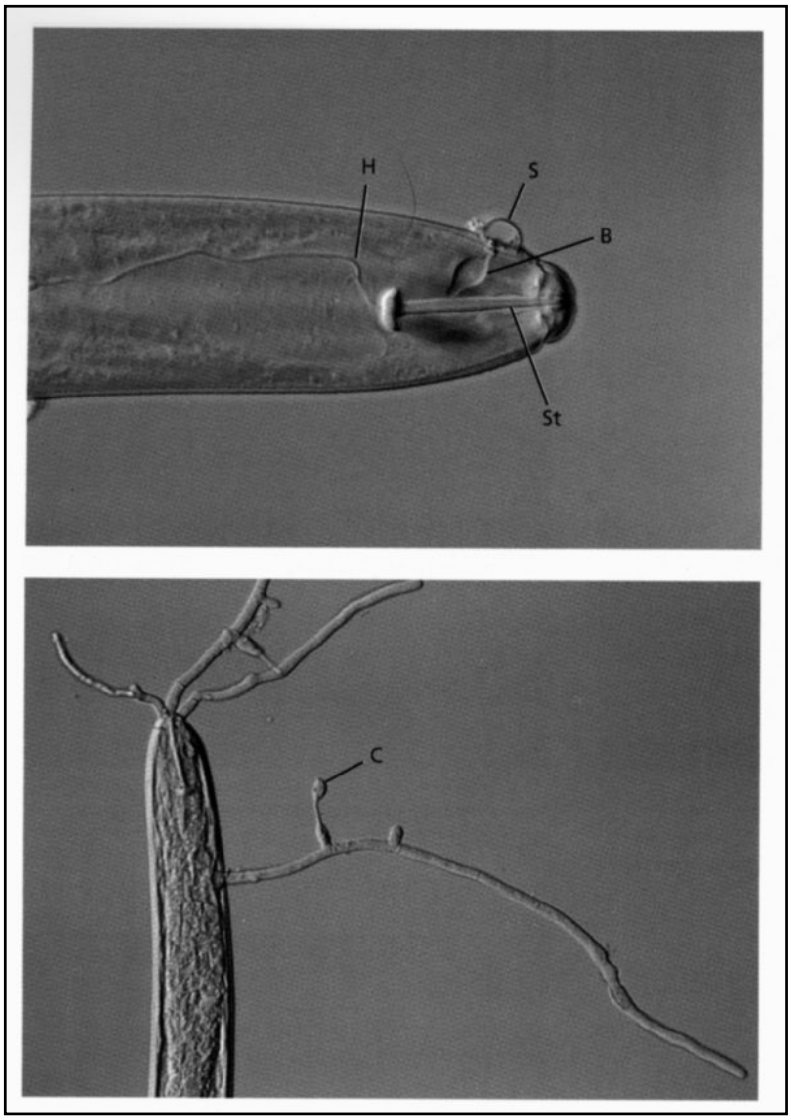


Fig 5.14. Endoparasitic nematophagous fungus attacking a plantparasitic nematode. Top: Head region of a plant parasitic nematode with a germinating fungal spore (s) showing an infection bulb (B) with an emerging hyphae (H). Observe the stylus of the plant parasitic nematode. Bottom: When the nematode has been consumed conidia forming hyphae grow out producing new infectious (sticky) conidia (c).

6.Mycorrhiza

What is mycorrhiza

Plants and fungi often co-operates. The most well known example of such co-operation is the lichens where an algae and a fungus cooperates and build up the lichen body. Most land plants are also dependent on co-operation with plants. The cooperation takes place between the plant roots and the fungus growing out into the soil. In most cases the symbiosis is mutualistic in that both partners gain on the relationship but it is not necessary (as with the orchid mycorrhiza.

Types of mycorrhiza

There are several types of mycorrhiza. The three most important types are the arbuscular mycorrhizas (AM) of herbaceous plants and some tropical trees, the ectotrophic mycorrhiza of forest trees and the endotrophic mycorrhiza of orchids. This division is based on the morphological characteristics of the mycorrhiza (fungal-filled root) (Fig 6.1).

