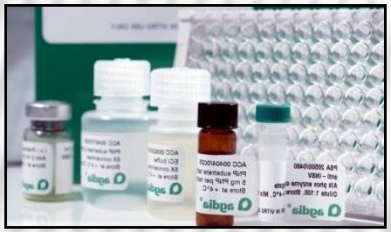
TWINNING PROJECT BA/12/IB/AG01

" Act. 3.3 „Train laboratory staff on laboratory methods for diagnosing harmful organisms"

Source: www.zeiss.com



Source: http://www.agdia-biofords.com

LABORATORY METHODS USED IN THE QUARANTINE MYCOLOGY

THIS PRESENTATION IS FOR ILLUSTRATIVE AND EDUCATIONAL PURPOSES ONLY

dr. Grażyna Szkuta
Main Inspectorate of Plant Health and Seed Inspection
Central Laboratory – Mycology Section, Toruń, Poland.

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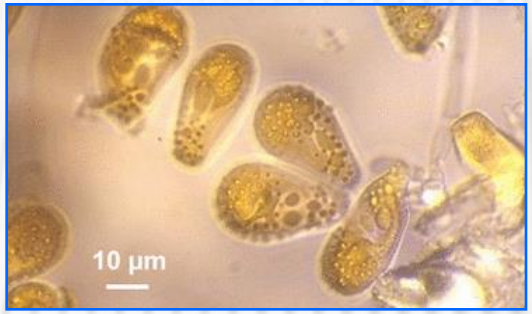
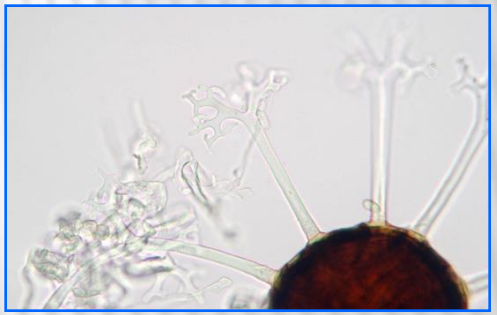
LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

Direct test with microscopy – this method is necessary for detection and identification of obligatory fungi and fungi-like organisms, e.g. smuts, rusts, downy and powdery mildew.

(source: https://wikipedia.org)



(source: http://www.eppo.int)



(source: http://www.bioimages.org.uk)

(picture: G. Szkuta)

(source: http://www.eppo.int)

LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

Moist chamber technique – this method is used for stimulation of fungi sporulation (e.g. immature fruits, lack of etiological signs, disease symptoms are observed only.

- Moist chamber is a container can consist the following materials: filter paper, lignin or cotton, cloth, sterile sand or soil that can be kept moist for several days even weeks.
- The specimen is placed on top of the moist material and left until fungi begin to grow on it.
- Moist chambers can be used for all kinds of materials: wood, leaves, old stems, corn stalks, bark, seeds, fruits.
- In order to obtain a pure culture of fungi sporulated in a moist chamber it is necessary to transfer mass of spores with sterile needle on apropriate agar media.

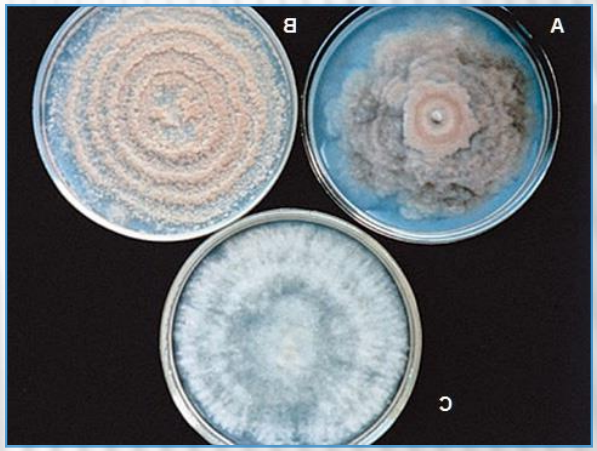
(pictures: G. Szkuta)

LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

Isolation (plating methods) – this method is used for separation of pathogens from their hosts in order to obtain a pure culture of a pathogen. Direct or indirect methods are used.

- Direct isolation is done when fungi are sporulated. In this case spores or fruits of fungi (picnidium, apothecium or perithecium) can be picked off with a sterile needle and put on a suitable agar media.

- If we do not observe sporulation of fungi or diseased border are seen we can use indirect isolation.



