Plant Defence Responses 2 – advanced

The British Society for Plant Pathology (BSPP) have put together this presentation to fulfil the requirements of the OCR exam specification in the UK, teaching about *communicable plant diseases* and specifically focussing upon Plant Defence Responses at A-level.

It is advisable to have viewed Part 1 in the series ahead of using this presentation as it takes more of an overview of the subject (and includes some more stunning images

You are welcome to adapt the slides in this presentation to suit your teaching style and specific curriculum requirements. These notes are designed to support you in the delivery of this presentation giving a commentary for each slide.

Slide 1

This presentation develops further the ideas introduced in Part I and in so doing, covers information required for the A-level programme of study.

Slide 2.

Why is an understanding of plant defence responses so important? There are 2 contexts in which the topic can be introduced – plant pathogens cause yield losses around the world affecting the livelihoods of millions of farmers, and efforts to control the diseases, are generally not fully effective and may have a potentially (negative) impact on the environment. So, a better understanding of plant defence responses and how to improve them can help increase world food production and help alleviate issues around global food security for a growing population. Plants ultimately underpin every single food web / food chain and healthy plants help us maintain wide biodiversity

Slide 3.

We need to think about an ongoing 'arms race' between plants and their pathogens. The first thing to remember is that remarkably, and fortunately, most plants are resistant to most microbes. The methods that plants employ to protect themselves are both passive and active and require the plant to 'recognize' the pathogen that is attacking it, so it can deploy the most appropriate defence response against it. We can think of these defences as falling into 3 broad groups – 1) preformed barriers and chemicals. 2) detection and associated signaling molecules that are involved in the initial recognition when a pathogen lands on the host plant. 3) Chemicals and barriers that are induced in response to perception of the attack.

Slide 4.

Here are the challenges facing a plant pathogen during its life-cycle.

Find a host plant and attach to it

Gain entry through the plant’s impermeable defences

Avoid the plant’s defence responses

Grow and reproduce

Spread to other plants

This presentation is going to focus upon those areas that are highlighted in **bold**, namely the plant defence response

Slide 5.

Plants are fixed in the ground and cannot escape attack by plant pathogens. Aphids, leaf hoppers, thrips and other insects are important vectors for viruses (the disease being passed by the insect when it feeds). Powdery mildew and other fungi form spores that can be carried by the wind. Many bacteria and fungal spores are transferred in water droplets (especially as a consequence of rain splash). Many soil-living nematodes, bacteria, fungi and oomycetes sense and respond to plant-exuded chemicals – movement in response to a chemical stimulus is known as chemotaxis. All these mechanisms put the plant at risk of attack from a wide-range of microbial pathogens.

Slide 6.

The relationship between the wax layer, the plant cuticle and plant cell wall is shown schematically in this slide. All these physical barriers, made up of different substances (wax, cutin and polysaccharide (cellulose), must be overcome by a pathogen before it can enter a plant cell. It’s a formidable challenge. Pathogens try to deploy an array of different enzymes and physical force (turgor pressure) to overcome these first defences – some of these are shown in the next slide.

Slide 7.

Pictured are examples of some of these processes. On the left, a spore of the powdery mildew fungus has produced a long germ tube which swells at the end to form a structure called an appressorium from which the fungus attempts to breach the epidermal cell wall by producing a narrow infection peg. In many instances, the fungus increases turgor pressure inside the appressorium to enable forceful penetration of the cell wall by the infection peg, aided by melanisation of the appressorium. In some cases, the fungus also produces cell-wall degrading enzymes to soften the cell wall and help the infection peg to penetrate. The Transmission Electron Microscope (TEM) image on the right, shows an appressorium of the bean rust fungus in close contact with the plant cell wall.

Slide 8.

Other infection strategies are used by pathogens to enter the host, ultimately trying to 'steal nutrients' from the plant to enable it to grow and reproduce. This is more information than your students need but we wanted to emphasise just how varied and complex is the battle between plant and pathogen. For example, the 'Avr' symbols represent different chemicals that are produced by fungi to try to prevent the plant from switching on its active defences when it recognizes that it is under attack. Some plants have evolved to be able to detect these 'Avr's' so that the plant is no longer fooled by the pathogen and so switches on its defences to become resistant.

Slide 9.

The plant cell wall is a very dynamic structure that as well as providing a physical barrier against pathogen entry, is a source of signaling molecules. Structurally it is made up of different mixes of polysaccharides: cellulose and hemi-cellulose with the secondary cell wall comprising almost entirely lignin, a phenolic polymer. It is the lignin that is believed to play a significant role in disease resistance in many cases.

Slide 10.

Bacterial/fungal cellulases may break down the cellulose but the lignin is more resistant. Lignification – strengthening of the cell wall – may prevent toxins and enzymes produced by the pathogen reaching the inside of the cell. Chemically the types of lignin vary between plant species but plants with higher lignin content are often more resistant. Staining for the presence of lignin or quantifying the amount accumulated in leaf tissue using spectrophotometric methods allows scientists to link the presence of lignin to pathogen attack. Lignin appears earlier and at higher levels in resistant plants in response to both fungal (stem rust on wheat) and bacterial attack (eg. in rice by *Xanthomonas oryzae* pv. *oryzae*).