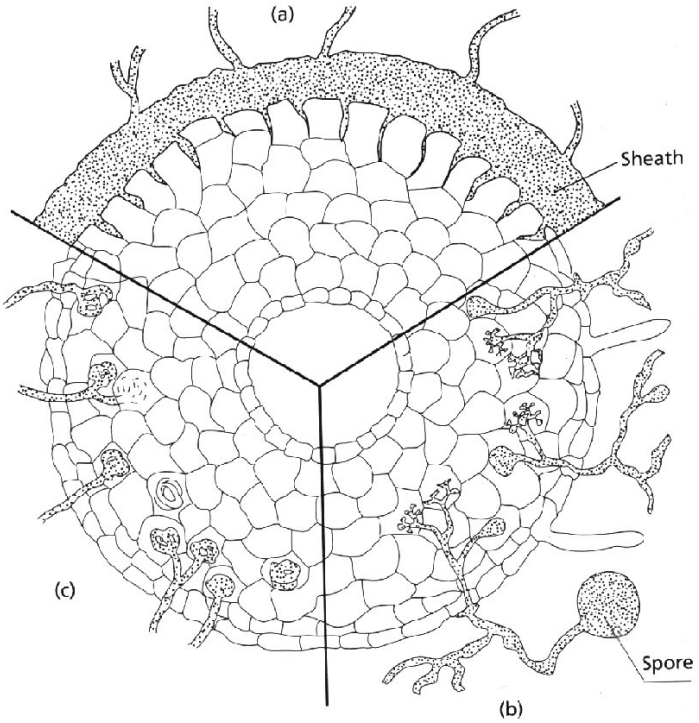


Fig 6.1. Diagrammatic representation of three types of mycorrhiza in transvers section of a root. a. Ectotrophic mycorrhiza of forest trees, showing a sheet of fungal tissue and limited invasion of the root by intracellular hyphae (the Hartig net). b. Arbuscular mycorrhiza of many herbaceous plants and tropical trees, showing arbuscules and vesicles in the cost cells. Large spores up to 400 um are formed on the hyphae that ramify in soil. c. Endotrophic mycorrhiza of an orchid, showing hyphal coils in the “host” cells.

Arbuscular mycorrhiza (AM)

Already the first land plants were equipped with this type of mycorrhiza and thus this association is at least 400 million years old. It appears as if AM mycorrhiza could have been a requisite for the colonisation of land by plants. Both plant and fungus gain on this co-operation. The fungus belongs to *Zygomycota*. It is biotrophic and thus cannot be grown without the plant. This is a great obstacle when studying the fungus since it cannot be grown on its own on sterile media. The closest to a controlled growth system that has so far been achieved is to grow the fungus on roots in excised root cultures. Spores of AM fungi are very large and spores of some species can be seen by the naked eye. The spores can survive for many years in soil and when they germinate they can support rather extensive hyphal growth. The host specificity is low and thus a AM species can cooperate with several species of plants. This is important since the fungus cannot grow without a plant root and thus have to be able to grow through soil to get in contact

and establish itself in a root. Most families of flowering plants have AM and are dependent on the association in nature. Exceptions are families of typical primary colonizers of bare soil *Juncaceae, Cyperaceae, Cruciferaceae, Chenopodiaceae, Caryophyllaceae* (many are weeds).

AM in the root

The hyphae of AM grow into the root and also penetrate the root cells. Inside the root cells the hyphae branch to form an arbuscule (a tree like structure) that works as an organ for nutrient exchange (Fig 6.2, left). Sometimes one can see lipid rich storage vesicles in the root as well (Fig 6.2, right). In the slightly older literature the AM mycorrhiza were called vesicular arbuscular mycorrhiza (VAM) but this name was dropped since not all AM form vesicles.