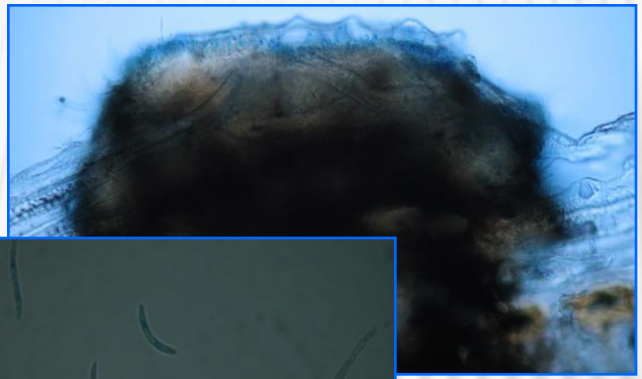
(source: http://www.eppo.int)

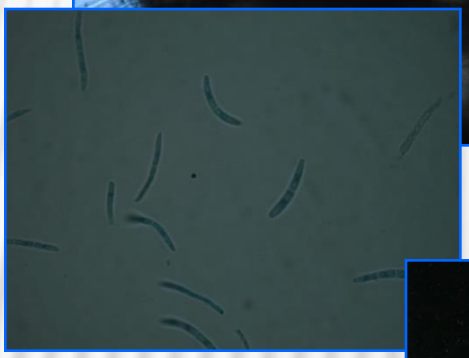
(picture: G. Szkuta)

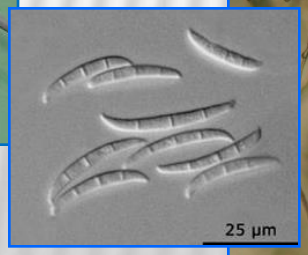
LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

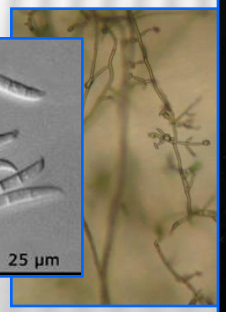
Cultural and morphological analysis – this method is used for study of culture pattern, type of conidiogenesis, sporulation, characteristics details of spores typical for the species, etc.

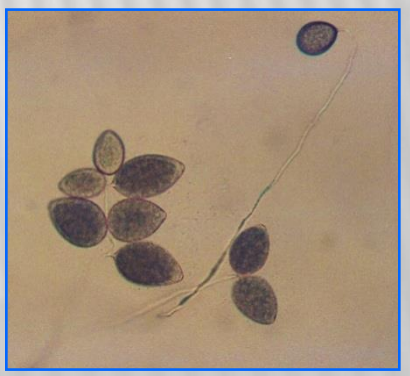
(source: https://wikipedia.org)











(pictures: G. Szkuta)

LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

Bating test - technique widely used for detection of *Phytophthora* species, *Ceratocystis platani*, etc.

Criteria for the ideal bait in *Phytophthora* bioassay according to Olaf Ribeiro are:

- Susceptibility to most if not all root-infecting *Phytophthora* spp.
- High sensitivity, especially when inoculum levels is low.
- The bait should be of reasonable size
- The bait should be fairly inexpensive
- Ready availability of baits, geographically and seasonally
- Convenience of use in setting up an assay and in subsequent isolating procedures.

(pictures: G. Szkuta)

LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

Pathogenicity test – the principle of this technique is the ability of an organism to cause disease symptoms.

Robert Koch established four criteria to identify the causative agent of a particular disease, these include:

- the microorganism or other pathogen must be present in all cases of the disease;
- the pathogen can be isolated from the diseased host and grown in pure culture;
- the pathogen from the pure culture must cause the disease when inoculated into a healthy, susceptible host plants;
- the pathogen must be reisolated and shown the same characteristic as the originally inoculated pathogen;

