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Tutorials Handout

Physiopathology

1st Year Master's in Applied Phytopharmacy, Semester 2

Course code according to the training framework : UEF 3

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Preamble

Plant pathophysiology is the science that studies disruptions in the normal functioning (physiology) of plants. It analyzes how pathogens (fungi, bacteria, viruses) or environmental stresses alter the main physiological functions of plants, such as photosynthesis, respiration or water absorption, leading to diseases. It is necessary to clarify the various pathogens that affect the plant. Furthermore, plants are confronted with a wide range of phytopathogenic microbes and pests. There are three main types of pathogens according to their trophic biology : biotrophs, necrotrophs and hemibiotrophs. Most of them have developed very specialized relationships with their host and are not or only slightly cultivable in synthetic medium nor are they saprophytic. To cope with this diversity of parasites, plants have developed an immunity system that allows them to react to pathogens through a wide variety of inducible responses. Indeed, despite the existence of constitutive barriers (plant wall, antimicrobial secondary metabolites etc.) conferring to the plant an effective general resistance, the induction of defenses more adapted to the infection, and to each type of parasite, is often necessary.

The teaching of this Tutorial Handout is scheduled for first-Year Master's students in Applied Phytopharmacy. It is designed to complement the Physiopathology subject scheduled for the first Year of the Master's program.

Supervised work in physiopathology aims to put into practice the knowledge acquired during theoretical courses. It is important to explain the interactions between phytopathogens and plants, as well as the types of resistance (passive and active) and genetic resistance (gene- to-gene).

This Tutorial Handout is intended for students enrolled in the first year Master's degree in Applied Phytopharmacy, in the field Agronomic Sciences, and even in other specialties of Nature and Life Sciences. It presents a series of research papers on phytopathogenic agents (fungi, bacteria), their relationship with the host plant and their infection mechanism, namely ; reminders on the concepts of parasitism and pathogenicity ; mode of action of phytopathogenic fungi ; effects of the pathogen on the physiological functions of the plant ; mechanisms of plant resistance to diseases and finally, substances produced by bacteria and their role in pathogenesis.

Wording

TD N1. Generalities : Reminders on the concepts of parasitism and pathogenicity

Exercise 1.

Fungi are responsible for very different diseases that it is not possible to describe globally.

1. Provide an explanation of the various stages of the infectious process.
2. Provide the title and legend for the figures below.

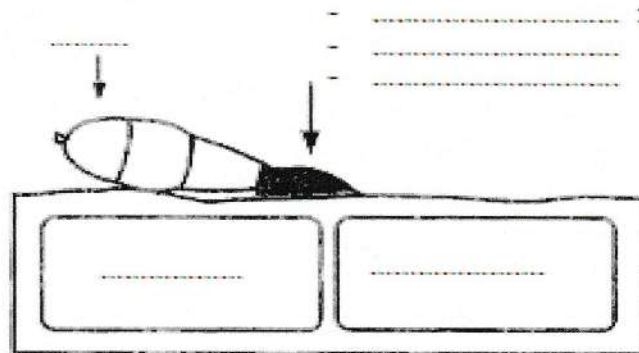


Figure 1 :

(Nichoison and Epstein, 1991)

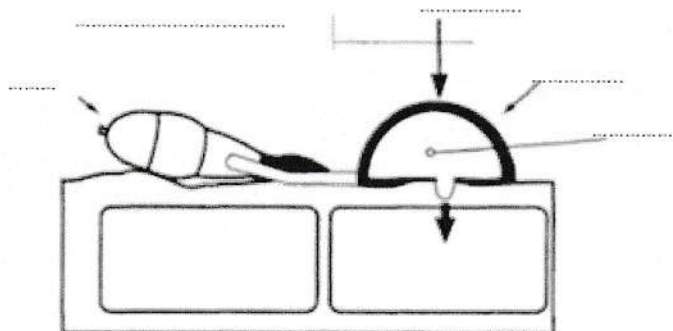


Figure 2 :

(Nichoison and Epstein, 1991)

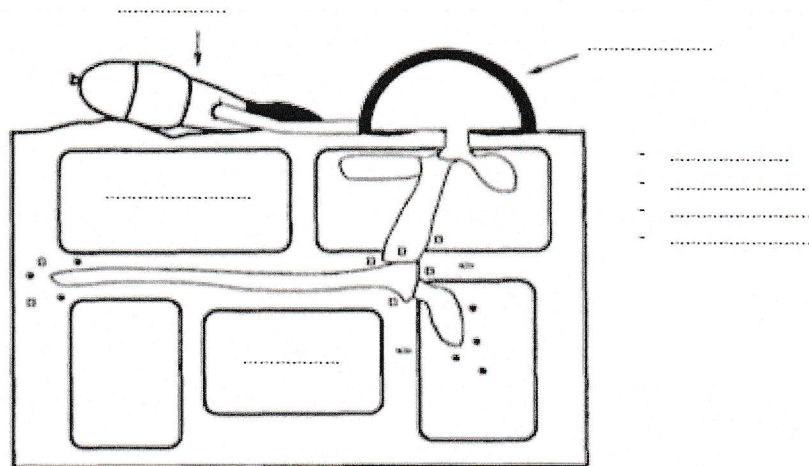


Figure 3 :

(Nichoison and Epstein, 1991)

Exercise 2

Check the correct answer.

A. In the case of monocyclic cycles, how many development cycles does a pathogen have ?
Can it have in one growing season ?

