Contents

Contents 2

Introduction 3

Suggested activities 5

Task 1 5

Task 2 6

Task 3, 4 and 5 8

Task 5 answers: 11

Task 6 12

Extension activities that are associated with the activities for stretch and challenge 13

Supporting information 13

Useful sites 14

This Topic Exploration Pack should accompany the OCR resource 'GCSE (9–1) Gateway Biology A Plant Diseases' learner activities, which you can download from the OCR website.

This activity offers an opportunity for English skills development.

Plant Disease

Introduction

In topic 6.3g, h and i, learners have the opportunity to explore the range of responses that plants have to the invasion of pathogens. Plants are subject to attack by many different pathogens, which can cause disease or damage to the plant. In section 6.3f, learners were introduced to three examples of plant disease:

- viral tobacco mosaic virus (*TMV*) tobacco mosaic virus disease
- fungal *Erysiphe graminis* barley powdery mildew
- bacterial *Agrobacterium tumafaciens* crown gall disease.

Although the importance of the study of animal disease is often clear for learners, the importance of studying plant disease is often overlooked. As plants are primary producers their role in the ecosystems cannot be underestimated. Any disease affecting plants can have a catastrophic effect on food chains/webs. It is important that learners are aware of the importance of the study for crops and food security but also for potential human medicinal properties.

In topic 6.3g, learners will look at the physical responses that plants can have towards infection by disease. In particular, focusing on the role of the leaf cuticle and cell wall as a first line of defence. This can be compared to the primary line of defence in humans. Learners will need to have a good recollection of the structure of the plant cell and the leaf so they can fully explain the adaptations plants can put in place as a result of pathogen invasion. Task 2 reviews the structure of a plant and leaf structure.

In topic 6.3h, learners will look at the chemical responses to invasion of pathogens. This can be compared to the immune response that humans have when a pathogen will invade. Task 1 focuses on highlighting these comparisons, particularly the passive and active responses of plants and animals. Some select examples of preformed and inducible antimicrobial plant substances will be given: the phytoanticipins and phytoalexins and the non-specific response of cell wall strengthening by deposition of callose, lignin and suberin. This topic is vast and it is important that learners are aware that studies in this area are still being carried out. Awareness of the potential of these plant antimicrobial substances for both human and veterinary medicine and transgenic crops should be covered.

Finally in topic 6.3i, higher level learners will identify the different ways that plant disease infection can be identified. This will look at simple observations of symptoms in the field by plant pathologists and by microscope examination of the sample, through to more advanced laboratory-based detection of the antigen or DNA from the pathogen using enzyme-linked immunosorbent assay (ELISA) and the polymerase chain reaction (PCR). Task 3, 4 and 5 look at the stages involved in carrying out these more technical observations. Learners commonly misinterpret the meaning of the term observation as just looking at the specimen. Task 6 highlights this misconception by giving learners a series of observations made when identifying plants infected with: *TMV*, *E. graminis*, *A. tumafaciens*.

Learning outcomes

1. Describe physical plant defence responses to disease
2. Describe chemical plant defence responses
3. Describe different ways plant diseases can be detected and identified in the lab and in the field (Higher).

Suggested activities

The following suggested activities look at particular aspects of this topic.

Task 1

Task 1 compares the primary and secondary responses of plants to the more familiar animal responses. Learners are given an empty print out of the table and then asked to sort the statements into the correct place. This also links to knowledge that will be required for section 6.3i-l. Have a discussion with learners about the difference between a primary and secondary response and what the difference is between active and passive immunity. Answers to the task are:

	Primary defences	Passive secondary defences	Active/induced secondary response
Plant	Barriers (e.g. waxy cuticle & cell wall)	Preformed antimicrobial substances – the phytoanticipins (e.g. glucosides, saponins)	Antimicrobial substances – phytoalexins (e.g. genistein or camalexin) Hypersensitive response – burst of oxygen to trigger antimicrobial response Antimicrobial enzymes (e.g. chitinases) Cell wall reinforcement (e.g. callose, lignin, suberin, cell wall proteins)
Animal (e.g. human)	Barriers (e.g. skin, hairs in nose, hydrochloric acid in stomach etc.)	White blood cells (e.g. phagocytes)	White blood cells (e.g. lymphocytes) Histamine release – to attract blood cell