Slide 11.

If the pathogen is able to enter the plant cell, there are a whole range of difficult challenges for it to overcome. These are listed here and will be considered individually in the coming slides. We will first learn about two very general plant defence responses: the production of callose and Reactive Oxygen Species (ROS). We will then consider some further responses, many of which are specific to particular plant-pathogen interactions.

Slide 12.

Callose is a high molecular weight β-(1,3)-glucan polymer: a polysaccharide. It is a major component of structures known as 'papillae' that form on the inside the plant cell wall, at the site of pathogen attack. They appear to be produced in all plants, as a general stress response and have been seen following wounding and in response to attack by insects and nematodes. The callose in the papilla is believed to provide a matrix into which other anti-microbial compounds can be deposited. Papillae are also known to contain arabinoxylan, cellulose and antimicrobial proteins and ROS.

Slide 13.

Schematic of cell wall apositions, papillae, known to contain callose.

(A) A fungal penetration attempt halted by deposition of a cell wall apposition (blue). Inset image illustrates a top-down view of the penetration site as typically visualized by light microscopy. (B) A successful penetration event in which the fungus has formed a haustorial feeding structure. The cell wall apposition materials form a neck-band or collar around the neck of the haustorium. (C) Tyloses serve a similar role in xylem vessels. Depending on their size and number they may 'clog' xylem vessels so preventing pathogen spread. They contain a variety of cell wall materials (cellulose, hemi-cellulose, pectin, lignin and phenolic compounds). (D). Light microscope of an infection peg from the powdery mildew fungus 'captured' in a papilla of the barley plant.

Slide 14.

This schematic should help students visualise where in the cell, callose papillae are formed and remind them that there must be some type of signalling pathway, from when the pathogen lands on the leaf surface via several intermediate steps that eventually result in the formation of an effective barrier to the pathogen. At the same time, this signalling pathway may have induced the formation of other responses, such as the formation of Reactive Oxygen Species (ROS).

Slide 15.

The production of Reactive Oxygen Species (ROS) is another active and general plant response, triggered not just by pathogen attack but as a response to both biotic and abiotic stresses. ROS have direct anti-microbial activity, but they are also necessary for triggering further defence reactions. It is good for students to realise that there are many and varied roles played by ROS, their specific role being influenced by the levels of these chemicals.

Slide 16.

The relationship between ROS and the hypersensitive response (HR) is also critical. The HR is only seen in plants and closely resembles the immune response seen in animals.

It is worth noting here that the OCR student guidebook incorrectly refers to this localized programmed cell death as 'necrosis'. This is not true, it is a hypersensitive response (HR) that is controlled by the plant. The term 'necrosis' generally refers to more extensive death of plant tissues that have usually been caused by the pathogen (through the action of toxins and enzymes) rather than the plant itself. HR is limited to a few cells around an infection structure and does not usually lead to visible necrosis (damaged and dead plant tissue) on the plant surface.

Pictured is a barley cell that has accumulated ROS (stained with DAB) and reacted hypersensitively, to attack by the powdery mildew fungus (not visible)

Slide 17.

We have already seen several examples of varied and active plant defence responses. Broadly these may occur in all plants. There are many more, often specific to a particular plant-pathogen interaction. These will be influenced by the method the pathogen uses to attack the plant. We will now consider some of these specific chemical responses. You may find it useful here to make an analogy with a football match between pathogen and plant. The plant may be set-up with a particular formation (defence responses). These work against the majority of pathogens (hence most plants are resistant). However, when playing a different team (different pathogen), this pathogen may employ a different formation that is able to overcome the plants defences (and cause disease).

Slide 18.

The following slides will give examples of terpenoids, phytoanticipins, phytoalexins, hydrolytic enzymes, defensins, phenolic compounds and alkaloids all of which have been implicated in different plant defence responses to microbial pathogens. To give an idea of the complexity involved in these interactions over 10,000 different structures alone have been described for the flavonoid group of phenolic compounds.

Slide 19.

Pupils may well have encountered scented plants capable of producing essential oils. Pictured is the surface of a mint leaf, photographed using a Scanning Electron Microscope. It shows the oil droplets that will contain menthol (structure shown) and menthone. As well as protecting the plant from microbes, these oils have also been utilized by humans seeking natural remedies and interested in their broader anti-microbial activity. This group of compounds are known as TERPENOIDS.

Slide 20.

Terpenoids can also act as powerful insecticides. Pyrethrin is obtained from *Chrysanthemum* plants; synthetic analogues belong to a widespread group of insecticides known as pyrethroids. Pine tree resin is also known to produce terpenoid compounds with insecticidal properties.

Slide 21.