1. Zero
2. One
3. Dozens

B. In a polycyclic cycle, what type of inoculum can be found ?

1. Primary inoculum only
2. Secondary inoculum only
3. Primary and secondary inoculum

C. What type of plant resistance to pathogens is the most durable ?

1. Monogenic resistance
2. Polygenic resistance
3. Transgenic resistance

D. How do we define the frequency of a disease ?

1. The frequency of a disease is the number of infected plants in a population ;
2. The frequency of a disease is the amount of infected plant tissue on a plant ;
3. The frequency of a disease is the number of times that the disease appears in the vegetative cycle of a plant.

Exercise 3

What is the significance of the following terms ?

- Biotroph
- Necrotroph
- Chlorosis
- Epinastie
- Tylosis
- Phosphorylation.
- Hypertrophic and hyperplasia
- Foliar abscission
- Haustoria or appressorium

Exercise 4.

1. To penetrate inside the host plant, phytopathogens must degrade wax, cutin, pectins, cellulose, hemicelluloses, lignin, and structural proteins. What is the reason ?
2. Phytopathogenic fungi produce enzymes of a deferential nature in order to degrade the plant cell wall. Could you explain the mechanism of pectinolytic enzymes ?

Exercise 5

1. Is the degradation of hemicelluloses carried out exclusively using enzymes such as hemicellulases, xylanase, and glucanase ? Justify the answer.
2. Is lignin necessary for the nourishment of fungal ? Do they have the ability to degrade lignin ?

TD N2 : Mode of action of phytopathogenic fungi

Exercise 1

Differentiate between toxins specific and non-specific to the host plant by giving an example for each category ?

Exercise 2

The melanization of the walls of the penetrating organs is necessary for the infection process. What is the reason ?

Exercise 3

The specificity between the plant and the pathogen depends on :

-
-
-

Exercise 4

Pathogens produce several enzymes such as xylanase, galactanase and arbinase to degrade different active substances or major compounds in the host plant. Demonstrate the target compound ?

Exercise 5

Explain the mechanism of action of phaseolotoxin toxin ?

Exercise 6

Why does wood lignin gradually degrade compared to hemicellulose and cellulose ?

TD N3 : Effects of the pathogen on plant physiological functions

Exercise 1

Analysis of the impact of *Fusarium* on transpiration. We measure the transpiration of two tomato plants : one healthy and one infected with *Fusarium oxysporum*. The results show a drastic drop in transpiration in the infected plant, well before the visible withering. Explain why transpiration decreases. What is the impact on water absorption by the roots ?

Exercise 2

Increased respiration in plants after fungal infection is a common physiological phenomenon. Unlike healthy tissues, infected tissues consume much more oxygen and release more carbon dioxide.

A study measures the O₂ consumption (ul/g tissue) on healthy barley leaves and leaves infected with rust (*Puccinia*), over a period of 10 days.

Compare the respiratory intensity of healthy and infected tissues.

Explain the link between increased respiration and the growth of the fungus. Why does respiration fall after the seventh day ?

Exercise 3

Infection by phytopathogens (fungi, bacteria, viruses) triggers a rapid response of the plant, disrupting its carbon balance in the first 24 hours. This disruption is generally characterized by a decrease in photosynthesis and an increase in cellular respiration, leading to a decrease in net carbon fixation.

A tomato leaf is inoculated with a pathogenic fungus (biotroph - oïdium). Net photosynthesis (NP) in $\mu\text{mol CO}_2$ is measured. m^{-2} for 48 hours.

Data : T0 (Inoculation) NP = 10

T 24 h : NP = 5

T 48 h : NP = 2

Calculate the percentage decrease in photosynthesis after 24 hours. Explain the probable cause of this decrease 24 hours before the appearance of visible symptoms (spots). Which metabolic process is diverted by the fungus for its own growth ?

Exercise 4

Can you explain in detail how pathogens influence plant growth ?

TD N4 : Mechanisms of plant resistance to disease

Exercise 1

What is the role of protease inhibitors (plant-phytopathogens) and how many categories are they subdivided ?

Exercise 2

Explain the process of hormonal deregulation in the host plant.

Exercise 3

Are the symptoms of a fungal infection similar to those of a bacterial infection ?

Exercise 4

Cite and explain the different types of resistance in plants.

Exercise 5

Explain the mechanism of pre-existing defenses in plants in case of vascular diseases.

Exercise 6

Determine the changes accompanying HR (hypersensitive resistance) in host plant cells during fungal or bacterial infection.

Exercise 7

In which cases does the plant form callose ?

TD N5 : Substances produced by bacteria and role in pathogenesis

Exercise 1

What physiological and morphological changes occur once the Ri gene from *Agrobacterium rhizogènes* is transferred into plant cells ?

Exercise 2

Grafting facilitates the insertion of *Agrobacterium tumefaciens* into plants. Grafting facilitates the insertion of *Agrobacterium tumefaciens* into plants.

Exercise 3

What are the consequences of activating syringomycin toxin synthesis during plant infection ?

Exercise 4

What is the distinction between coronatine toxin and syringomycin during plant infection ?

Solutions

TD N1. Generalities : Reminders on the concepts of parasitism and pathogenicity

Reminder of course

Phytopathogenic fungi mainly attack the aerial parts of plants, but some fungi present in the soil infect the roots, or are introduced by the underground parts of plants into vascular systems, and therefore colonize the entire plant by moving up the xylem vessels, causing diseases such as damping off, vascular rot, or root rot.