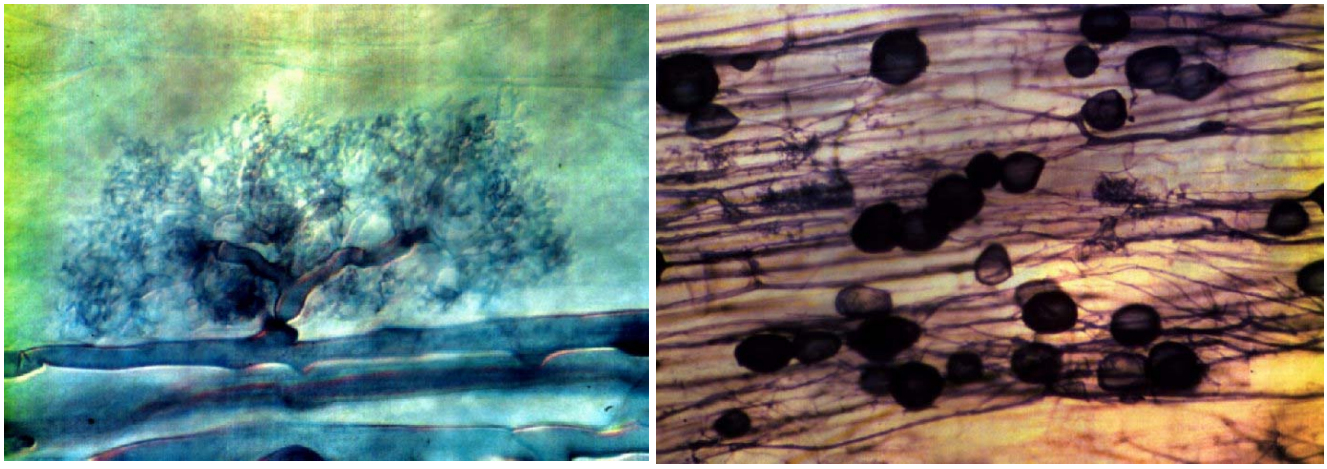


Fig 6.2. AM hyphae entering cells forms tree like highly branched absorptive structures called arbuscules inside the root cortex cells (left). Storage structures, vesicles, can also be formed inside the root cells by some AM fungi (right).

Ectotrophic mycorrhiza (EM)

Trees in temperate and boreal areas have ectotrophic mycorrhiza. The fungi forming these mycorrhiza belongs to the basidiomycota Many of these fungi are the ones that produce edible fruitbodies in forests. The mycelia cover large areas and might connect many trees even of different plant species (Fig 6.3). The specificity for different trees are not very high for many of the fungi but there are those fungi only able to form mycorrhiza with one tree species.

EM in the root

The fungi interacts with the short roots of the trees. The roots becomes stunted and short often coralloid. A thick sheath of interweaved hyphae, the mantle,

surrounds the stunted root tips. From the mantle hyphae grow into the root cortex between the root cells forming the so called Hartig net.

Fig 6.3. Plant-mycorrhiza growing on thin peat layers showing the extensive EM mycelium extending out in the soil from the stunted plant roots. The hyphae closest to the root tip forms hyphal strands that connect the roots with the expanding mycelial front. The network can as in this experiment connect two plants with each other. The metal cup in the picture was used to give the system radioactive phosphorus. This phosphorus could then be detected throughout the fungal system including both plants.

Orchid mycorrhiza

Orchids have a special relationship with fungi. These plants have also mychorrhiza although the symbiosis is not mutualistic. It is the plant that parasitizes the fungal partner. There are even orchids without chlorophyll that are entirely dependent on the fungus for the supply of both carbon/energy and minerals. The fungi involved are species of *Rhizoctonia* (basidiomycota) and even the plant parasitic

Rhizoctonia solani have been shown to form mycorrhiza with orchids under experimental conditions. Since the relationship between the plant and the fungus is not mutualistic this type of mycorrhiza is not considered further.