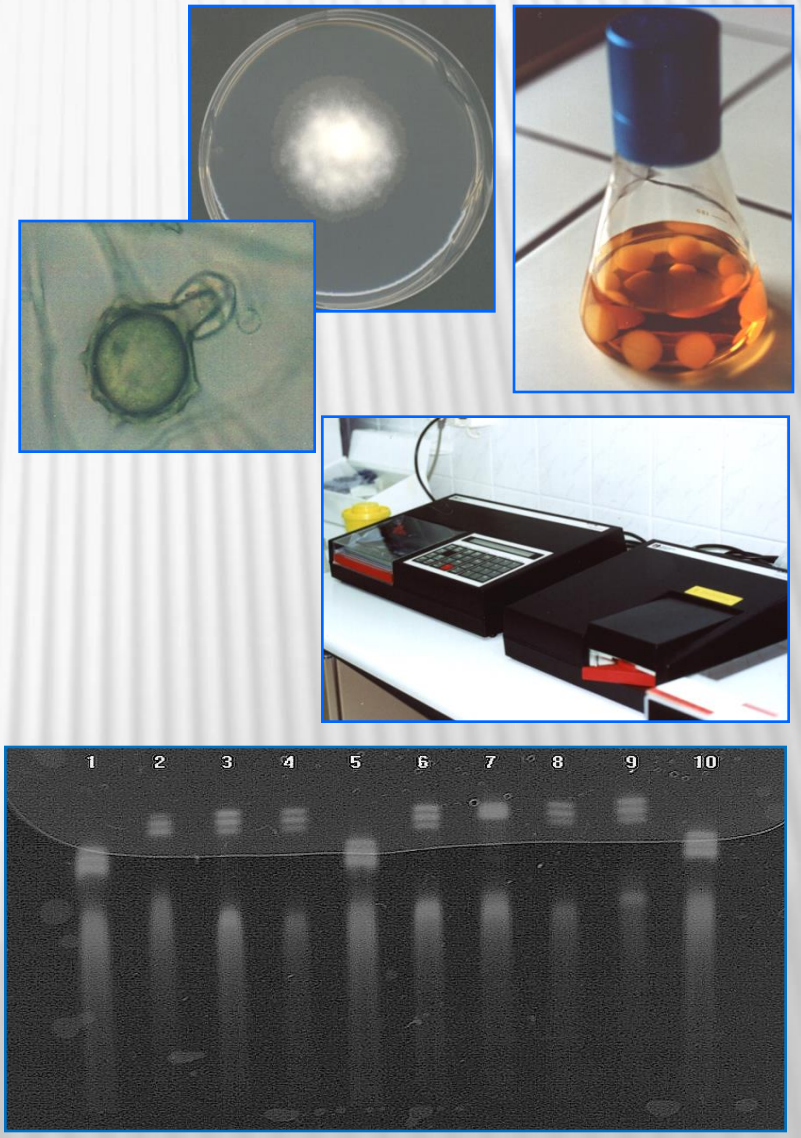
(pictures: G. Szkuta)

LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

Isozyme analysis – this method is used for identification of *Phytophthora* species (mainly hybrids).

- The principle of isozyme analysis is using specific, sensitive histochemical reactions to detect position of products of low copy genes (enzymes) on a polyacrylamide gel after electrophoresis.

- The differential electrophoretic mobility of enzymes makes it possible to visualize the enzymes on gels as specific bands corresponding usually to allelic products.

