MASARYK UNIVERSITY, FACULTY OF SCIENCE

DEPARTMENT OF BOTANY AND ZOOLOGY

FUNGAL ECOLOGY

(sometimes with special regard to macromycetes)

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

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

INFECTION BY PHYTOPATHOGENIC FUNGI

Fungal **parasites of plants** belong to many different groups.

Infestation of plants by a parasite (pathogen) causes changes in the development, growth or physiological processes of the host and can lead to death of tissues or the whole organism; externally visible signs of infection are called **symptoms**.

Examples of symptoms caused by some fungi:

• **rot** is a common manifestation of the action of wood-decaying fungi, but also herbs can be rotten by fungi; in principle, it is a breakdown of the host tissue caused by the pathogen enzymes;

• **resin outflow** may be an accompanying symptom of infestation by wood-decaying fungus (sometimes the only one visible symptom, if the rot is hidden);

• **wilting** occurs if water loss is greater than intake of the plant – if the pathogen disrupts the root system, or if the conduction of water from the roots to the tops of the shoot is disturbed (e.g. dieback of treetops in the case of tracheomycosis);

• combination of the two previous cases can lead to **damping-off** (sudden death of seedlings, caused e.g. by *Pythium*, *Phytophthora* /*Oomycota*/, *Fusarium* or *Rhizoctonia* – we can see that species of this genus, mentioned in mycorrhizal symbioses, can also be plant parasites);

• uncontrolled growth or differentiation of the tissue (cell hypertrophy or hyperplasia) leads to formation of **witches' brooms**, **galls** or „cancerous" **tumors** (burrs/burls) – the causative agents are parasites which have the ability to affect hormonal balance in the plant;

Witches' brooms of *Taphrina betulina* in the canopy of *Betula pubescens*

http://cs.wikipedia.org/wiki/Soubor:Betula_pubescens_Taphrina.jpg

• **conidiomata** in the plant tissue, which open in various ways (sporodochia, acervuli, pycnidia), are formed by many imperfect fungi;

• **colour changes** can have a number of causes, infestation (not only by fungi, e.g. so-called mosaics are of viral origin) and physiological changes; it is important for plants, whether the photosynthesis is reduced due to degradation of chlorophyll or reduction of its formation (or leaf fall);

• some groups of fungi (especially obligate biotrophic parasites) are characterised by specific symptoms, often denoted by the common name of the group (rust, smut, powdery mildew).

The elimination effects of parasites can be of different levels – according to this, the parasites can be divided into several groups:

- „killers“ cause wilting or death of the whole plant (seedling or adult plant);
- „debiliators“ cause lesions or chronic infections;
- „castrators“ affect flowering and fruit production, but vegetative growth is affected only minimally.

The effect very much depends on the particular species of plant and parasite and on the conditions in which the plant grows (from optimal through suboptimal to highly stressful).
Parasitic effects on particular plants can also affect the surrounding environment and the organisms living there, for example in litter (production of phenolic substances in defence) or populations of mycorrhizal fungi (when reducing photosynthesis, the plant produces fewer exudates).

Let us describe the **infection process** in detail – course of the infestation is influenced by traits of the pathogen, the host and external environmental factors; if any of this prevents a successful attack, the infection will not occur..

Pathogen factors:
virulence, amount of infectious particles, their effectiveness and ability to survive.

Host factors:
health status (necrotrophs tend to attack debilitated individuals, while biotrophs need a healthy host),
susceptibility to the pathogen, developmental stage (some pathogens infect adult plants, some others infect seedlings),
population structure (in general, the extent of infection is directly proportional to the density and inversely to the distance of individuals of the infested species, monocultures are ideal stands for extensive infection).

Environmental factors:
temperature (higher temperature usually shortens the incubation time and the time from the infection outbreak to sporulation of the pathogen),
moisture (important especially for organisms whose spores germinate in the water, thus they need plants "wet" from rain or dew),
light, aeration, pH and nutrient availability (for fungi spreading in soil),
wind (in case of air propagation),
environmental pollution (acts indirectly, weakening plants).