Task 2

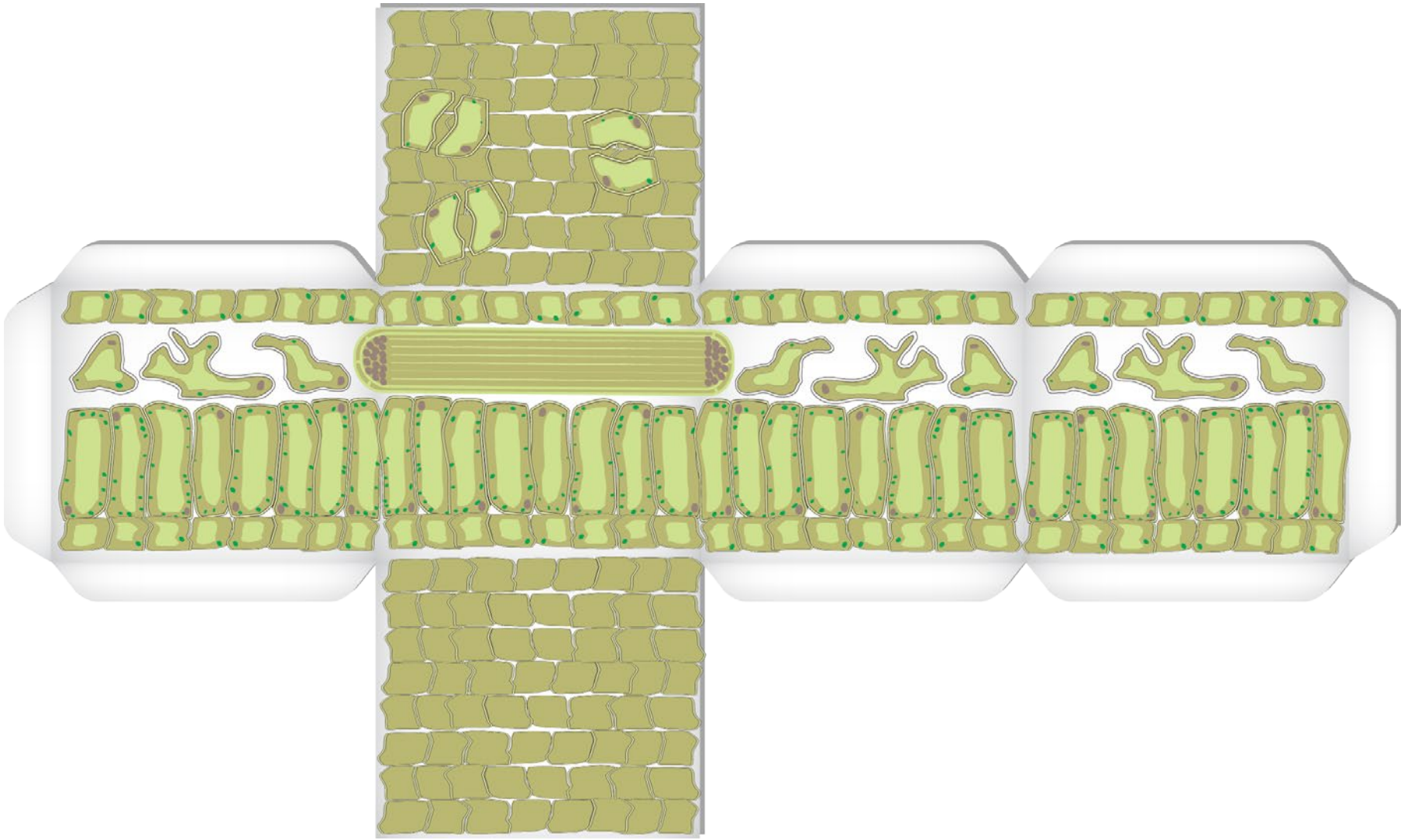
This activity is to make a 3D structure of a leaf section. You may find it useful to have microscopes and TS leaf section slides available as an extension to this activity. This will enable learners to identify the main sections of the leaf. Learners may be more familiar with the function of the cuticle in preventing water loss. It is important that at this stage, learners become aware of their role in primary defence for a plant against pathogens.

