Koch's postulates for plant pathogens

Apples infected with *Monilinia fructigena*, the cause of brown rot

British plums infected with *Monilinia fructigena*, the fungus also causes brown rot of other fruits

Classification:
Kingdom, Fungi; **Phylum (Division),** Ascomycota; **Class**, Leotiomycetes; **Order**, Helotiales; **Family**, Sclerotiniaceae; **Genus**, *Monilinia*; **Species,** *M. fructicola.*

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Introduction

During the late 19th century a bacteriologist called Robert Koch laid down a set of rules for confirming that an organism is the cause of a disease. These are now known as 'Koch's postulates'.

When a plant becomes infected with a fungus (or any other disease causing microorganism), it is likely to become weakened and therefore more susceptible to infection by other microbes. So how do us plant pathologists work out what pathogen has caused a particular disease? We have to apply Koch's postulates to the disease.

To determine Koch's postulates:

(a) the organism must be consistently associated with the lesions of the disease;
(b) the organism must be isolated from the lesions and grown in pure culture;
(c) the organism from pure culture must be re-inoculated into the healthy host and must cause the same disease as was originally observed;
(d) the organism must be re-isolated into culture and shown to be identical to the organism originally isolated.

Aim:
To demonstrate Koch's postulates using apples infected with the brown rot fungus, *Monilinia fructigena*,.

Length of practical:
Four double lessons (60-120 minutes each session).

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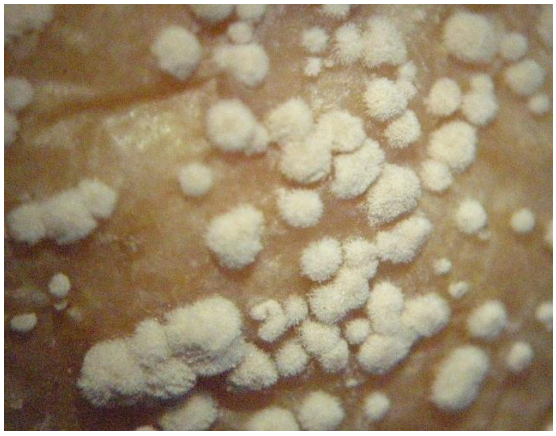
Koch's postulates: brown rot

To determine which microbe caused that fuzzy stuff on your apple you must:

1. Describe the symptoms you see on the infected apple and isolate the suspected fungus pathogen responsible.
2. Isolate the fungus in pure culture (this means grow the fungus on its own, away from the host plant (apple) and without any contaminating microorganisms).
3. Use the fungus that you isolated in pure culture to inoculate a healthy apple.
4. Record the symptoms that develop on the healthy apple following infection with the cultured fungus. Are your observations the same as recorded previously?
5. Re-isolate the fungus and check that it is the same as observed initially.

Materials needed

1. Apples infected with *Monilinia fructigena*
2. Slides and coverslips
3. Cotton blue stain
4. Stereo & compound microscope
5. Imersion oil
6. Sterile scalpels
7. Potato dextrose agar (PDA) plus antibiotic
8. Fresh apples



A close up of the infected apple showing pustules (which contain spore bearing structures) *of Monilinia fructigena.*

Practical Session 1

Session 1:

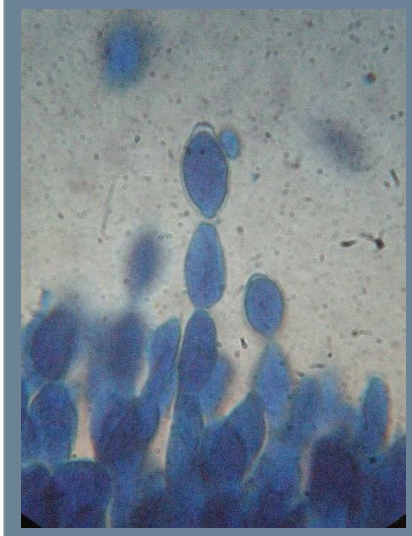
You are provided with:

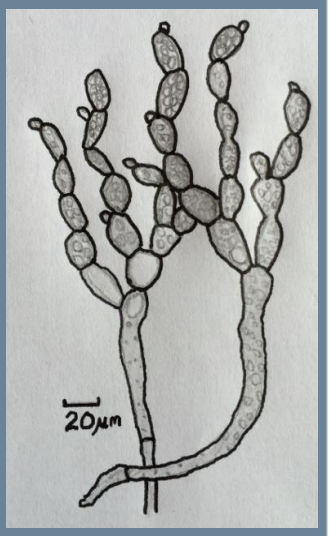
- *An apple infected with the fungus Monilinia fructigena;*
- *Three plates of Potato dextrose agar (PDA) supplemented with antibiotic (to control bacteria);*
- *Microscopes, slides, cover slips and cotton blue stain*