The initial contact of the parasite with the host is referred to as **inoculation**; chemical signals are often applied – compounds excreted from the stomata, or exudates on the surface of leaves or roots.

The infection begins by spore germination on the surface of the host body; the germination is also often stimulated by substances in fluid drops on the plant surface (so-called infectious drop, substances excreted by the plant, e.g. carbohydrates, amino acids, vitamins, salts, minerals) – spores of some species germinate only if these substances are available, they would not germinate in clean water. The so-called **infection density** is important – the minimum amount of germinating spores needed for a successful infection.

Fungi enter the host tissues from different environments (they can also spread through water, by overwintering in seeds or through vectors – most often insects with a piercing or sucking mouthparts, penetrating plant tissues), but there are two basic ways of infection – from soil and air.

Airborne infection can only come in the form of spores on the aboveground parts of plants – the „life success“ of such parasites is fully dependent on the effectiveness of spore release and spread and subsequent reinfection, especially in host-specific species. For example, these fungi include rusts, smuts, powdery or downy mildews.

Some pathogens actively penetrate cells and tissues (formation of appressoria and haustoria), others use natural openings (stomata, hydatodes), places without compact epidermis (stigma) or open wounds.

Direct penetration into cells is exerted by fungi capable of overcoming cell walls by pressure and enzymatic equipment. For successful infection, the parasite must overcome the superficial layer – the epidermis. Fungal parasites have cellulases, unlike pathogenic bacteria and viruses. Some fungal species secrete a sticky substance of a proteinaceous nature, with which their spores or appressoria can „stick“ to the epidermis.

On the surface of the epidermis, a cuticle formed by fatty substances (cutin is a polymer of fatty acids) is a waterproof and water-repellent layer – this is not usual way for „intruders“ (although some fungi are able to overcome the cuticle, e.g. *Botrytis*).

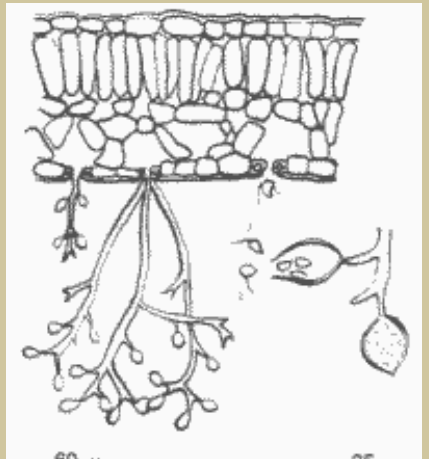
It is easier to enter the leaves through the stomata; here, however, the fungus must both be able to locate the stoma and to „recognise“ when it is open => a form of chemotaxis is applied, a reaction to the oxygen released through the stomata during photosynthesis. Similarly, „susceptible“ sites on the leaf surface may be lenticels or hydatodes (pores for gas exchange or water excretion, i.e. guttation).

In the phyllosphere (leaf surface) the pH is usually 7–8 and parasitic fungi are adapted to this, while spores (usually conidia) of saprotrophic species do not germinate here; if the pH in the phylosphere drops below 6, chances of the parasitic fungi sharply decrease. Rain can help to decrease the pH, while on the contrary, a solid fall or a higher intensity of plant assimilation can contribute to increasing the pH.

The solid „barrier“ is the tree bark – if it is not disturbed, it provides reliable protection, as most saproparasitic species are not able to overcome the intact bark *(see later)*.

Unprotected areas of injury are a good „gateway“ to infection; on the other hand, in the case of natural phenomena such as leaf or fruit fall, the plant „has to treat“ it by creating a separating tissue.