During infection, fungi produce specialized structures such as haustoria that allow them to extract nutrients from the host cell without significantly disrupting its function. , depending on the symptoms observed, different names : gray rot, mildew, bacterial wilt, ... The observation of symptoms already makes it possible to glimpse the host-parasite relationship in its diversity and specificity. The infection of plants by parasites requires the use of their pathogenic power. In the case of microorganisms, this pathogenic power is exerted through various molecules called effectors (enzymatic, toxins, and other determinants). They ensure the degradation of plant cell surfaces, allowing both the installation of the parasite and its supply of carbon and nitrogen sources.

Exercise 1

1. The different stages of infectious processes

The stages of infectious processes can be explained as follows :

A. Spore adhesion and penetration into the plant

Fungal spores carried by water, rain or wind, land on the surface of plants. From their first contact, the spores strongly adhere to the surface of the plant, probably with the help of sticky glycoproteins and polysaccharides. After their adhesion, the spores germinate and emit germ tubes that run along the surface of the plant by modifying it with the help of plant wall-degrading enzymes (proteases, pectinases, cellulases and cutinases).

In most cases, mycelial hyphae from spore germination rapidly differentiate a penetration organ, the appressorium. In order to be functional, appressorium needs to be melanine-coated. This pigment is a polyphenol whose monomer (dihydroxynaphthalene) is synthesized from acetate by very specific enzymes that have been characterized and are the target of so-called antipenetrating fungicides. This melanization of the walls is necessary for the establishment of a strong pressure inside the appressorium (80 bars in *Magnaporthe grisea*), which allows the forcible penetration of a mycelial hyphae through the wall of the epidermal cell.

B. Recognition by the host plant

The host specificity of some fungi depends on their ability to produce selective toxins. These toxins secreted during and after penetration are toxic only to the host plant of the pathogen. After its penetration into the host plant, the fungus can be identified by the plant's recognition systems. This plant-fungus recognition step is essential for the success of the infection, because only fungi that have not been recognized by the many resistance genes of the plant will be able to continue their infectious process. This recognition involves on the one hand an avirulence gene of the fungus and on the other hand a resistance gene of the plant (gene for gene relationship).

C. Colonization of the plant -host

When the fungus escapes the plant's recognition system, it can continue its progression in the tissues. This colonization step is the least well known, despite numerous studies (identification of wall-degrading enzymes and toxins). During this phase, the fungus is in a nutritional deficiency condition.

D. Sporulation

The final stage of the infectious cycle is when the fungus differentiates between its asexual and sexual reproductive organs, releasing spores that will then spread the disease. This phase of sporulation.

2. The title and legend for the figures

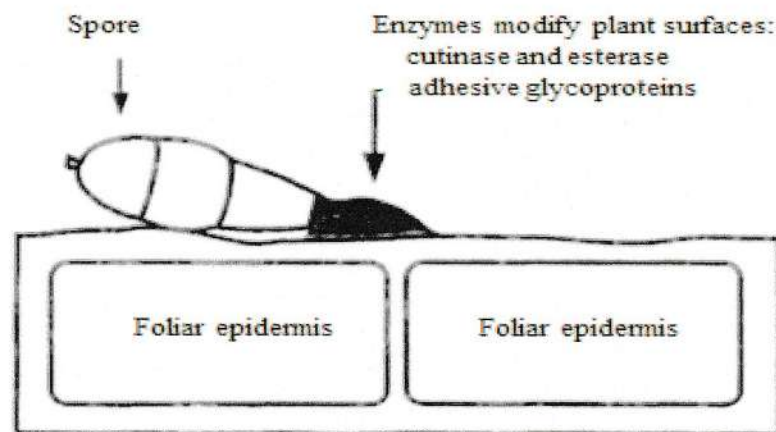


Figure 1: Adhesion of spores on the leaf surface (Nichoison and Epstein, 1991)

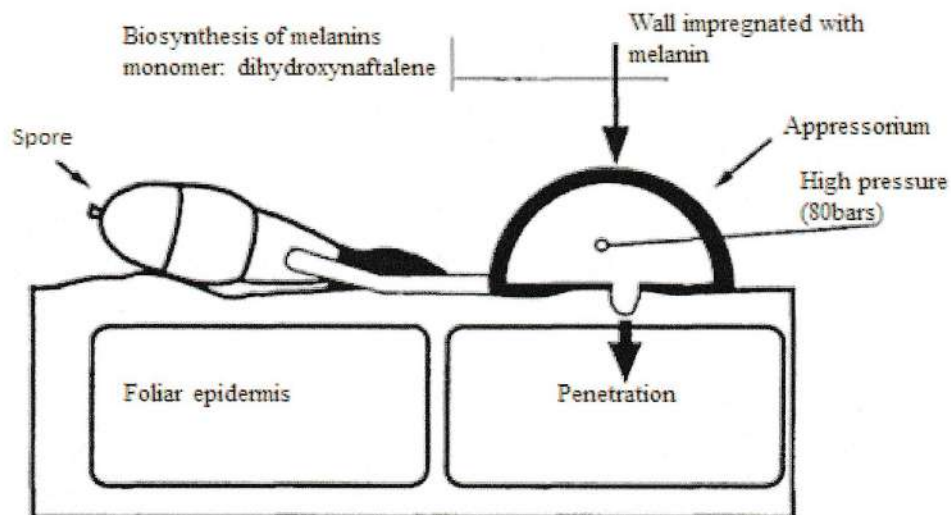


Figure 2 : Penetration and functioning of the appressorium of the pathogenic fungus (Nichoison and Epstein, 1991)