As an extension activity, the learners could compare the leaf cuticle to human skin and emphasise its role as a barrier by producing another net model of the skin. The learners should be able to detect that both of the sections have many layers, including an epidermis at the surface. They have good transport to the area in vascular bundles and blood vessels.

Learners can use ‘Help sheet 1’ included in the activity document to guide them through this task.

A leaf

Make a 3D model leaf section. Draw in detail at least one palisade cell.



Task 3, 4 and 5

Task 3, 4 and 5 allows learners to explore the main molecular methods that can be carried out to identify plant disease. These include the more advance methods of ELISA and PCR. There are a number of animations and activities online that can support this task and give the learners more of a visual idea of the process. These can be found in the useful sites section at the end of this document. Learners may ask how an antibody is isolated for the ELISA test when plants do not produce antibodies. This is because antibodies can be obtained from the plasma of an animal, commonly a rabbit, after it has been injected with the plant virus/or isolated plant pathogen antigen.

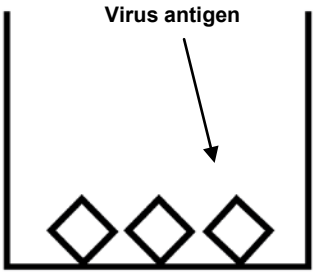
Please note that there are many different ways to perform an ELISA. The simplest is an antigen-trap ELISA (or PTA ELISA). This is the easiest ELISA to explain to learners. Other methods require more than one antibody and will necessitate the description of more than one host animal and antibodies that detect other antibodies.

Sample answers for these tasks can be found on the following pages:

Task 3 answers:

Add plant sample

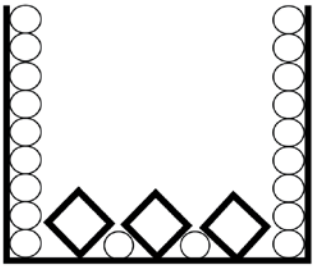
1.



WASH 1

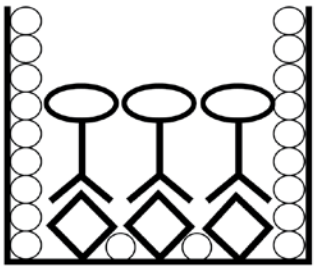
Block

2.



Add anti-plant virus specific antibody complexed with enzyme

3.



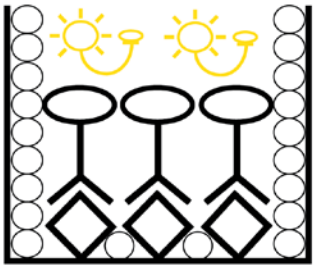
Antibody attached to antigen

WASH 2

Add substrate. Detect colour change.

WASH 3

4.



Colour change

Task 4 answers:

1. What does PCR stand for?

Polymerase chain reaction.

2. What is the purpose of PCR?

A procedure that allows you to make billions of identical copies of a section of DNA.

3. What are the 4 nucleotides that make up DNA?

ACGT

4. What is a primer?

A short piece of DNA made in the laboratory. They are designed to match the ends of a DNA segment that you want to make copies of. You will need 2 primers; one for the beginning and one for the end of the DNA segment.

5. What is the role of DNA polymerase?

DNA polymerase is an enzyme that reads the DNA code and makes a copy of it. The DNA polymerase starts attaching nucleotides at a primer.

6. Why is the DNA heated to 95°C?

To separate/unzip the DNA double helix into two separate single stranded DNA molecules.

7. What property must a PCR tube have?

Designed to withstand even heat distribution.

Task 5 answers:

1.

Obtain sample (eg blood at scene of crime) →

2.

Extract DNA using a commercially available kit ↓

3.

Add primers (TWO), nucleotides and DNA polymerase to the DNA sample in an Eppendorf tube ←

4.

Add the tube to the thermo cycler ↓

5.

Denature DNA at 95°C

```
ATGCGTAGGGCTTAGCTTTCGGATTCTTCTTGCTATTC
TACGCATCCCGAATCGAAAGCCTAAGAAGAACGATAAG
                    ↓
ATGCGTAGGGCTTAGCTTTCGGATTCTTCTTGCTATTC
                    +
TACGCATCCCGAATCGAAAGCCTAAGAAGAACGATAAG
```

6.