Stimulation of spore germination can be chemical (spore germination of *Bremia lactucae* stimulated by germination of lettuce seeds) or it can depend on environmental factors (oidia of powdery mildews germinate better in dry conditions; on the contrary, high water content in air stimulates release of oomycete zoospores – generally, here it depends on water availability, whether a hypha germinates from the sporangium or zoospores are released) or does not depend on them (zoospores of *Pseudoperonospora humuli* sought open stomata for their encystation, even though they were offered a „dummy“ where chemotaxis was out of the question). Chemotactic recognition of the host is genetically encoded in specialised parasites; the same can be true, when the plant detects a parasite attack and triggers defence mechanisms.



http://www.eickelmann.de/Seiten/Hopfen/Wissen/PfSchutz/SchadbildFM.html

After germination from the spores, fungi form special adaptations to penetrate the host tissue and cells: the fungus first forms a surface appressorium with an adhesive substance on its surface => then it forms (according to abilities of particular species) cutinases, cellulases, pectinases and proteases to penetrate the cuticle and cell wall => at the same time (or alternatively) a penetrating hypha can grow from the appressorium – a thinned protrusion, which is pushed by the pathogen's cell into the host cell by an increased turgor (*see in General Mycology, chapter Vegetative thallus*). Thus, penetration into the cells is realised by mechanical pressure (in some fungi up to 0.5 MPa = 5 atmospheres) or by enzymatic disruption of the cell wall. *Course of the infection see later (at obligate parasites).*

In contrast, parasites **infecting plants from the soil** have an easier task – the underground parts of plants are in permanent contact with soil full of various microorganisms, usually with a more or less stable supply of water and nutrients. Moreover, it is sufficient for these fungi to grow as hyphae, they do not need to undergo the spore germination process for infection; in contrast to „air travellers“ they have a limited possibility of movement and spread over a greater distance.

Examples are e.g. wood-decaying basidiomycetes (*Heterobasidion, Armillaria*) or simpler organisms spreading in the form of zoospores or planozygotes (*Plasmodiophora, Synchytrium*).

Roots do not have a hydrophobic layer on the surface, they are usually covered with mucus from exudates <= the fungi apply chemotropism, attraction of zoospores (*Phytophthora*) or directed growth of the germ tube.

Exudates can also stimulate the formation of infectious structures (rhizomorphs of *Armillaria*) or directly stimulate germination even after long-term dormancy (sclerotia of *Sclerotium cepivorum* react specifically to exudates from the roots of *Allium* species – this kind of „compatibility“ of the fungus with the host is genetically determined <= a small genetic change can cause an inability to attack the host or development of a resistant host race). It might seem that „more exudates => higher chance of infection“, but ... the release of nutrients also promotes the growth of populations of saprotrophs in the rhizosphere, which in turn makes infection more difficult for parasites; moreover, saprotrophs tend to be stronger both in competition for nutrients and in the production of antibiotics.

The most easily attacked are the thin-walled cells of the growing root (root caps) or root hairs – on the other hand, older sections of the roots are later covered with a crust of dead cells, through which the individual hyphae have no chance to pass (only a few fungi can do this, e.g. Honey fungi via rhizomorphs).

In *Gaeumannomyces*, „runner hyphae“ are formed – these are hyphae that grow ectotrophically over the root surface, branch, anastomose (in the extreme they form a mycelial „cover“) and subsequently thin penetrating hyphae grow out of them (up to one hypha to each cell).

The specialty of *Plasmodiophoromycetes* is penetration of the cell wall by „spiny“ protrusion of the cell (sessile zoospore).

Compared to the phyllosphere, pH In the rhizosphere is commonly acidic – calcification is a practical defence against fungi adapted to a lower pH.