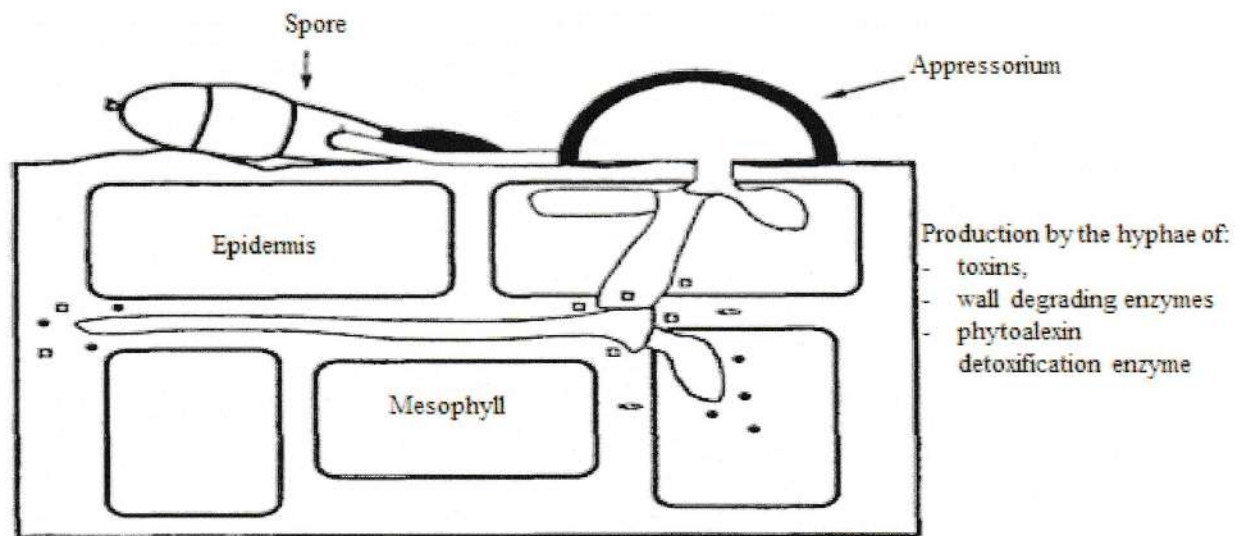


Figure 3 : Colonization of the leaf by mycelial hyphae and sporulation (Nicholson and Epstein, 1991)

Exercise 2

- A. one
- B. Primary and secondary inoculum
- C. Polygenic resistance
- D. The frequency of a disease is the number of infected plants in a population

Exercise 3

Definition of terms

- Biotroph : (obligate parasite) develop in close association with living cells. Specialized hyphae (haustorium) penetrate the cell wall and induce invagination of the cell membrane, example, powdery mildew agents, cereal rusts, mildew agents.
- Necrotrophic : produce enzymes or toxins to kill the host plant cells in front of the infection area. Hyphae develop between dead cells, ex: agent of gray rot *B. cinerea* - Chlorosis: yellowing of plants due to lack of chlorophyll.

- Epinastie is the phenomenon of downward curvature of a plant's leaves due to differential cell growth between the upper and lower petioles in response to certain stresses. (e.g. immersion, viral attack, nutritional deficiency, ethylene intake).

Tylosis : Tyloses are growths on the parenchyma cells of the xylem vessels of the secondary heartwood. When the plant is stressed by drought or infection, tyloses fall from the sides of cells and "dampen" vascular tissue to prevent further damage to the plant. (Included phloem differentiates from parenchymal mass within the xylem and tyloses formation is stimulated.).

- Phosphorylation : Phosphorylation consists of the addition of a phosphate group to an organic molecule, which increases its potential energy. In molecular biology, oxidative phosphorylation is the process that phosphorylates ADP into ATP using the energy released by oxidation.

- Hypertrophies and hyperplasias : the first is due to an increase in cell volume, the second to cell division. These phenomena can lead by growth inequalities to windings, blisters present on leaves, tumors or galls present on twigs and roots.

- Foliar abscission : abscission is the natural physiological process by which an organ (most often a leaf, a flower, a fruit), or even a part of the flittering plant (called abscisil) detaches itself from a plant or its root.

Haustoria or appressorium: A haustorium is a penetrating organ of a parasitic fungus, which inserts into the tissues of the host plant to absorb water and nutrients contained in the cells. Appressorium is a system used by fungi to penetrate a host cell (plant) ; after sporulation, the spore will adhere to the surface of the plant and will germinate. This leads to the formation of an appressorium, which by exerting mechanical pressure will pass the pectocellulosic wall and adhere to the plasma membrane of the host cell.

Exercise 4

1. Fungal pathogens must pass through the cuticle (covered with a layer of wax) and then the cell walls of the various plant organs. For this, pathogens must degrade wax, cutin, pectins, cellulose, hemicelluloses, lignin, and structural proteins. For take their nutrients from the parenchymal cells.

2. Phytopathogens produce enzymes such as : cutinase with high amounts after activation by a few cutin monomers. Resulting in cutinase gene activation, this enzyme degrades the plant cutin and allows the pathogen to penetrate into tissues.