Benefits of the symbiosis

AM and EM gives both partners in the symbiosis benefits compared to growth on their own.

Benefits to the fungus

- The fungus gets carbon/energy from the plant. Since the supply of carbon/energy is large and not from the soil the fungus can make an extensive mycelium and effectively compete with other fungi confined to the soil and the energy/carbon in the soil.
- Often the plant supplies the fungus with other metabolites and vitamins.

For AM both these points are compulsory since the AM fungi are obligate biotrophs.

Benefits to the plants

- The fungi forms the effective root surface for the plants giving then a very large surface are for nutrient uptake. The nutrients of most importance are nitrogen and phosphorus. Phosphorus is boud very hard in the soil and does not diffuse easily. The organisms nee a very large surface area and the hyphae grow into contact with the nutrients more than the nutrients diffuse to the hyphae. The uptake of both nitrogen and phosphorus can come from sources unavailable for plant roots (see shortcutting nutrient cycles below). The fungi might use their capacities for producing extracellular enzymes and acids to degrade organic material and dissolve minerals. There are also indications that the fungus can obtain nutrients by attacking saprotrophic soil organism.
- Plants having mycorrhiza are often more resistant to drying conditions. There seems to be a better availability of water to the plant. The larger surface area of the fungus and the hyphal ability to penetrate into very small capillaries probably causes this.
- Plant roots gets a protection against pathogens and toxins. When it comes to EM this protection could be simply physical. The plant is not available to the pathogen since the normally rather vulnerable root tips are behind the fungal mantle. AM plants as well as EM plants both show better resistance against even foliar pathogens. This could of cause be caused by the better nutrient status of mycorrhizal plants but the plant seem to be on more “alert” and better defends itself against pathogenic fungi.
- AM have been shown to negatively affect neighbouring non-mycorrhizal plants. Thus the fungus appears to act like a pathogen against non-

mycorrhizal plants and aiding its plant symbiont in the competition for space and nutrients (Fig 6.4).