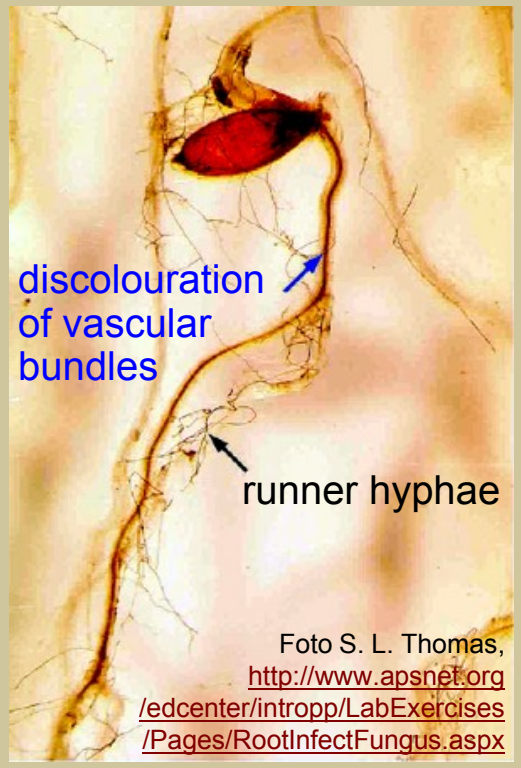
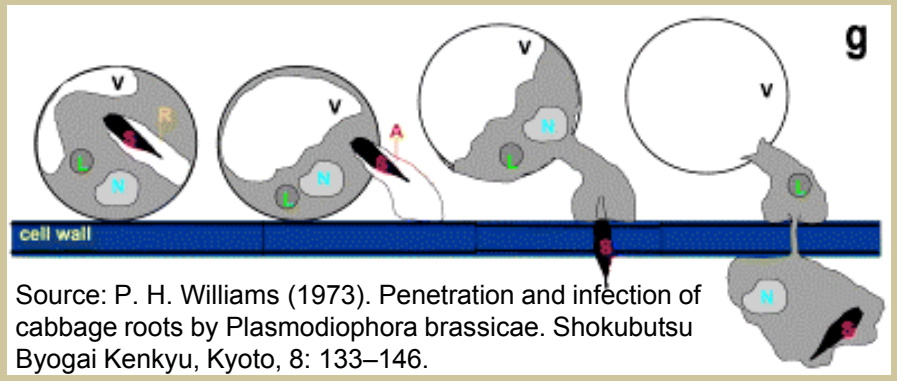


Foto S. L. Thomas, http://www.apsnet.org/edcenter/intropp/LabExercises/Pages/RootInfectFungus.aspx



Source: P. H. Williams (1973). Penetration and infection of cabbage roots by Plasmodiophora brassicae. Shokubutsu Byogai Kenkyu, Kyoto, 8: 133–146.

Top: „runner hyphae“ of *Gaeumannomyces graminis* infecting small roots of *Agrostis*.
Bottom: sessile zoospore of *Polymyxa graminis* (*Plasmodiophoromycetes*) penetrating the host cell wall; **A** – appressorium, **S** – „spine“, **N** – nucleus, **L** – lipid droplet, **V** – vacuole.

The transfer of a parasite from one host plant to another and the subsequent infection is referred to as **chain of infection** – it can be continuous or interrupted.

A continuous chain is common, for example, in the tropics, where the parasite has the opportunity to infect new individuals throughout the year – either directly or through vectors (typically insects) or using alternative hosts (usually related species that can also be infected by the parasite; if the infection does not break out in them, they also actually act as vectors, albeit plant-based).

An interrupted chain, on the other hand, is a common phenomenon in areas where favourable and unfavourable seasons (warm/cold, wet/dry) alternate and the growing season of the host plants is limited to only part of the year – naturally, their pathogens have to adapt.

Fungal parasites use different **survival options** – facultative parasites can live saprotrophically, obligate parasites usually form resting stages: oospores, teliospores, chlamydospores, sclerotia, some ascomycetes also form fruitbodies with ascospores (e.g. *Erysiphales*).

A clever way of survival is so-called latent infection – e.g. in *Ustilaginales*, a seed is infected, but the fungus remains in a dormant phase and its growth is activated only with seed germination => then hyphae grow through the developing plant.

