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How do pathogens attack plants?

Pathogens have evolved the ability to exploit substances produced by plants and have therefore also developed ways to attack plants in order to utilise these substances. However, plants have many structral and biochemical defense mechanisms which the pathogen must breach. In order for a pathogen to successfully infect a plant it must firstly arrive at the plant, attach to the plant, penetrate, obtain the plant's nutrients, establish and disperse while at the same time neutralising the defense actions of the plant. These activities are achieved by mechanical force or by secretions of chemicals that affect the metabolic host processes.

Plant pathogens have one of three modes of nutrition which determine the way the pathogen attacks the plant. Necrotrophs such as *Botrytis cinera* attack with the intention of killing the host. They are highly opportunistic, have a wide host range, release many enzymes and feed as they grow. Biotrophs do not want to kill their host as they require a living host to complete their life cycle. Enzyme secretion is much lower and more controlled and they only begin to feed once inside the host. Examples include wheat stem rust and powdery mildew fungus. Hemibiotrophic nutrition is where pathogens begin being biotrophic but as they begin to run out of food they adopt nectrophy. The rice blast fungus is an example.

Pathogen attack begins with the pathogen arriving at the plant, which can occur in a number of ways including wind, water, vectors and diseased stock. The force of the wind can blow spores such as rust spores to a plant; water can transport bacteria which can arise through the incorrect management of irrigation systems, vectors such as bumble bees can carry the fire blight disease and diseased stock will cause other stock to become diseased. With pathogens in the soil, plant roots may grow so that they arrive at the pathogen instead of the other way around. In addition chemotaxis which is where pathogens are attracted to plants due to chemicals produced by the plant can also help the pathogen to arrive at the plant. This is particularly associated with pathogens that have motile stages, which means that they can move towards the plant in response to stimuli. For example, border cells that originate from the root cap meristem have been shown to affect bacterial and fungal plant pathogens; Acetetosyringone, which is a phenolic compound secreted from wounded plants such as tobacco chemo-attracts *Agrobacterium tumefaciens* bacteria to attack them which causes crown gall.

Once the pathogen has arrived at the plant it must attach to it so that it is not washed or blown off. This can occur either passively for example by becoming trapped in the surface hairs of the plant or actively by the pathogen secreting an extra-cellular matrix and sticking to the plant. Adhesion to the plant may also be necessary for germination of the pathogen. As the majority of substances that the pathogen requires are in the protoplast of the plant cell, pathogens need to enter the plant. They can either enter through natural openings, wounds or by using enzymatic and/ or mechanical force to penetrate the host's barriers which there are, unfortunately for the pathogen, many.

Plants have a number of natural openings that mean that the plant's exterior barriers do not have to be breached. These include stomata, nectaries, hydathodes and lenticels. Stomata and nectaries are utilised by fungi and bacteria especially when they arrive by wind or rain. This is illustrated by the bacterial pathogen that causes wide fire of tobacco entering via the stomata and the pathogen that causes Fire Blight that commonly enters through nectaries. Hydathodes are structures that have guttering. When there is high humidity, droplets are exuded which can become contaminated by pathogens. Wounding is generally needed by pathogens that enter via nectaries. The Urwinea bacterium that is carried by a bumblebee vector enters in this way.

Pathogens that do not utilise the natural openings of the plant have to instead penetrate the plant. Before the mechanisms which pathogens have adopted to enter plants are discussed, the actual structure of the plant will be described to explain what the pathogen has to get through.

The most outer layer of a plant is normally the waxy cuticle that is made up of an outer layer of wax and an inner layer of cutin. Wax is highly hydrophobic and made of long chain hydrocarbons and a small amount of lipids. Cutin is made up of smaller chains of hydrocarbons with more lipids for example serine esterase. The underground parts of the plant are covered with a more complex substance called suberin. Under the cutin and suberin plants have a cell wall that is made of cellulose and constructed in two layers: the primary and secondary cell wall. Cellulose is made of 1-4 beta linked glucan which form chains known as microfibils which are linked by hydrogen bonds and are embedded in an amorphous phase. The secondary cell wall also contains hemicelluloses. The plant cells are held together by a substance called pectin which makes up the middle lamella. Pectin is made up of straight chains of galacturonic acid residues that are joined by alpha 1-4 links. Lignin is an important substance of woody tissues.