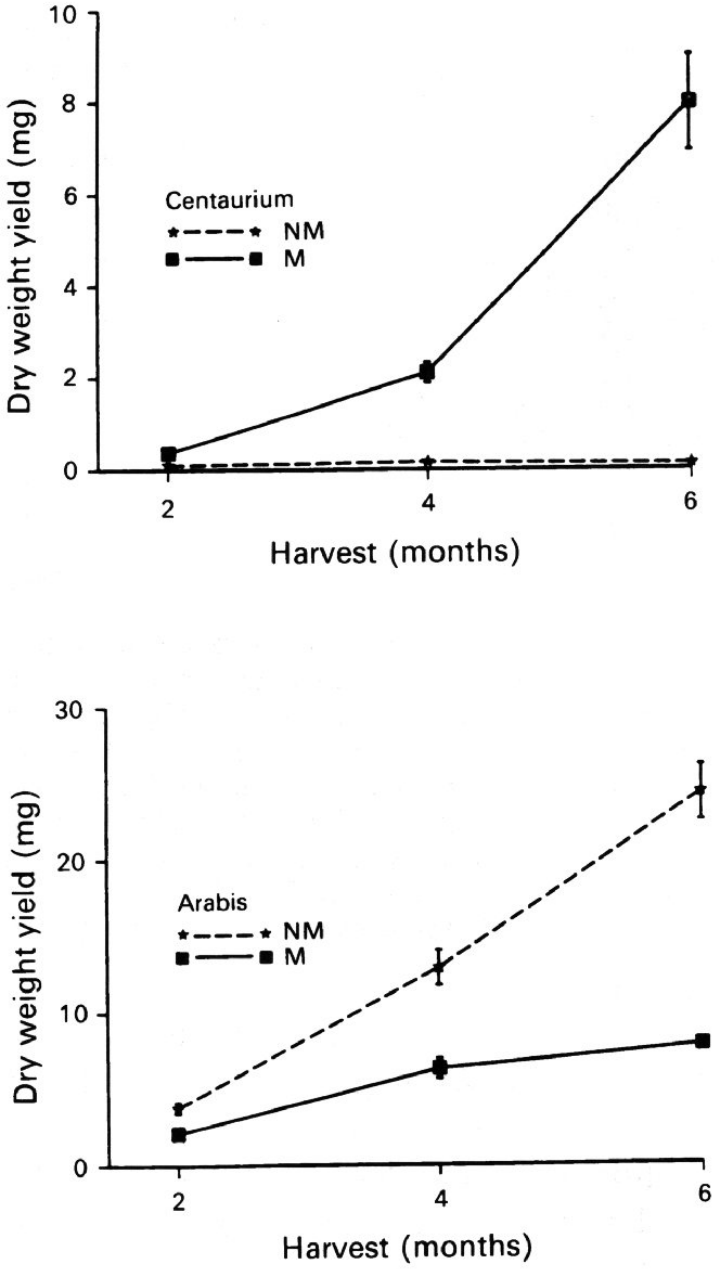


Fig 6.4. If a mycorrhizal plant is planted adjacent to another mycorrhizal plant the usual positive response to the mycorrhiza is seen as a dry weight increase (top solid line). If on the other hand a nylon mesh hinders the fungus from connecting to the plant no such response is detected (top dashed line). If on the other hand a

non-mycorrhizal plant is planted adjacent to a mycorrhizal plant it grows better when the nylon net is present (bottom dashed line) than when the mycorrhizal fungi can contact the roots (bottom solid line)

Use of complex nutrients and the "shortcutting" of nutrient cycles

AM and especially EM gives the plant access to non-soluble complex nutrients in the soil. Traditionally when considering nutrient cycling we think of a primary production by a plant, followed by the death of plant, mineralisation of the plants by microorganism activities, release of nutrients to the soil solution and then uptake of these soluble nutrients by plants (Fig 6.5). There is also a small amount of nutrients liberated from insoluble pools in the soil by weathering. Mychorrizas introduce shortcuts in these cycles by making it possible for the plant-fungal system to get nutrients directly from dead plant material by actively decomposing it.

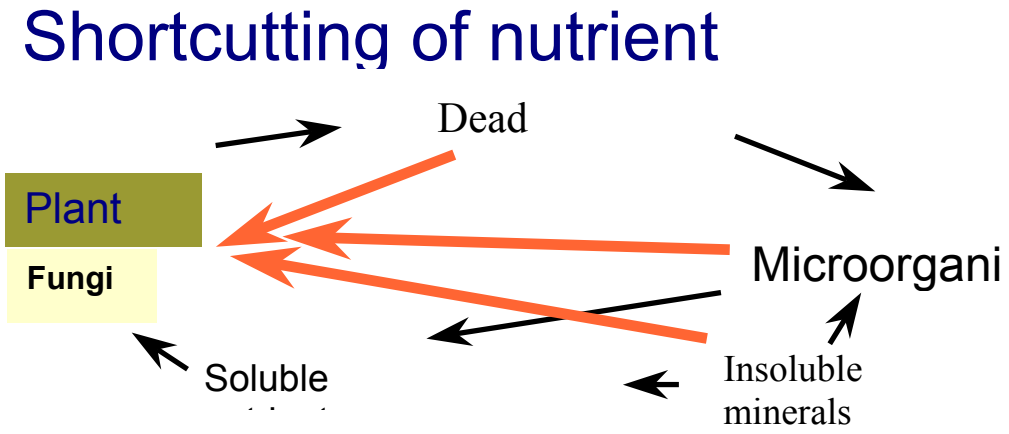


Fig 6.5. Black arrows shows the "normal" cycling of nutrients and orange arrows how the AM fungus shortcuts the "normal" cycle to the benefit of the plant-fungal system.

The mycorrhizal symbiont could also directly attack other soil organisms and use their nutrients (Fig 6.6).