The parasite propagules which „start“ the infection in the new growing season are referred to as the **primary inoculum**, while the **secondary inoculum** are the infectious particles which spread the infection further through the season.

Distribution of infected plants in the population can tell a lot about source of the infection and nature of its transmission – it is rarely completely uniform (except when the entire batch of seeds is affected in cultivated crops) or completely random (here it is probably an infection of a few individuals of the fungus which randomly attacked a few individuals in the stand and there is no further spread).

Clusters of infected plants or certain places with a concentration of infection, spreading from the primary inoculum, are typical for pathogens spreading in soil (not only fungi, but also nematodes) or through slow vectors (e.g. aphids).

If a gradient is evident, source of the infection is apparently outside the affected stand; this case most likely indicates the vectors capable to quickly pass longer distances (flying insects), and the gradient steepness is usually directly correlated with proximity of the source of infection.

Affection of barley culture in a limited area, typical of soil pathogens – in this case there was a parasitic affect of *Rhizoctonia* sp. (as can be seen, the well-known mycorrhizal fungi can be both a „good servant“ and a „bad master“...).

http://www.agric.wa.gov.au/ikmp/images/rhizocto_webpic.jpg

DEFENCE MECHANISMS OF PLANTS

The parasite penetrates the cell wall either using extracellular enzymes or mechanically (osmotic pressure in the cells reaches up to 5 MPa in some cases), a combination of both is certainly possible.

Most often, the „attack“ is iniciated by production of enzymes which degrade components of the cell wall; there is a „succession“ – pectinolytic enzymes are followed by hydrolases, cellulases, etc. However, plants are not defenceless, they can produce **enzymes** which degrade the fungal cell wall (chitinases, glucanases); both plants and fungi can also produce inhibitors of the „enemy“ enzymes.

The most effective mechanism of plants is storing **proteins** in their cell walls, which inhibit the activity of polygalactorunases (pectinolytic enzymes), affecting them selectively (they do not affect other enzymes) but non-specifically (they inhibit polygalactorunases of different fungi – hence they do not need many different inhibitors to be resistant to different fungi).

Host and pathogen can also „fight“ each other by producition of **toxins**:
– in plants, these are so-called **phytoanticipins** and **phytoalexins**
(the difference is that phytoanticipins are substances present in plants at all times, while the production of phytoalexins is a response to action of the pathogen; to make it not so simple, substances that are phytoanticipins in one species can be phytoalexins in another species);
– only necrotrophic fungi, of course, produce toxins, for example fusaric acid (pathogenic *Fusarium* species belong to strongly „callous“ necrotrophs).

Plants are often subjected to attempts at attack by various pathogens, but only a few attempts are successful. Its **immunity (resistance)** plays a role in whether the plant resists the attack of the parasite; a resistant host is able to either completely withstand the attack or slow down the development and reproduction of the parasite (or its infectious particles).

Immunity can be manifested in different stages from preventing the germination of diaspores to preventing the further spread and reproduction of an already „settled“ fungus; there are usually several partial reactions (whose effects can be „merged" into a collective response) at the molecular, cellular or tissue level. Just as pathogenic fungi have genetically determined „abilities“ (genes encoding the production of enzymes or phytoalexin inhibitors), plants have genetically determined immune mechanisms (genes encoding the production of proteins ensuring the detection of elicitors and the triggering of a defence reaction). Immunity can be distinguished:

– **non-specific** – a wider scope, often against different parasites, but on the other hand, it can be more easily overcome by toxins or suppressors of specific parasite;

– **specific** – it is evolved in a specific host species against attack by a specific parasite (possibly only in a certain cultivar or against a certain race of the parasite or both at once, depending on the genetic dispositions of both); this type is usually developed beyond the non-specific immunity, and specific elicitors and selective enzymes and suppressors (inhibitors) are applied.