Cool to 55°C this allows the primers to anneal

```
ATGCGTAGGGCTTAGCTTTCGGATTCTTCTTGCTATTC
TACGCA               +
                                CTATTC
TACGCATCCCGAATCGAAAGCCTAAGAAGAACGATAAG
```

7.

Heat to 72°C the optimum temperature for the enzyme

```
ATGCGTAGGGCTTAGCTTTCGGATTCTTCTTGCTATTC
TACGCATCCCGAATCGAAA→
                    +
                    ←CGGATTCTTCTTGCTATTC
TACGCATCCCGAATCGAAAGCCTAAGAAGAACGATAAG
```

8.

You have now turned one piece of DNA into two

```
ATGCGTAGGGCTTAGCTTTCGGATTCTTCTTGCTATTC
TACGCATCCCGAATCGAAAGCCTAAGAAGAACGATAAG
                    +
ATGCGTAGGGCTTAGCTTTCGGATTCTTCTTGCTATTC
TACGCATCCCGAATCGAAAGCCTAAGAAGAACGATAAG
```

9.

Now repeat steps 5-7 34 times and you will have made 9 billion copies

Task 6

Task 6 looks at the term 'observation' and how this is used in the field for disease diagnosis. It is important that learners are made aware that observations are not simply things that are seen with their own eyes but plant pathologists often use: hearing, feeling, smelling. Learners are given a number of observed 'clues' to match to the specific plant disease. It is important that the learner has covered the main points about the three diseases in 6.3f.

Match the observations to the plant disease.

Barley powdery mildew	Crown Gall disease	Tobacco mosaic virus
1. Circular, powdery white spots on upper surface of the leaf 2.  4. Presence of fungus *Erysiphe graminis* 9. Fungal hyphae are produced on both upper and lower leaf surfaces	3. As galls grow, plants often become stunted, weak and may eventually die 7. Presence of bacterium *Agrobacterium tumefaciens* 8. Tumour-like growths above soil level 10. 	5. 'Mosaic'- like mottling and discolouration on the leaves 6. 

Extension activities that are associated with the activities for stretch and challenge

1. More able learners could look into investigating the following question:

Why do they put mint in toothpaste? Would garlic be better?

A suggested practical is available at: http://www.markedbyteachers.com/as-and-a-level/science/investigation-into-the-antibacterial-properties-of-mint-and-garlic.html

2. Learners investigate the beneficial properties to humans of the drug digitalis found in foxgloves. This is naturally produced by the foxglove as a defence toxin.

Supporting information

There are a number of responses of plants to defence against pathogens. Some of these chemical responses are still being researched and only a few samples are given in Task 1. Phytoanticipins and phytoalexins are introduced as two groups of chemicals produced in an attack from plants. A good way to remember their action is that phytoanticipins are 'anticipating' the response; they are already present and are generalised. The phytoalexins are induced once a pathogen has invaded. A basic level of understanding of the structure of microorganisms will allow learners to access the impact of the antimicrobial enzyme chitinase, which acts to break down fungal cell walls and prevent invasion. In addition to this, it may be useful to compare the roles of the hypersensitive oxygen burst released from plants to the release of histamine in humans in triggering the specific secondary response in plants and animals.

Useful sites

Website summarising generalised plant defence.
http://www.apsnet.org/edcenter/intropp/topics/Pages/OverviewOfPlantDiseases.aspx

Easy access information about ELISA techniques.
http://www.apsnet.org/edcenter/intropp/topics/Pages/PlantDiseaseDiagnosis.aspx
http://www.elisa-antibody.com/ELISA-Introduction
https://www.boundless.com/microbiology/textbooks/boundless-microbiology-textbook/immunology-applications-12/diagnostic-immunology-148/enzyme-linked-immunosorbent-assay-elisa-748-10812/

This animation discusses the main process of ELISA. This could be watched before completing task 4.
http://highered.mheducation.com/sites/9834092339/learner_view0/chapter18/elisa_enzyme-linked_immunosorbent_assay.html

This website has an animation on how PCR is completed. Learners can then relate this to how plant disease is detected.
http://www.isaaa.org/resources/publications/pocketk/22/default.asp
http://learn.genetics.utah.edu/content/labs/pcr/

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