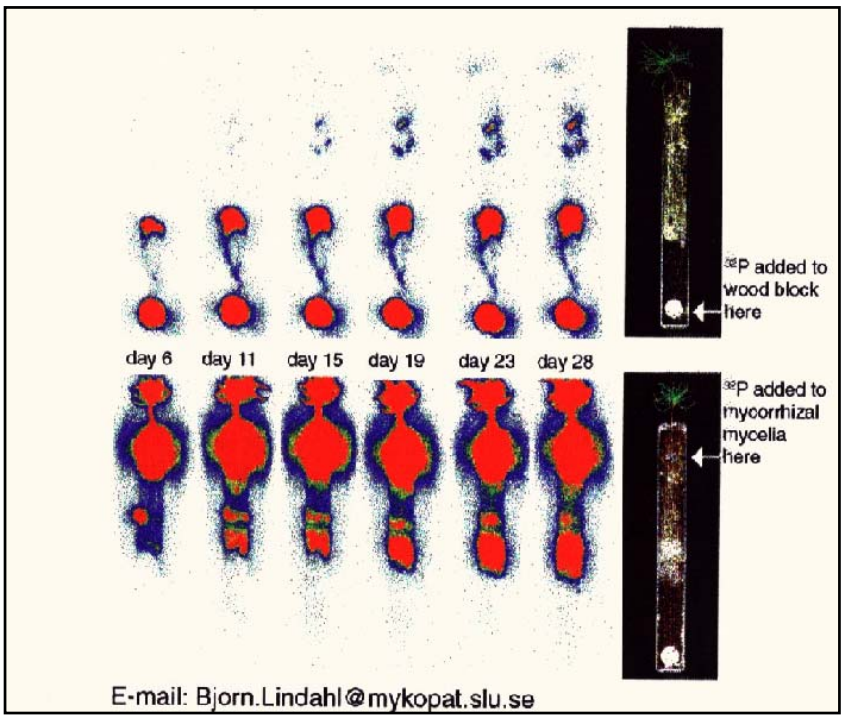


Fig 6.6. Demonstration that a mycorrizal fungus can “steal” mineral nutrients from a wood decomposing fungus in soil. Two fungi were growing in the system. A mycorrhizal fungus growing out into the soil from the small pine seedling meeting a wood decomposing fungus expanding out into the soil from a wood block. In the top series labelled phosphorus was added to the wood block. It can then be seen that the phosphorus quickly enter the wood decomposing fungus and gets translocated to the hyphal front were it meets the mycorrhizal fungus. The mycorrhizal fungus can then take the nutrients from the wood decomposing fungus and translocate it up to the plant. If on the other hand the label is given to the mycorrhizal fungus (below) the nutrients is spread within the mycorrhizal mycelium and does not pass over to the wood decomposer.

EM fungi can also get hold of nutrients from rock mineral by active weathering of rock minerals. The fungus might even do this from rather inert rock materials like granite where it has been shown that mycorrhizal fungi can etch tunnels through the stone mineral (Fig 6.7 and 6.8).

Fig 6.7. Mycorrhizal mycelium growing on granite rock surface.