Druhou možností je rozlišení imunity přirozené a získané.

Natural immunity is given genetically, it was evolved in the course of evolution; we distinguish two types:

• **active immunity** means the ability to defend against an invading infection:

– action of the aforementioned enzymes, proteins or toxins;

– formation of protective necroses;

• **passive immunity** means factors which do not allow the pathogen to penetrate the tissue:

– anatomical-morphological (also referred to as physical): firm cuticle, wax coating, strong cell walls to thick tissue (bark); vertical orientation of the leaves (on which the water runs off quickly) also reduces the success rate of pathogens with motile stages (zoospores);

– chemical: composition of cell-juice, production of substances preventing spread of the pathogen – already mentioned phytoanticipins (e.g. saponins, glycosides, terpenoids, tannins) are usually enclosed in vacuoles in an inactive form and are activated by the action of alien enzymes;

– functional and physiological: movement of stomata, rate of wood growth or wound healing – it is important against wood-decaying fungi.

Acquired immunity is a state that arises after infection as a result of ongoing responses (here we adopt a broader sense of the term, including both immediate responses, which can directly stop the parasite, and long-term changes, which lead to permanent resistance to it). This immunity can be induced artificially by vaccination (use of avirulent strains of pathogenic species is optimal), delivery of certain substances – this is called plant immunisation.

Different types of reactions, which can be covered by the term acquired immunity:

• **change of permeability of the plasmalemma** (transfer of K^+, H^+, Ca^{2+} ions) and production of reactive compounds (H_2O_2), capable of directly killing some microorganisms;

• **hypersensitivity** – rapid death of cells at the site of attack and the surroundings („scorched earth tactics“) is effective against biotrophs (source of their nutrition at the site of attack is eliminated), but ineffective (if not accompanied by another „immune reaction“) against necrotrophs, possibly non-pathogenic fungi – therefore this type of response rarely occurs non-specifically, in the vast majority of cases it is a specific response to a particular pathogen;

Hypersensitive reaction in tobacco leaf.

Photo Kent Loeffler, http://www.plantpath.cornell.edu/PhotoLab/Stills/TobaccoLeaf.htm

• **production of phytoalexins** (see above), in principle these are low-molecular antibiotics capable of both directly killing fungi or bacteria and ensuring subsequent resistance against possible further infection by the same pathogen. Over 350 phytoalexins are known in more than 100 plant species (a number of plants have a mixed „poison cocktail“ against various pathogens). Phytoalexins are formed both in the attacked cell and in neighbouring ones (they are transported in vesicles); in some plants, they are also created preventively, stored in vacuoles in an inactive form and quickly released when needed.

The formation of phytoalexins is usually induced by the presence of so-called **elicitors** – substances of alien origin which the plant recognises and triggers a defence response; we can distinguish:

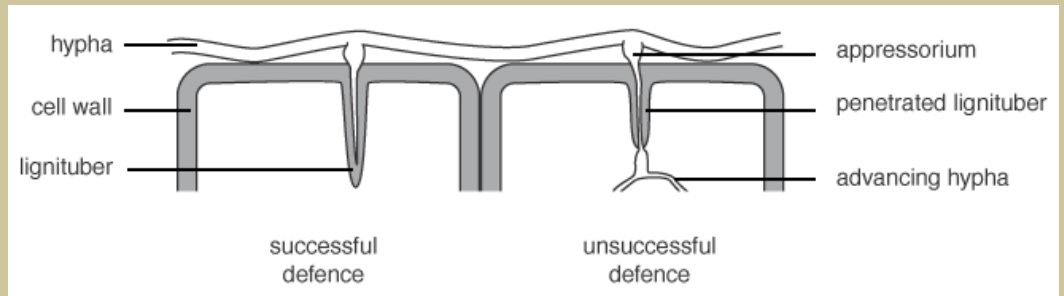
– biotic elicitors (fragments of the cell walls of fungi and bacteria, hydrolytic enzymes, peptides, glycoproteins, fatty acids and especially oligosaccharides) can act non-specifically (the plant sometimes also reacts to a fragment of its own cell wall; however, a non-specific reaction can be beneficial in the case of necrotrophic parasites, when an almost harmless fungus can induce resistance even against strong pathogens) and highly specific (evoke a response against a specific type of pathogen – this case includes substances whose production is related to the action of avirulent strains during immunisation);