Here is an example of a triterpenoid molecule (bound to a sugar) that is found in roots of the oat plant. It is known as avenacin and has been shown to possess anti-microbial activity, protecting the roots against attack by a number of pathogens. It is also an auto-fluorescent molecule allowing scientists to visualize its presence *in planta* using uv light. These molecules are present in the plant ahead of any pathogen attack, so are known as PHYTOANTICPINS: they are <u>a preformed</u> antimicrobial compound.

Slide 22.

A second PHYTOANTICIPIN is α-tomatidine, found in tomato plants and active against the fungus *Septoria lycopersici*. The structure of α-tomatidine and the symptoms caused by the fungus on a tomato leaf are shown.

Slide 23.

Many of the chemicals associated with plant defence are <u>actively synthesised</u> by the plant in direct response to pathogen attack (requiring energy) – these are known as PHYTOALEXINS. An example is Camalexin, synthesised from the amino acid tryptophan and made in the leaves of *Arabidopsis thaliana*, a member of the Crucifer family, <u>after</u> attack by a pathogen. *Arabidopsis* is a so-called 'model species' for Brassica crops.

Slide 24.

Hydrolytic enzymes represent another group of chemicals that help protect some plants against attack by pathogens by helping to breakdown the structural integrity of the invading pathogen. They may be active in the extracellular spaces. Three examples are given: chitinases (breaking down chitin in fungal cell walls); glucanases (breaking down glucans in cell walls of oomycetes) and lysozymes capable of degrading bacterial cell walls.

Slide 25.

Defensins are: small, highly stable, cysteine-rich peptides (small proteins) known to occur in both plants and animals, and shown to possess widespread anti-microbial activity. Interestingly, most plant defensins are anti-fungal, while those expressed in mammalian systems are primarily anti-bacterial.

Investigations into the role of defensins has focused upon the demonstration of anti-fungal activity in vitro (against pathogens grown on agar plates), although some are better characterised such as the potent seed defensins RS-AFP1 and Rs-AFP2 from radish (*Raphanus sativus* Anti-Fungal Proteins 1 and 2) . Seeds are well protected with a hard seed coat, however upon imbibing water leading to germination, the seed coat softens putting the seedling at risk of colonisation from a wide variety of soil living pathogens. Defensins are believed to provide protection as the seed germinates and grows. Other defensins have been found in other plant parts and from many plant species.

Slide 26.

Phenolic compounds are a very large class of secondary metabolites. These compounds vary structurally from simple molecules such as Scopoletin – a coumarin - comprising a phenyl ring linked to a benzene ring (giving a 1-benzopyran structure), to more complex polymers seen in later slides. Growth of the rubber tree in the Amazon basin is hampered by a fungal disease known as South American leaf blight (*Microcyclus ulei*). Scopoletin has been shown to be induced in partially resistant clones of the tree with the degree of resistance related to the rapidity and intensity of scopoletin accumulation

Slide 27.

The flavonoids are another large class of phenolic compounds, many members of which possess anti-microbial activity. A role in disease resistance has been shown for anthocyanin and catechin (note the phenyl ring), protecting crab apple against Cedar apple rust (*Gymnosporangium yamadai*). In varieties of apple with low levels of these compounds, disease symptoms (such as those shown) are common. Human health benefits have also been shown for many flavonoid molecules found in a diverse range of foods. They are potent scavengers of Reactive Oxygen species, reducing the cellular damage caused by these compounds.

Slide 28.

Also worthy of note, particularly in plant-insect interactions, are the condensed tannins. Tannins are the reason for the residues left in cups of tea, but at certain doses can be lethal to insects.

Slide 29.

Finally, the alkaloids. Another large diverse class of compounds this time all containing nitrogen, and including many compounds the names of which should be familiar to your students. As well as its anti-fungal and insecticidal uses, coffee plants use their own plant chemicals, in this case caffeine, as a way of gaining a competitive advantage over other plants for space and nutrients, a phenomenon known as allelopathy.

Slide 30.

We have covered lots of information in the preceding slides, and this table summarises that information reminding students of the key **defence compounds** they are required to learn about. The details of the specific interactions we have cited is probably beyond their needs but serves to emphasise the specificity of these responses. (F) - fungus

Slide 31.

This slide serves to summarise visually the examples we have highlighted earlier of plant defence responses. We have simplified our understanding of these processes quite significantly, and even the information in the next slide (closer to our current understanding) - which tries to compare plant and mammalian systems will be a simplification again of the complex interactions between plants and pathogens. Those students who wish to read more about this fascinating and rapidly advancing area of biology should be directed to the further reading on the related Information Sheet.

Slide 32.

The detailed information in this slide is not required by students. It serves to emphasise that both plants and animals first DETECT and then RESPOND to pathogens in many different ways through a variety of signalling and biochemical pathways. Plants lack a true immune system (since unlike mammals, they lack mobile defender cells and a somatic adaptive immune system), they do however rely on the innate immunity of each cell and on systemic signals coming from pathogen infection sites.

Slide 33.

SUMMARY.