Method:

1. Carefully record the symptoms of the disease. Examine the fruit externally. What do you see?
2. Slice through the infected apple. Are there any colour and texture changes in the infected fruit? Observe the symptoms using a microscope and compare them with a healthy apple (control).
3. Using a sterile scalpel blade, carefully remove a pustule (containing reproductive structures of the fungus) from the infected apple and place it on a microscope slide. Place a drop of cotton blue stain onto the pustule and cover with a coverslip. Lightly press down the cover slip and use a circular motion to squash the pustule. This is best done using the end of a pencil. Take care not to crack the coverslip. Record your observations.
4. Using a sterile scalpel blade isolate small pieces of tissue from the edge of the infected apple and place one piece of tissue at the centre of each plate of PDA.

A healthy apple (control) on the left compared with an apple infected with *Monilinia fructigena* on the right.





Spores (conidia) of *Monilinia fructigena* under high magnification and an artist's impression of spore bearing structures with conidia

The plates will be incubated at 25°C and returned to you in session 2.

Practical Session 2

Session 2:

You are provided with:

- *Isolation plates from session 1;*
- *Healthy apples;*
- *Sterile scalpel;*
- *Microscopes, slides, cover slips, cotton blue stain.*

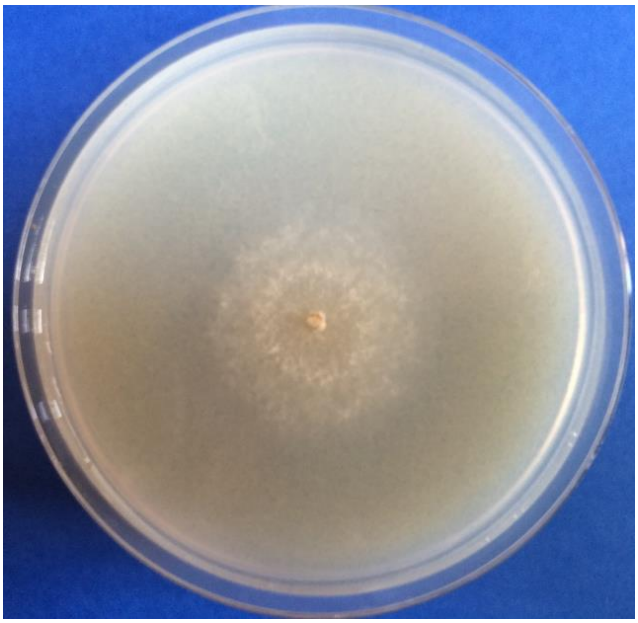
Method:

1. Carefully examine the plate cultures of the micro-organism isolated from the apples.

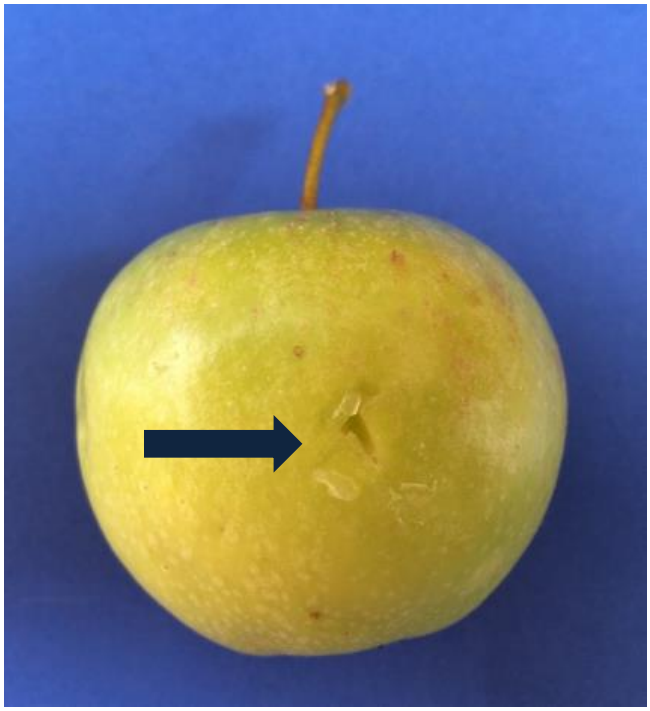
2. Inoculate a fresh apple with the micro-organism that you have isolated. Sterilise the apple surface by wiping with 70 % ethanol, remove a piece of fungal colony with a sterile scalpel and insert it into the healthy apple.