The exact role of pectinases is oriented towards the terminal bonds of the pectin chain and releases simple units of galacturonan, on the other hand, the endopectinases cut the chain at random sites and release shorter chains.

Cellulases or cellulolytic enzymes produced by plant pathogens degrade cellulose by releasing glucose. Cellulases perform different enzymatic reactions :

C1 : is a cellulase that attacks native cellulose by splitting cross-links between chains,

C2 : is a cellulase that attacks native cellulose and cuts it into shorter chains,

C3 is a group of cellulases that degrade short cellulose chains into cellobiose (disaccharide),

β -glycosidase : is an enzyme that eventually breaks down cellobiose into glucose.

□ The degradation of proteins is carried out by proteinase, protease or proteolytic enzymes by releasing amino acids. The degradation of host plant proteins results in disorganization and dysfunction of host cells.

□ Lipids are degraded by pathogen enzymes called lipases or lipolytic enzymes. The hydrolysis of host lipid molecules releases fatty acids that appear to be directly used by pathogens.

□ Regarding antimicrobial substances such as saponins, they are degraded by enzymes called saponinases.

Exercise 5

1. Pathogenic fungi can produce degradation enzymes like hemi cellulase... In order to degrade hemicelluloses, they can also degrade hemicelluloses by radicals such as reactive oxygen, the hydroxyl group, and proline-rich proteins (PRPs), glycine-rich proteins (GRPs).

2. Lignin is a biomolecule, in fact a family of polyphenolic polymer macromolecules (an aromatic complex polymer), which is one of the main components of wood along with cellulose and hemicellulose. Lignin is present mainly in vascular plants and in some algae. Lignin is the most resistant polymer to enzymatic degradation. For this reason, phytopathogens are unable to break down this molecule for use.

TD N2. Mode of action of phytopathogenic fungi

Reminder of course

Plant pathogens (fungi, bacteria) use a complex biochemical arsenal to infect the host plant, mainly composed of cell wall degrading enzymes and toxins (specific or not). These agents act synergistically to weaken the physical structure of the plant and disrupt its cellular metabolism. Because of their efficient entry into the host, plant pathogens are also capable of producing various enzymes, such as cutinases and lipases (pectolytic enzymes). These enzymes play an essential role in the success of infection in their hosts, making them important virulence factors. They also produce several toxins that play a key role in their virulence.

Exercise 1

Non-host-specific toxins are capable of partially or completely reproducing disease symptoms on host plants and also on other plants that are not normally hosts for their pathogens. Unlike specific toxins, they have the distinction of being toxic to a wide range of plant species, whether or not they are hosts of the disease. Non-specific toxins are not essential to the pathogen, such as :

- The cercosporine
- Phytotoxine coronatine
- Solanapyrones

In regards to the specific toxins :

- The toxine-T
- The toxine victorine
- The HC toxin

Exercise 2

Melanization of the walls of the penetration organs (appressoria) is crucial for the infection of fungal plant pathogens because it allows to maintain an extremely high mechanical turgor pressure, necessary to pierce the cuticle and the host cell wall. Melanin is deposited in the cell wall of the appressorium, making it impermeable to solutes. This impermeability allows the accumulation of very high concentrations of solutes inside, creating a high osmotic pressure that turns into a physical pressure greater than 80 bar (8 MPa). This force allows appressoria to cross the cuticle and cellulosic wall of the plant. Melanin acts as a shield against plant defense reactions, such as the production of reactive oxygen species (ROS) and hydrolytic enzymes.

Exercise 3

The specificity between the plant and the pathogen depends on :

- The virulence of the pathogen
- The physiological state of the host
- The host's food

- The host's age
- The morphology of the host
- Presence of toxic compounds

Exercise 4

Plant pathogens secrete enzymes to degrade the plant cell wall, allowing them to access nutrients and colonize the host plant. Xylanase, galactanase and arabinase specifically target the structural polymers of this wall, called hemicelluloses and pectins.

Exercise 5

Phaseolotoxin is a phytotoxin produced by the bacterium *Pseudomonas syringae* pv. *phaseolicola*. It is a major virulence factor that allows the bacteria to spread systemically in the host plant, causing symptoms of chlorosis (yellowing). Once secreted in the plant, phaseolotoxin is cleaved by peptidases from the plant to release a smaller and highly active molecule called octicidin, which inhibits the enzyme ornithine tran-carbamylase.

Exercise 6

Lignin in wood degrades much more slowly than cellulose and hemicellulose because of its complex, three-dimensional chemical structure that resists the action of microorganisms. While cellulose and hemicellulose are broken down by hydrolysis, lignin degradation requires complex oxidative processes. Unlike cellulose, which is a single linear chain, lignin is an amorphous and three-dimensional phenolic polymer. Its structure, composed of phenylpropane units, is bound in a non-repetitive manner, which makes enzymatic attack difficult.

Lignin is a hydrophobic network (it repels water), which limits the access of water and hydrolytic enzymes (used by fungi and bacteria) to the wood structure, slowing down its decomposition.

TD N3. Effects of the pathogen on plant physiological functions

Reminder of course

Any pathogen that attacks green aerial tissues affects the crop yield. In general, the harmful effects of pathogens can be directly attributed to the destruction of photosynthetic tissues. This is the case for chlorosis, defoliation, leaf spots, necrosis, etc. Such destruction in green tissues reduces the interception of solar radiation and consequently the photosynthetic capacity of the plant. In other cases, photosynthesis may be reduced due to the inhibition by pathogen toxins of enzymes that are involved in photosynthetic activity.