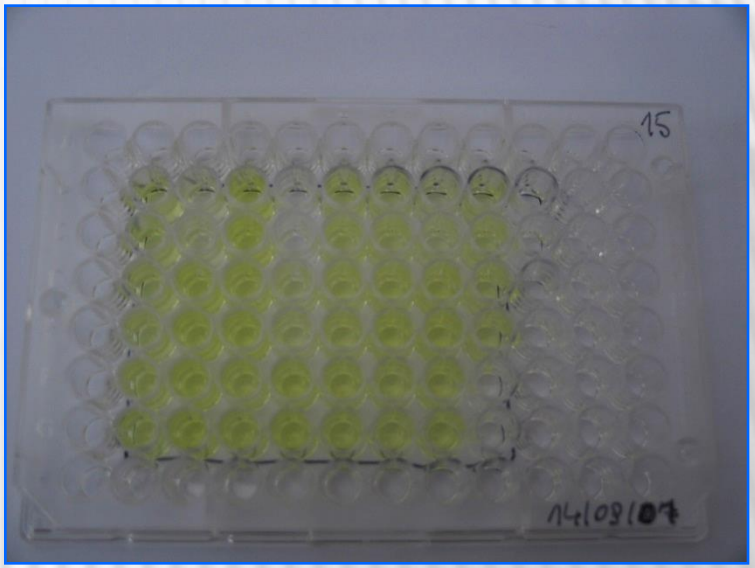


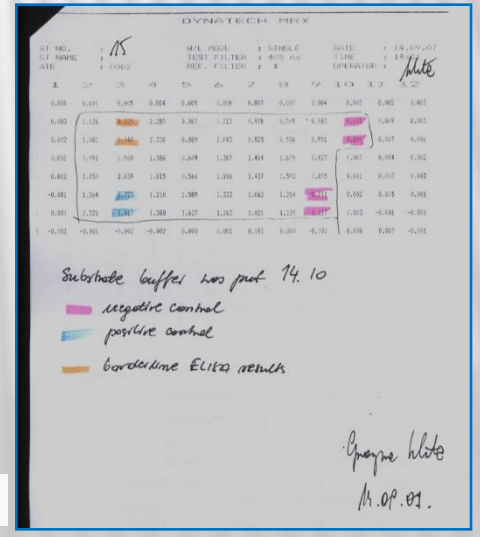
(pictures: G. Szkuta)

LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

ELISA test – this technique was used for *Colletotrichum acutatum* identification.

- This method combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme.
- ELISA can provide a useful measurement of antigen or antibody concentration.
- There are two main variations on this method: The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen.





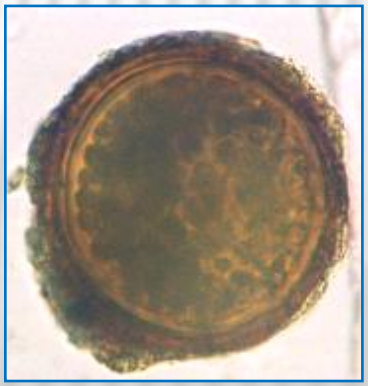
(pictures: G. Szkuta)

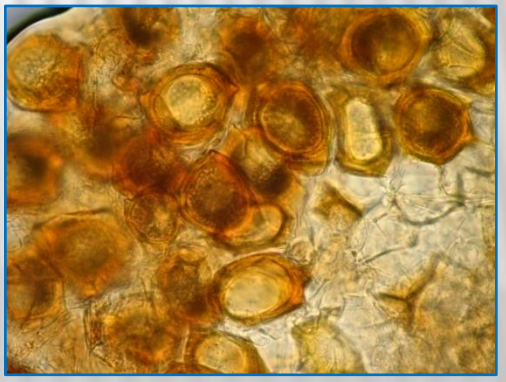
LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

Wet-sieving and centrifuging flotation in saturated solution of calcium chloride – this method is used for detection of *Synchytrium endobioticum* resting spores in soil samples.

(source: (http://www.johnmorris.com.au)

(source: www.labindex.pl)

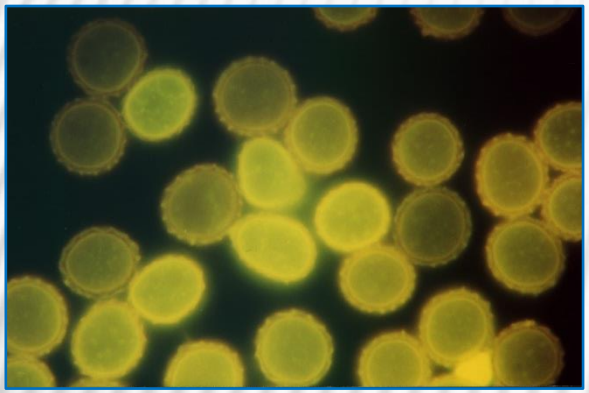


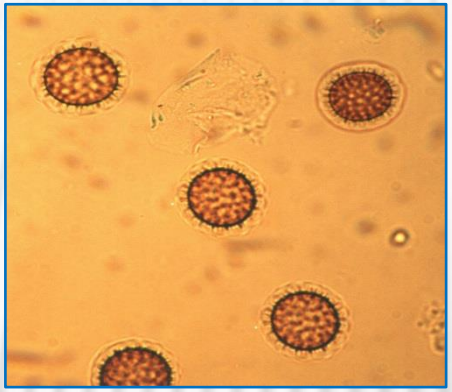


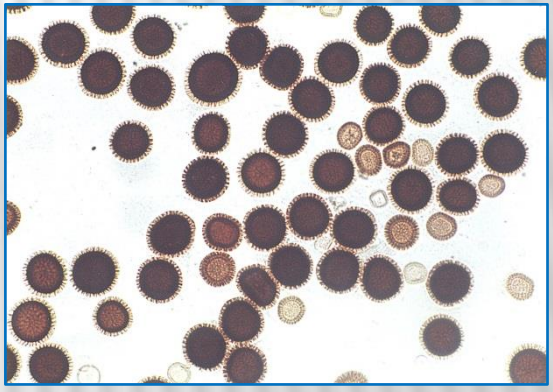
(pictures: G. Szkuta)