– abiotic elicitors can also act inductively: heavy metal ions (e.g. mercury), UV radiation;

• **change in the structure** and chemical **composition of the cell wall**:

– strengthening of the cell wall – increase in cell activity (flow of cytoplasm, accumulation of building materials) in the place of attempt to penetrate the cell, and addition of e.g. lignin or melanin, formation of proteins or enzymes involved in their synthesis;

– deposition of callose etc. from inside the cell wall in the place of attempt to penetrate => a papilla (thickening between the cell wall and plasmalemma) or a lignituber (protrusion into the cell, it envelops the already penetrating hypha and, in case of a successful defence, encloses it inside);



http://bugs.bio.usyd.edu.au/learning/resources/PlantPathology/infection/image_pages/lignituber.htm

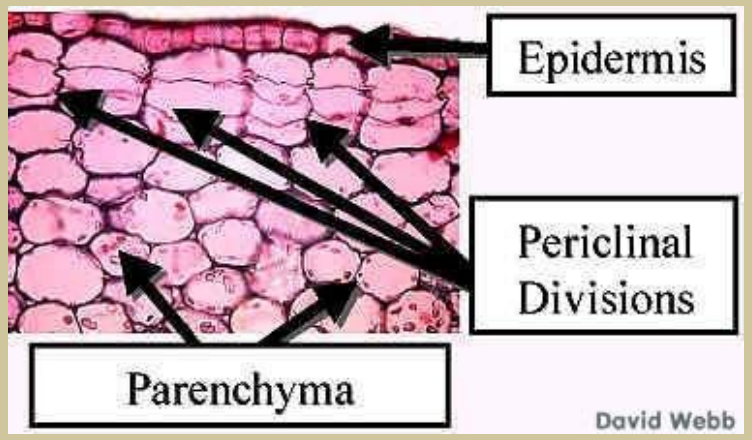
– hydroxyproline-rich glycoproteins (HRGP) are a normal part of the cell wall, but their production increases in infected and surrounding cells => the cell walls become thickened;

• formation of substances ensuring **active defence at the cellular level**:

– tannins (e.g. present in bark) are phenol polymers, which bind to enzymes and inhibit them;

– chitinases and glucanases break down the cell walls of fungi (some also break down the peptidoglycan walls of bacteria, and can also degrade callose, which belongs to glucans too);

• **formation of periderms**, secondary tissues around the affected areas – renewed division of cells impregnated with suberin in bark and wood => separation of the affected area from the healthy tissue; similar effect can be achieved by formation of gelatinous „gum“ or tyloses inside the wood – protrusions of xylem parenchyma cells, which can clog vessels (however, it has a double-edged effect, preventing spread of the parasite, but also reducing water flow in the plant);



Periclinal division of parenchyma cells is characteristic for formation of periderm (it is also formed naturally during formation of bark, replacing the epidermis in secondary thickening).
Photo David Webb, http://biology.about.com/library/weekly/aa030101a.htm

• **Systemic acquired immunity** can be defined as a case where an attack by a certain pathogen in a certain place triggers a reaction in the whole plant, which can, as a result, provide defence against a wider spectrum of pathogens; the principle and functioning are not yet fully understood, but signal about the attack is transmitted from the site of infection through phloem (a key role of salicylic acid in interaction with bound proteins was found in places of necroses, but the direct mediator spreading the signal throughout the plant is not yet known); rather than an immediate response, ensuring immunity against later attack is important in this case.