In diseased plants, the water regime can be disrupted either at the absorption level - the roots are attacked and no longer function - or at the transport level. Xylem occlusion is due to freezing resulting either from enzymatic degradation of cell walls or the production of extracellular polysaccharides or glycopeptides by the parasites themselves. Such changes in host plant activities are due to their infection by pathogens.

Exercise 1

The fungus develops in xylem vessels (xylem-invading pathogen), which obstructs the flow of raw sap. The plant reacts to water stress by closing its stomata to limit water loss, thereby reducing transpiration. Perspiration creates a suction (tension) that pulls water from the roots. If transpiration decreases (stomatal closure), root water uptake also decreases (reduction in raw sap flow).

Exercise 2

Here is a comparison of respiratory intensity based on study data : Initial phase (Days 1 to 3) : The respiratory intensity is identical between healthy and infected leaves (50 ul/g). At this stage, the infection is latent and does not yet alter the host's metabolism.

Active infection phase (days 4 to 7) : A clear divergence is observed. While healthy leaves remain stable (about (55 ul/g), the O₂ consumption of infected leaves flies off to (150 ul/g), nearly 3 times more than that of healthy tissues. This sharp increase coincides with the onset of symptoms and reflects the intense metabolic activity of the fungus as well as the plant's defense response.

Sporulation phase (Days 8 to 10) : The respiratory intensity of infected tissues drops sharply to (80 ul/g), while remaining higher than that of healthy tissues (55 ul/g). This decrease is explained by the degradation of leaf tissues (necrosis) following the depletion of resources by the parasite during its reproduction (sporulation).

Link between the increase in respiration and the growth of the fungus (Days 4-7). The drastic increase in oxygen consumption ((O₂) from 50 to 150 ul/g) (days 4-7) corresponds to the active colonization phase by Puccinia. The fungus develops inside leaf tissue (intercellular mycelium), which requires considerable energy. The infected wheat plant increases its own respiration to produce energy necessary for the establishment of defense mechanisms (synthesis of phenolic compounds , defense enzymes).

The decline in respiration (from 150 to 80 ul/g) after day 7, despite maximum sporulation, is explained by senescence and tissue destruction. At this stage (8-10 days), the fungus has completed its active growth phase and is moving to massive sporulation (release of uredospores). Metabolic building activity (growth) is replaced by spore production. Rust pustules burst, destroying the epidermis and causing leaf cell death (necrosis/chlorosis). Because the tissue is dead or badly damaged, it no longer breathes actively. The energy demand

of the plant collapses because it can no longer defend itself, and the tissue becomes a dead zone for sporulation.

Exercise 3

1. Initial value (T_0) = $10 \text{ } \mu\text{mol CO}_2 \cdot \text{m}^{-2}$

Final value ($T_{24\text{h}}$) = $5 \text{ } \mu\text{mol CO}_2 \cdot \text{m}^{-2}$

Calculates:

Difference: $10 - 5 = 5$

Division: $5/10 = 0.5$

$$\text{Reduction percentage} = \left(\frac{\text{Initial value} - \text{Final value}}{\text{Initial value}} \right) \times 100$$

Net photosynthesis decreased by 50% after 24 hours.

Note : At $T_{48\text{h}}$ ($2 \text{ } \mu\text{mol CO}_2 \cdot \text{m}^{-2}$), the total decrease relative to T_0 is 80%.

2. The early decrease in net photosynthesis (NP) before the onset of symptoms in a plant infected with a biotrophic pathogen such as powdery mildew is explained by two major physiological mechanisms:

A. Stomatal closure (defense response) from the early stages of infection (recognition of molecular patterns of the fungus), the plant triggers an immune defense response. One of the first signals is the production of abscisic acid (ABA) or reactive oxygen species (ROS).

Stomata close to limit entry of the pathogen. Effect on NP : Closure drastically reduces the entry of CO_2 into the leaf, which limits the biochemical phase of photosynthesis, even if the tissues still appear healthy.

B. The diversion of resources : Although powdery mildew is biotrophic (it keeps host cells alive to feed on), it installs haustoria (suction organs) in epidermal cells. The infected area goes from a "source" status (which produces sugars) to a "sink" status (which consumes or loses sugars to the fungus). The plant increases its cellular respiration to produce the energy required for its defense mechanisms (synthesis of PR proteins, wall strengthening).

As **NP = Gross Photosynthesis - Respiration**, the increase in respiration causes the measured net value to fall.

3. The metabolic process diverted by the fungus for its own growth is the flow of carbon from photosynthesis (the transport of carbohydrates or photosynthates).

A. A biotrophic fungus like powdery mildew needs the living host cell. It uses specialized structures (haustoria) to pump the sugars produced by the plant before they are used by it.

B. By taking carbon on a massive scale, the fungus transforms the leaf (which is normally a "source" of energy) into a "sink" (consumer).

C. The drastic drop in the PN you observe (from 10 to 2 in 48 hours) is explained by two phenomena related :

- The fungus directly consumes the fixed CO₂.
- The accumulation of sugars at the point of infection (not exported to the rest of the plant) triggers a retro-inhibition of photosynthesis, signaling the plant to stop producing energy that it no longer controls.