3. If time permits, remove a small amount of the fungal culture (using a sterile scalpel) and place on a microscope slide. Stain with cotton blue and observe under the microscope following the same method as described previously. Look particularly for the characteristic conidia (asexual spores) of *Monilinia*.

The apples will be incubated at 25^{o}C and returned in session 3.



Monilinia fructigena growing on PDA agar (4 days post inoculation at 25^{o}C)



Healthy apple inoculated with fungal plate culture from session 1 (as shown above). The arrow indicates point of inoculation.

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Practical Session 3

Session 3:

You are provided with:

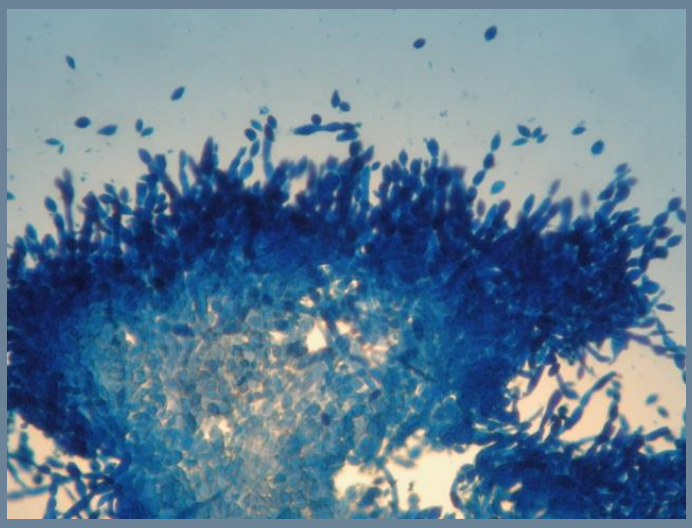
- *The apples which were inoculated in session two with the micro-organism isolated on PDA plates from the rotted apples supplied in session one;*
- *Sterile scalpel*
- *Microscopes, slides, cover slips, cotton blue stain.*
- *3 plates of potato dextrose agar (PDA) supplemented with an antibiotic (to control bacteria).*

1. Compare the symptoms of disease in the inoculated apples with those recorded in session 1. Are they identical, or are there differences?

2. You must now re-isolate the micro-organism from the apple inoculated in session 2, to show that it is identical to the organism originally isolated.

The plates will be incubated at 25^{o}C and returned in session 4.

Apple inoculated with a pure culture of *Monilinia fructigena* shows symptoms of brown rot. Arrow shows point of inoculation.



A conidial pustule of *Monilinia fructigena*, stained with cotton blue and squashed under a cover slip. The pustule contains spore bearing structures of the fungus and was viewed using a compound microscope (x10 magnification).

Practical Session 4

Session 4:

You are provided with:

- *The re-isolation plates inoculated in session 3.*

1. Carefully examine the cultures.
2. Use the skills that you have learnt in the previous sessions to examine the cultures using microscopy.
3. Look for the characteristic spore bearing structures.

Safety Notes

- No eating, drinking or chewing in the laboratory
- Wear a lab coat or apron
- Keep lids on cultures when not in use
- Wear disposable gloves when using cotton blue stain.
- Wash hands before and after the practical work.

Monilinia fructigena is deemed low risk; however, it is advisable to wear a face mask when dealing with sporulating fungal cultures.

All Images are credited to: Dr Ali Ashby

Assessment:

In the assessment, students address the following questions:

1. Decide whether you have proven Koch's postulates for this disease.
2. What additional experiments would you wish to perform in order to further substantiate your conclusions?

Useful references:

Monilinia fructigena data sheet from CABI: (http://www.cabi.org/isc/datasheet/34747).

Diagnostic fact sheet for *Monilinia fructigena* from the USDA: http://nt.ars-grin.gov/taxadescriptions/factsheets/index.cfm?thisapp=Moniliniafructigena

Note:

The preferred scientific name for this fungus is *Monolinia fructigena*; however, it is sometimes referred to as *Sclerotinia fructigena* (*Sclerotinia fructigena* (Pers.: Fr.) J. Schröt. 1893). Its common name is brown rot.

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