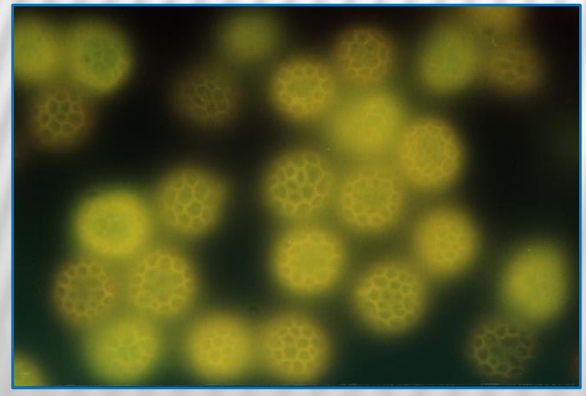
LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

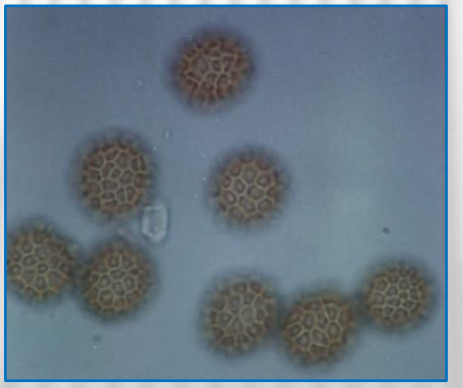
Washing and centrifuging – this method is used for detection of many fungal pathogens carried on the surface of seed as the teliospores of smuts and bunts, including *Tilletia indica* i other *Tilletia* spp., the oospores of downy mildews and various spore stages of rust fungi.









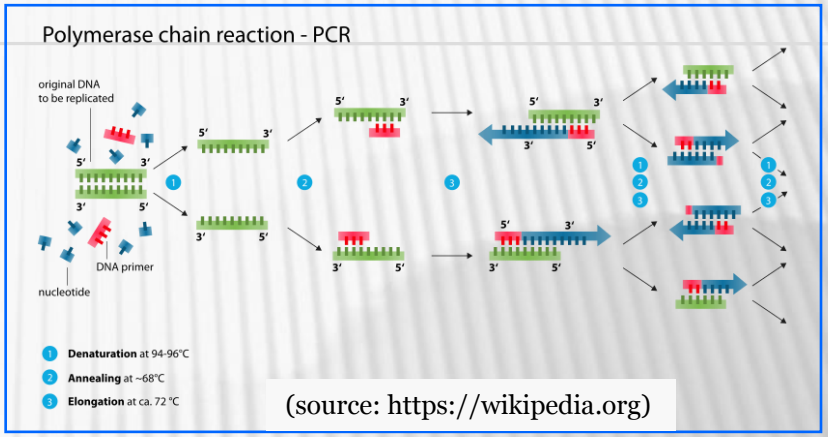


(pictures: G. Szkuta)

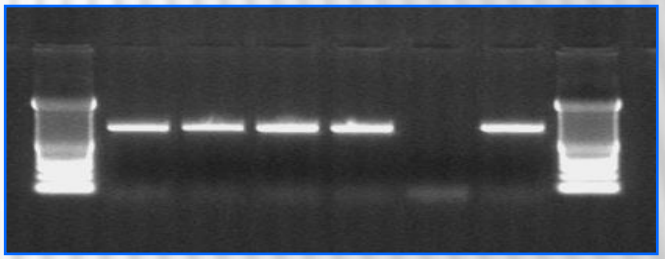
LABORATORY METHODS USED IN QUARANTINE MYCOLOGY