Exercise 4

Pathogens (fungi, bacteria, viruses, nematodes) influence the growth of plants by diverting their resources, disrupting their physiology and altering their hormonal development, which generally leads to a slowdown in growth (dwarfism), deformities, or death. This influence manifests itself through complex attack mechanisms and energy-intensive defense responses for the plant.

- By causing necrosis (leaf spots), chlorosis (yellowing) or defoliation, fungi and bacteria reduce the photosynthetic leaf area, thus limiting the production of sugars necessary for growth.
- Bacteria and fungi (e.g. *Fusarium*) colonize the xylem and phloem, blocking water, mineral and nutrient transport. This causes the plant to wilt, desiccate, and die.
- Biotrophic pathogens (which live on a living host) establish specialized structures (haustorium) to "pump" directly the plant's sugars and amino acids, depriving young growing tissues of these resources.
- Dwarfism : Viruses and certain fungi disrupt the production of gibberellins, resulting in reduced stem elongation.
- Root rot : Fungi such as *Pythium* or nematodes destroy the root system, reducing the plant's ability to absorb water and nutrients, resulting in very low aerial growth.

TD N4. Mechanisms of plant resistance to disease

Reminder of course

The interaction between a plant and potentially pathogenic microorganisms is one of nature's constants. The development of a disease is possible only if the pathogen manages to overcome the various host barriers. However, this situation remains exceptional because the microorganism is most often unable to infect the plant due to the existence of a system of preformed defenses. The other levels of resistance are characterized by the involvement of induced (or activated) defense mechanisms. These result from the initial recognition between the plant and the pathogen, which can be general or specific. It follows an activation of signal cascades leading to the induction of defense genes and the synthesis of several proteins and other molecules that create an unfavorable environment for the invasion of the parasite. The signals emitted during defense can subsequently extend to adjacent cells, leading to local acquired resistance (LAR), then spread throughout the entire plant, thus preventing potential secondary infections. We then speak of systemic acquired resistance (SAR).

Exercise 1

Protease inhibitors (PIs) are defensive proteins characterized by their ability to inactivate proteolytic enzymes of endogenous or exogenous origin. On the one hand, they protect the plant against uncontrolled proteolytic activity and, on the other hand, they ensure the control of exogenous proteases secreted by herbivorous insects or pathogens. They are subdivided into four categories according to the type of proteases they associate with :

1. Serine protease inhibitors, widely distributed in plants.
2. Cysteine protease inhibitors, also abundant in plants.
3. Metalloprotease inhibitors, relatively rare in plants.
4. Acidic protease inhibitors, which are rarely found in plants.

PIs play an important role in the defensive arsenal of plants.

Exercise 2

The main mechanisms of PR proteins :

1. Inhibition of spore germination
2. The formation of papillae
3. Strengthening of the plant cell walls
4. Enzymatic degradation B. 1,3-glucanases and chitinases hydrolyze the structural polymers (chitin and glucan) of the fungal cell wall, ...
4. Release of glucan monomers that act as fungal elicitors.

Exercise 3

Symptoms of fungal and bacterial infections on plants can be similar, including wilting, leaf spots, rot and yellowing. Fungi produce pustules or felting, while bacteria cause more angular spots delimited by the veins, seeps, or decay smells. Both can cause decay, but bacterial rot is often softer and has a strong odor.

Exercise 4

There are mainly two types of resistance in plants

1. Natural Defenses (Constitutive vs Induced)

A. Preformed Resistances (Passive) : Physical and chemical barriers existing before the attack : thick cuticle, wax, cell walls, involving preformed constituent barriers.

B. Induced Resistances (Active) : Reactions triggered after detection of the aggressor, involving newly formed barriers in response to stress.

- Hypersensitive Reaction (HR) : The plant causes local cell death to "starve" the pathogen.

- Systemic Acquired Resistance (SAR) : Generalized defensive response to the entire plant after a first local infection.

2. Genetic Resistance (Qualitative vs Quantitative)

A. Qualitative (or vertical) resistance : Linked to one or a few major genes. It is often total against a specific strain (gene-for-gene).

B. Quantitative (or horizontal) resistance : Controlled by many genes (polygenic). It is partial, slowing down the development of the disease without eliminating it completely.

Exercise 5

Preexisting (or passive) defenses in plants are the first line of resistance against vascular diseases, acting even before the pathogen enters tissues. They are particularly crucial against soil fungi and bacteria (such as *Fusarium* or *Verticillium*) that seek to invade the xylem, the sap transport system.

1. Preformed Physical Barriers : Plants use their physical structure to prevent the entry or limit the spread of the pathogen.

- Reinforced Cell Walls : Thick and lignified cell walls, especially in the xylem, provide mechanical resistance to penetration by fungal mycelium.

- Tyloses (Pre-existing or rapid) : Although often induced, certain tylose structures (cell protrusions) can be preformed or formed very quickly from parenchyma cells associated with vessels, physically blocking the spread of infection in the xylem.

- Pores of the punctuation membranes : The pore size of the xylem walls is often too small to allow fungal spores to pass through, limiting their horizontal propagation.

2. Preformed chemical defenses :

- Saponins : These molecules, stored in tissues, are capable of destroying the cell membranes of pathogens.
- Other compound : Tannins, alkaloids and flavonoids are also present to prevent the growth of phytopathogens.

Exercise 6

Plant defense seems to intervene with HR. They include :

1. The accumulation and release of reactive oxygen species (ROS).
2. The activation of phenol oxidases and the oxidation of phenols.
3. The accumulation of enzymes such as peroxidase and lipoxygenases,...