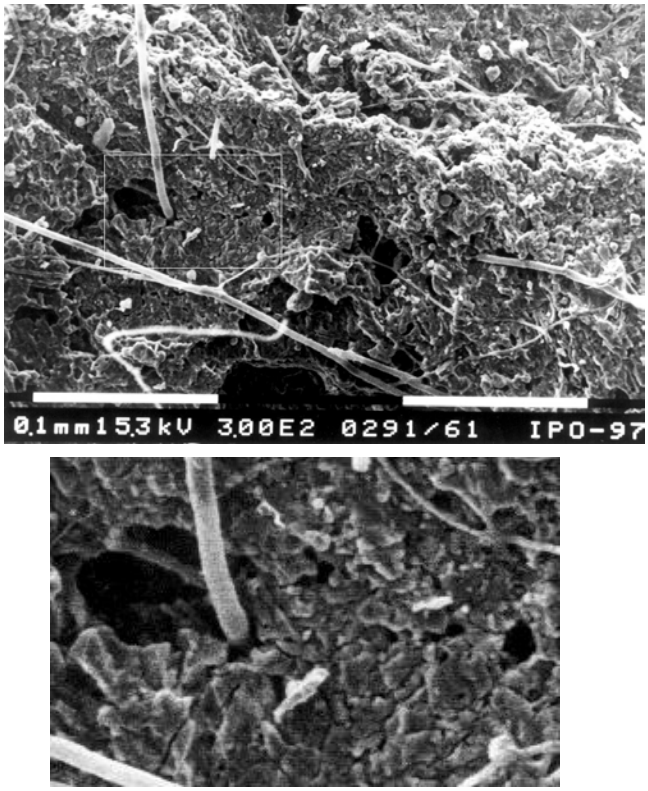


Fig 6.8. Mycorrhizal hyphae etching tunnels in granite. Top image showing many hyphae growing ofver the surface and bottom image a close up of the hyphae penetrating into the rock.

Other consequences of mycorrhizal symbiosis

As stated before a mycorrhizal mycelium might connect several plants of the same species but also plants of different species Fig (6.9). It has been shown that this also happens in nature and that all kinds of nutrients can flow between the plants including carbon fixed by photosynthesis. If a plant is linked to other plants by a common mycorrhiza it has been experimentally shown that carbon can flow from a plant in the sun to a shaded plant. These experiments have only been done by radioactive pulse-labelling and it is still debated if this flow is of any significance to the plant systems. There is thus a possibility that plant ecosystems are much more dependent on the below-ground hyphal network than we have traditionally thought.

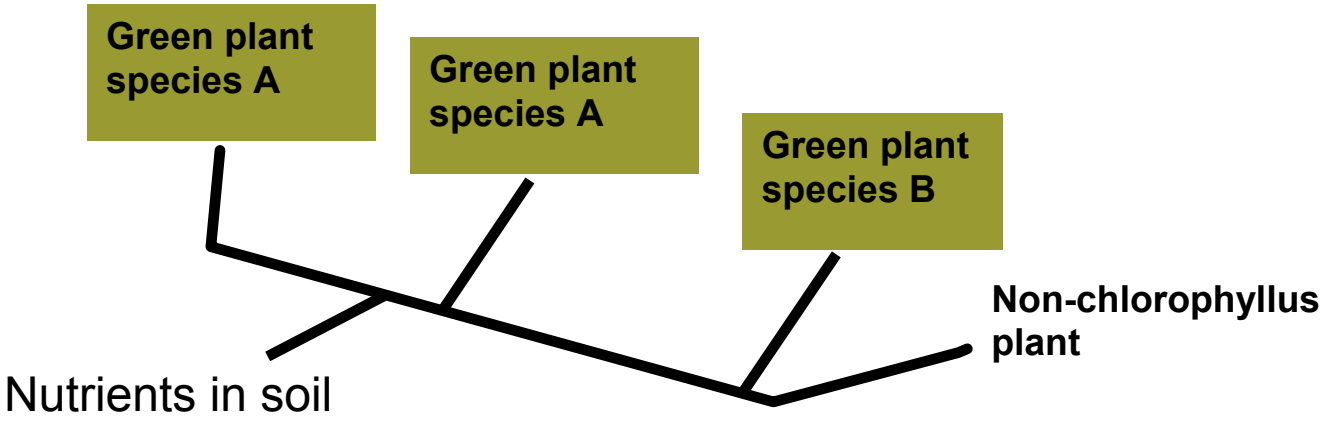


Fig 6.9. Schematic drawing of a hyphal network connecting different plant species, including a non-photosynthetic plant totally dependent receiving nutrients from the fungus.

Final words

"...under agricultural field conditions, plants do not, strictly speaking, have roots, they have mycorrhizas"
Comment by the plant pathologist Stephen Wilhelm

This statement applies to most plants under most natural conditions and not just agricultural plants.