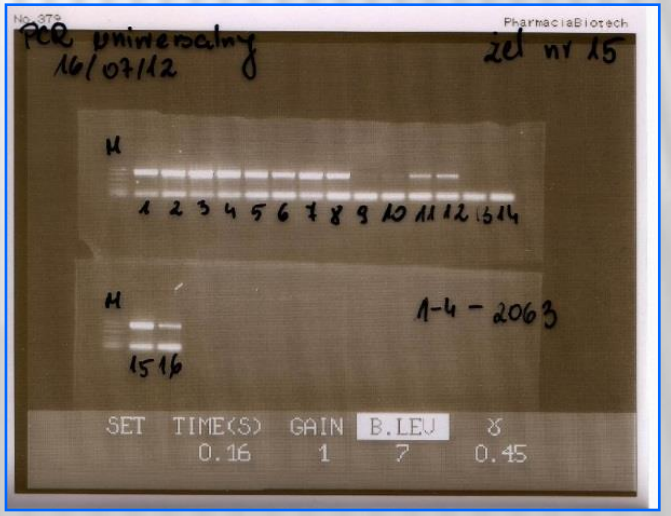
Polymerase Chain Reaction (PCR) is a powerful method for identification of fungi and fungi-like organisms.

This procedure is carried out entirely biochemically, that is, in vitro. PCR was invented by Kary Mullis in 1983. He shared the Nobel Prize in chemistry with Michael Smith in 1993.
PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3` end of a custom-designed oligonucleotide when it is annealed to a longer template DNA. Thus, if a synthetic oligonucleotide is annealed to a single-stranded template that contains a region complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3` end to generate an extended region of double stranded DNA.



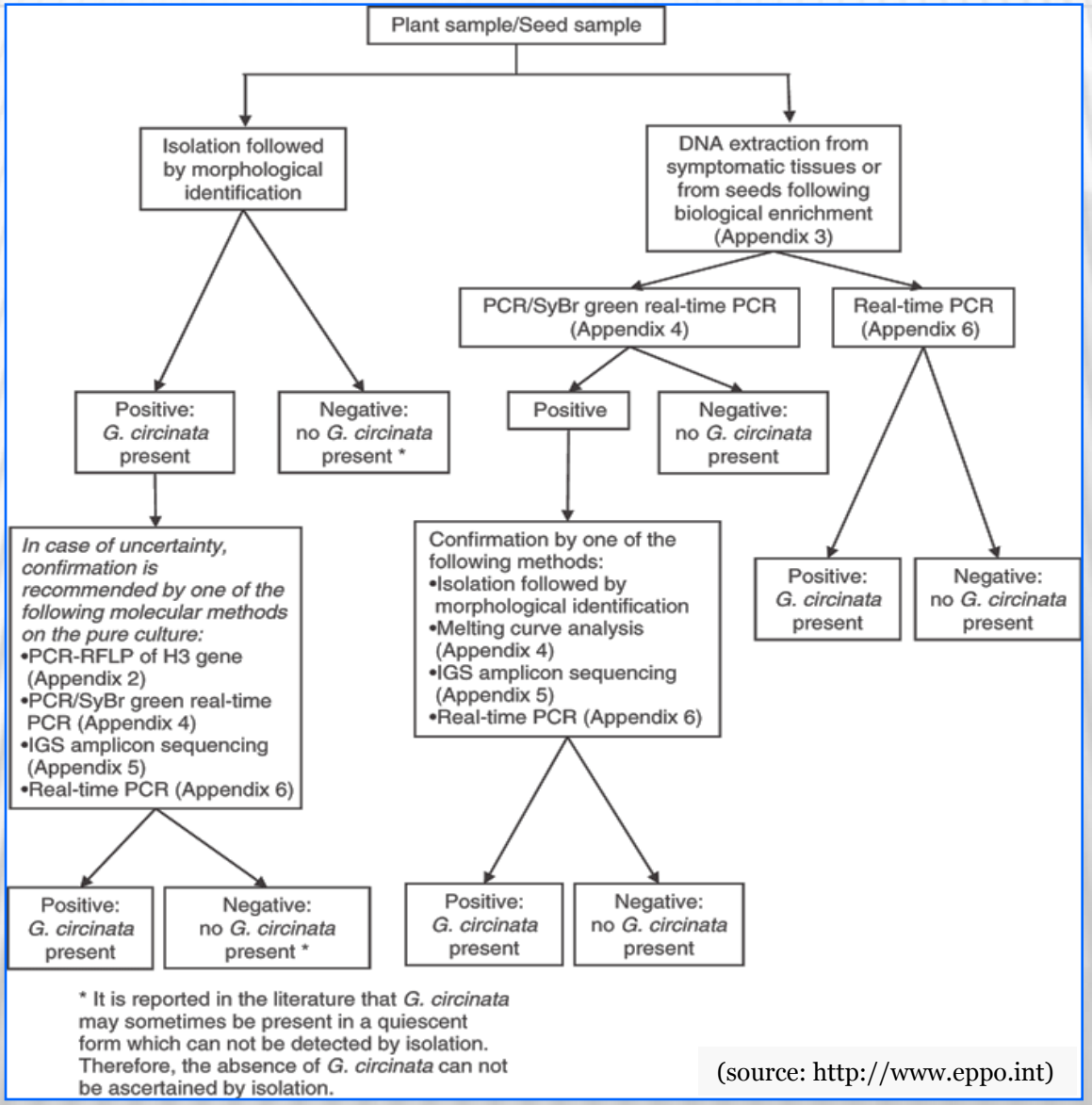
(source: https://wikipedia.org)