There are a number of factors of the plant which determine the process of pathogen propagule germination and the differentiation of infection structures. These include hydrophobicity, hardness, chemical signals, topology, mechanical force and enzymes. Hydrophobicity is the repelling of water and is measured by the contact angle of the water droplet with the surface. The more hydrophobic the higher the contact angle. The nature of the plant affects the formation of structures such as appressoria (explained later) of the fungus *Magnaporthe grisea* which causes rice blast disease. Hardness is a factor because pathogens generally require a hard surface. *M. grisea* has been shown to only form appressoria when in contact with a hard surface. Chemical signals from the host plant have been shown to influence pathogens. *Colletrotrichum* species, for example which infect ripe fruit such as bananas and tomatoes are influenced by the chemical ethylene by forming multiple appressoria. By recognising ethylene, the pathogen can attack at the most appropriate time. The topography of plant surfaces influence pathogens that are able to sense structures. Rust fungi for example, which enter the host plant via stomata seem to be very sensitive to minute alterations in the surface and spacing between the ridges that occur around stoma with a ridge of 0.5μm being the optimal for appressoria development. 0.5μm is found to be the height of the guard cells that surround the stomata of the fungus' host plant *Phaseolus vulgaris*.

Pathogen attack on plants is achieved by mechanical force, chemical activity or by a combination of both. Only some fungi, nematodes and parasitic plants use mechanical force. In the case of parasitic plants and fungi, adhesion to the plant is achieved firstly by the formation of intermolecular forces between the plant and pathogen. Sometimes, spores, which land on moist surfaces, adhere by the formation of an adhesion pad that is helped by enzymatic action. In addition, adhesive substances which pre exist on the spore will allow adhesion once hydrated. Once an appropriate site is found, adhesion is followed by the radical or fungal hyphal tip increasing in diameter to form a flattened, bulb-like structure known as an appressorium that increases the degree of adhesion. A penetration peg then extends from the appressorium, and penetrates the cuticle and cell wall. Once the penetration peg has passed through the cuticle it increases in diameter. The best example of a pathogen that forms appressoria is the rice blast fungus, *Magnaporthe grisea*. The *M. grisea* appressoria forms at the end of germ tubes in response to a hard, hydrophobic surface, requires water and responds to chemical signals of waxes and lipids from the plants surface. In addition, the appressoria has a melaninised cell wall. In the case of nematodes, entry by mechanical force is achieved by the use of a stylet which is an extremely hard and thin. Adhesion is achieved through suction and penetration is achieved by muscular movements that force the sylet into the plant in a probing in and out manner. Nutrients are then extracted through the stylet. Once pathogens have achieved entry, the cell wall is softened by pathogen secreted enzymes

Chemical attack is a much more common mechanism with pathogens having four types of chemical weapons; enzymes, toxins, growth regulators and polysaccarides. All pathogens can synthesise these chemicals except for viruses and viroids that instead are able to stimulate the plant to make them. For each of these chemicals, plants have corresponding defenses. Enzymes are faced with suberin, anti fungal enzymes and host deactivation of the enzymes; plant enzymes can detoxify pathogenic toxins and plants have biochemical defenses such as phytoalexins and HR to combat growth regulators. It can therefore be appreciated that plants and pathogens are in constant battle to defend and attack.

Enzymes are large protein molecules that act as natural catalysts for all the chemical reactions in a living cell with each enzyme being specific for each reaction. Enzymes are the most common from of chemical

attack by degrading the components of the plant cells. The first components that need to be degraded are the plant's surface layers of wax, cutin and suberin.

Views concerning waxases are unsure. Some people dismiss them and conclude that wax layers are penetrated by mechanical force only, however electron microscope work has shown zones of clearing suggests that some pathogens such as *Puccinia hordei* are able to produce wax degrading enzymes. There are many fungal pathogens and a few of bacterial pathogens that secrete cutinases to degrade the main component of the cell wall: cutin. Cutinase breaks the ester linkages between the cutin molecules releasing monomers and oligomers and can either be constitutive or inducible. Fungi produce small amounts of cutinase all the time, which upon contact with cutin, release monomers. The release of monomers enters the pathogen cell which causes the cutinase gene to be expressed resulting in a 1000X increase in cutinase production. Cutinase can also be triggered by fatty acids in the wax.