Release of signal molecule in and around plant cells.

Exercise 7

The plant forms callose in response to an infection to strengthen its cell walls by accumulating glycoproteins and blocking the entry of pathogens. This physical barrier, often induced by immunity to limit the fungal spread (diseases).

TD N5. Substances produced by bacteria and role in pathogenesis

Reminder of course

Several bacterial species, plant pathogens, have been reported and described, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Erwinia amylovora*, *Agrobacterium rhizogenes* and *A. tumefaciens*. *P. syringae* is essentially a necrogenic agent that causes more or less localized destruction of parenchymal tissues.

As is well known, plants secrete several hormones to maintain their cellular balance. In the case of an infection, it will confront stress; we find that certain genes encoded by T-DNA from *Agrobacterium* allow the synthesis in the plant cell of plant hormones, which is attributed to an undesired balance of hormones in the host plant. Moreover, toxin production appears to be an important element of pathogenicity. So, it seems that the syringomycin produced by *P. syringae. pv* disrupts physiological functions localized within the plasma membranes of host cells, causing cell lysis and plant tissue necrosis.

Exercise 1

The main phenotypic and physiological changes observed :

- Hairy Roots formation : This is the most spectacular modification. It is manifested by the appearance of adventitious roots, very branched, thin and fast growing, at the site of infection.
- Morphological changes : Transformed roots show a distinct phenotype, plagiotropic growth (develops horizontally), an absence of geotropism, and a strong branching capacity.
- Synthesis of opines : Transformed cells produce specific molecules called opines (agropine, manopine, etc.), which are used as a source of nitrogen and carbon by the bacterium *A. rhizogenes*.

Exercise 2

The infection of plants by *Agrobacterium tumefaciens* (agent of crown gall) is strongly promoted by grafting, mainly because this operation creates an open wound that serves as a gateway for the bacteria. The main effects and consequences of this process :

- Tumor formation at the graft site : The bacteria take advantage of the grafting injury to insert part of its DNA (T-DNA) into plant cells, causing uncontrolled cell proliferation. This results in woody, brown and irregular growths, often called "galls" or tumors, at the weld area.
- Failure of the graft/rootstock junction: The tumor that develops at the union prevents the normal formation of the callus and the fusion of tissues (xylem and phloem) between the rootstock and the scion.
- Wasting and weakening : Infection can lead to poor graft feeding, causing growth retardation, yellowing of the leaves, or even death of the transplanted plant. Systemic infection : Once established, the bacteria can persist systemically contaminating the entire plant. The infection of plants by *Agrobacterium tumefaciens* (agent of crown gall) is strongly promoted by grafting, mainly because this operation creates an open wound that serves as a gateway for the bacteria. The main effects and consequences of this process :

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- Hormonal disruption : The bacterium transfers part of its own DNA (T-DNA) into the plant genome, forcing plant cells to divide anarchically, producing excess growth hormones (auxin and cytokinin).
- Production of opines : Tumor cells are forced to produce compounds called opines, which only the bacteria can use as a source of nutrients.
- Susceptibility to secondary infections : Since the gall's tissues are often broken down, they serve as a gateway for other pathogens (fungi, bacteria).

Exercise 3

The consequences of this interaction during plant infection are mainly : Activation of virulence (Synergy) : The perception of specific phenolic glycosides by the bacterium activates the *syrB* gene (and other genes in the *syr* cluster), inducing the production of syringomycin.

- Increase in necrosis symptoms : The mass-produced toxin causes tissue degradation (necrosis) and characteristic folate spots, promoting the colonization of intercellular space.
- Host cell membrane alteration : Syringomycin targets the plasma membrane of plant cells, increasing K^+ , H^+ , and Ca^{2+} fluxes, leading to cytolysis (cell death) and ionic regulation failure.
- Facilitation of invasion : The toxin helps in the multiplication of bacteria and their systemic movement in the plant, increasing the severity of the disease, although it is not strictly necessary for basic pathogenicity.

Exercise 4

The main difference between coronatine and syringomycin, two phytotoxins produced by the bacterium *Pseudomonas syringae*, lies in their mode of action and their specific role during infection:

Coronatin acts as a hormonal manipulator (mimicking a plant hormone), while syringomycin acts as a membrane-destroying agent.

1. Coronatin (COR) : It is a structural and functional analogue of jasmonic-isoleucine acid (JA-Ile), a plant hormone. It "tricks" the plant by activating its defense signaling pathways, but inappropriately.

Role in infection (Reopening of stomata) : It forces the stomata (pores on leaves) to open, allowing bacteria to enter the leaf. Suppression of immunity : By activating the jasmonate pathway, it indirectly inhibits the salicylic acid pathway, which is the main defense against *P. syringae*.

Symptoms : Widespread chlorosis (yellowing) and hypertrophy of the tissue.

2. Syringomycin (SR) : It is an amphipathic lipodepsipeptide that inserts into the plasma membrane of plant cells.

Role in infection (pore formation) : It forms ion channels (pores) in the membrane, causing ions (potassium, calcium) and nutrients to leak.

Necrosis : It destroys host cells (cytolysis) to release nutrients that the bacterium uses to multiply (necrotrophic lifestyle).

Bio-surfactant : It helps the bacteria spread to the surface of tissues.

Symptoms : Necrosis (dead, brown or black tissue) and rot.

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