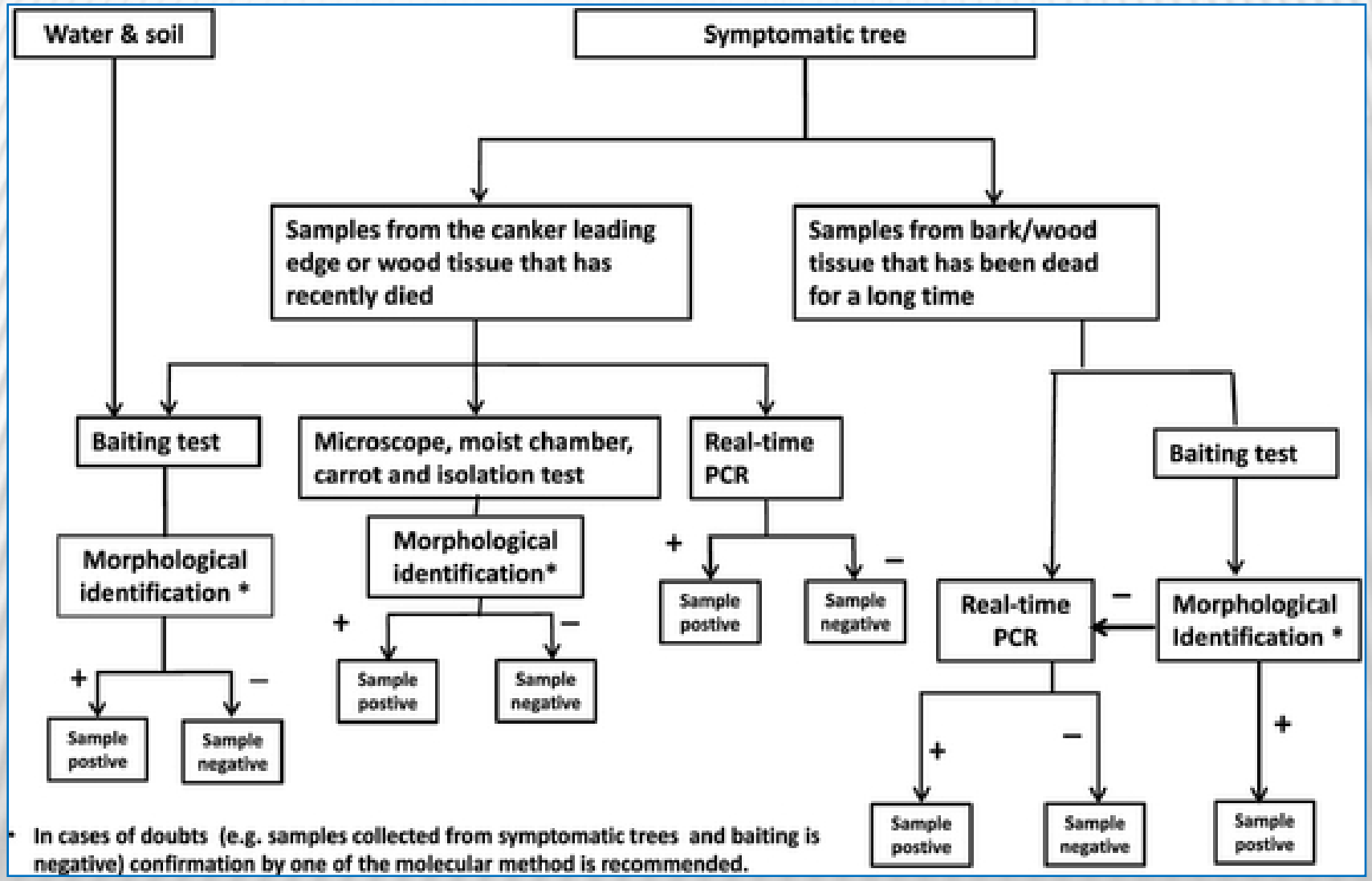
(pictures: G. Szkuta)