Pectic substances are the main component of the middle lamella that holds the cells together. They are also present in the primary cell wall. Pectic degradive enzymes are known as pectinases or pectolytic enzymes. Pectin methyl esterases remove small branches of the pectin chain. This does not affect the pectin length but does affect the solubility, which makes it susceptible to other enzyme attack. Polygalacturonase – chain splitting pectinase adds a water molecule which hydrolyses the linkage between 2 galacturonan molecules. Pectin lyases or transeliminases split the pectin chain by removing a molecule of water. As with cutinases, pathogens seem to constantly produce small amounts of pectinases that release galacturonan monomers upon contact with pectin that causes autocatalytic induction that enhances the synthesis of pectinases resulting in the release of more pectinases. Catabolic repression stops the enzymatic production once it gets too high. The action of these enzymes causes maceration, separating the cell walls, causes occlusion of vessels, weakens the primary cell wall and upsets the osmotic balance of the cell causing it to lyse which all allow entrance for the pathogen. Diseases caused by pectinases are those causing soft rot of tissues.

Cellulose is the main component of the cell walls. Cellulases are enzymes that break the glucose chains with different cellulases breaking the chain at different points. They are formed by fungi, bacteria and nematodes and cause the softening and eventual collapse of the cellular structure. Hemicelluloses break down hemicellulose.

Lignin is found in the middle lamella, in the cell wall of xylem vessels and in the fibres that strengthen plants. Only a small number of microorganisms have the ability to degrade lignin of which fungi mainly of the basidiomycete variety are the only reported type of pathogen. White rot fungi produce ligninases to degrade lignin.

Substances contained in plant cells are degraded by proteinases (protein degrading), amylases (starch degrading), lipases and phospholipases (oil and fat degrading).

The second main chemical attack is by toxin release which is either host or non-host specific. Toxins directly act on living host protoplasts and either kill or seriously damage the plant cells. Toxins are released in low concentrations and are very poisonous. Host cells are affected by alteration of the permeability of the cell membrane or by plant enzyme interference.

Non-host specific toxins

Toxin	Organism	Disease	Mechanism of action and symptoms
Tabtoxin	Bacterium-Pseudomonas syringae	Wild fire disease of tobacco	In the cell it releases tabtoxinine which inactivates glutamine synthetase leading to the build up of toxic ammonia. Uncouples photosynthesis and

			photorespiration and causes necrotic spots on leaves
Phaseolotoxin	Bacterium- Pseudomonas syringe pv. Phaseolicola	Halo blight of bean	Binds to and inactivates ornithine carbamoyltransferase causing reduced growth of new leaves, apical dominance interference
Tentoxin	Fungus- Alternaria alternata	Chlorosis in seeding plants	Binds to and inactivates an energy transfer to chloroplast protein and inhibits photophosphorylation causing chlorosis

Host – specific toxins

Toxin	Organism	Disease	Mechanism of action and symptoms
Victorin	Fungus – Cochliobolus victoriae	Leaf blight	Toxin is produced and carried to the leaves and kills entire plant
T toxin	Cochliobolus heterostrophus	Southern corn leaf blight	Acts on mitochondria of susceptible cells and inhibits ATP sythesis
HC toxin	Cochliobolus carbonum	Leaf spot disease of maize	Mechanism not known

Growth regulators are plant hormones and include auxins, gibberellins, cytokinins and ethylene. They work in small concentrations, are usually synthesised at a distance from the site of action and promote the syntheses of m-RNA molecules which leads to the formation of specific enzymes. Plant pathogens can produce more of the same growth regulators, inhibitors of the growth regulators or new growth regulators which causes imbalances in the hormonal system of the plant.

Growth Regulator	Organism	Disease	Mechanism of action
Auxin	Pseudomonas solanacearum	Bacterial wilt of solanaceous plants	Increases plasticity of cell wall and inhibits ligninfication
Gibberellins	Gibberella fujkuroi	Foolish seedling disease of rice	Rapid growth
Cytokinin	Rhodococcus fascians	Crown galls	Prevents genes from being switched off and activating previously switched off genes
Ethylene	Pseudomonas solanacearum	Yellowing of fruit	Chlorsis, leaf abscission, increased cell membrane permeability

Polysaccarides are of the least important but are significant in wilt diseases of plants. There are also thought to be suppressors that suppress the defense mechanisms of the plant.

In conclusion, pathogens attack on plants is achieved through many mechanisms that vary depending on the pathogen and plant.

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