FUSARIUM CIRCINATUM – EPPO DETECTION SCHEME



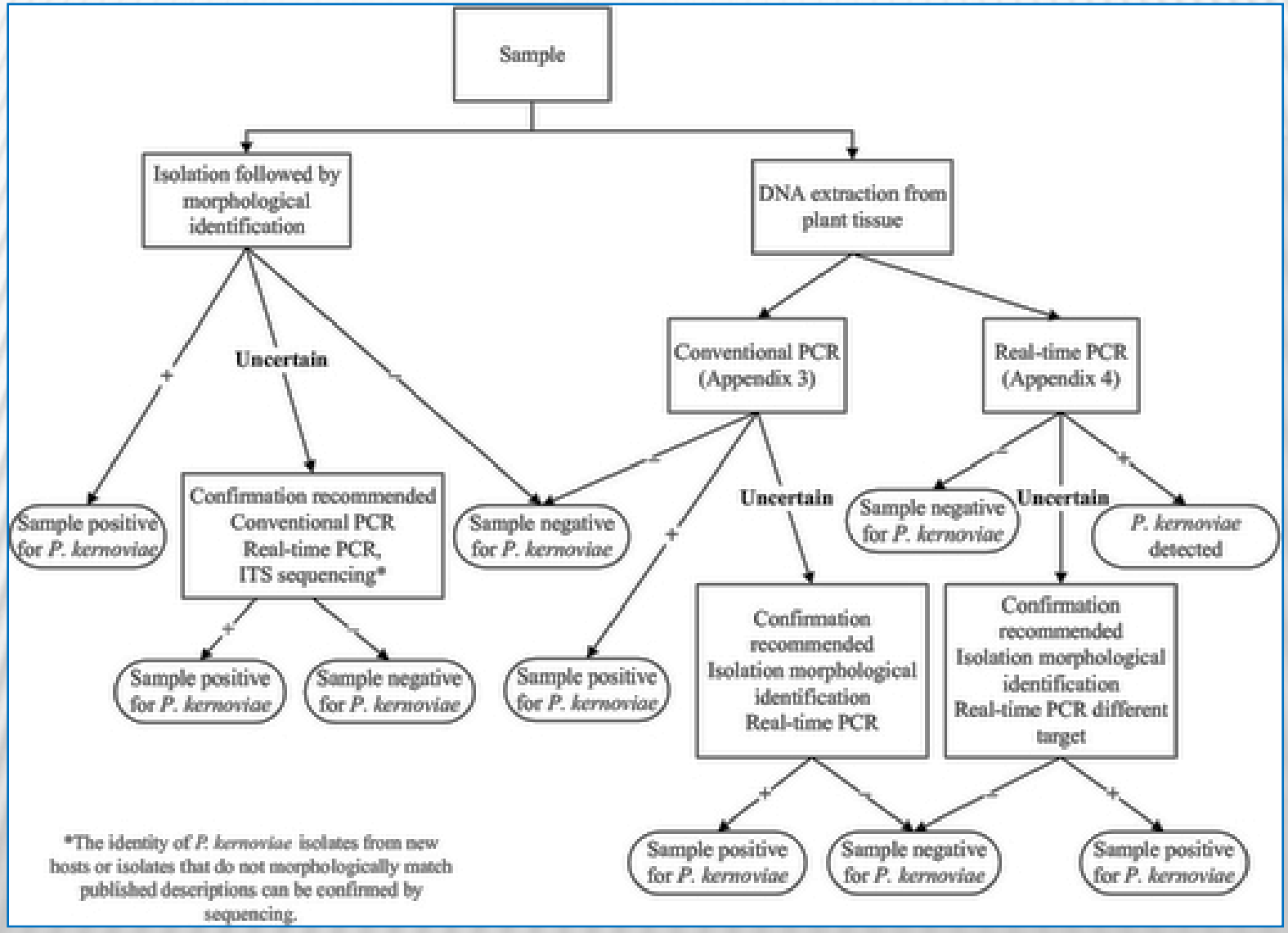
(source: http://www.eppo.int)

CERATOCYSTIS PLATANI – EPPO DETECTION SCHEME



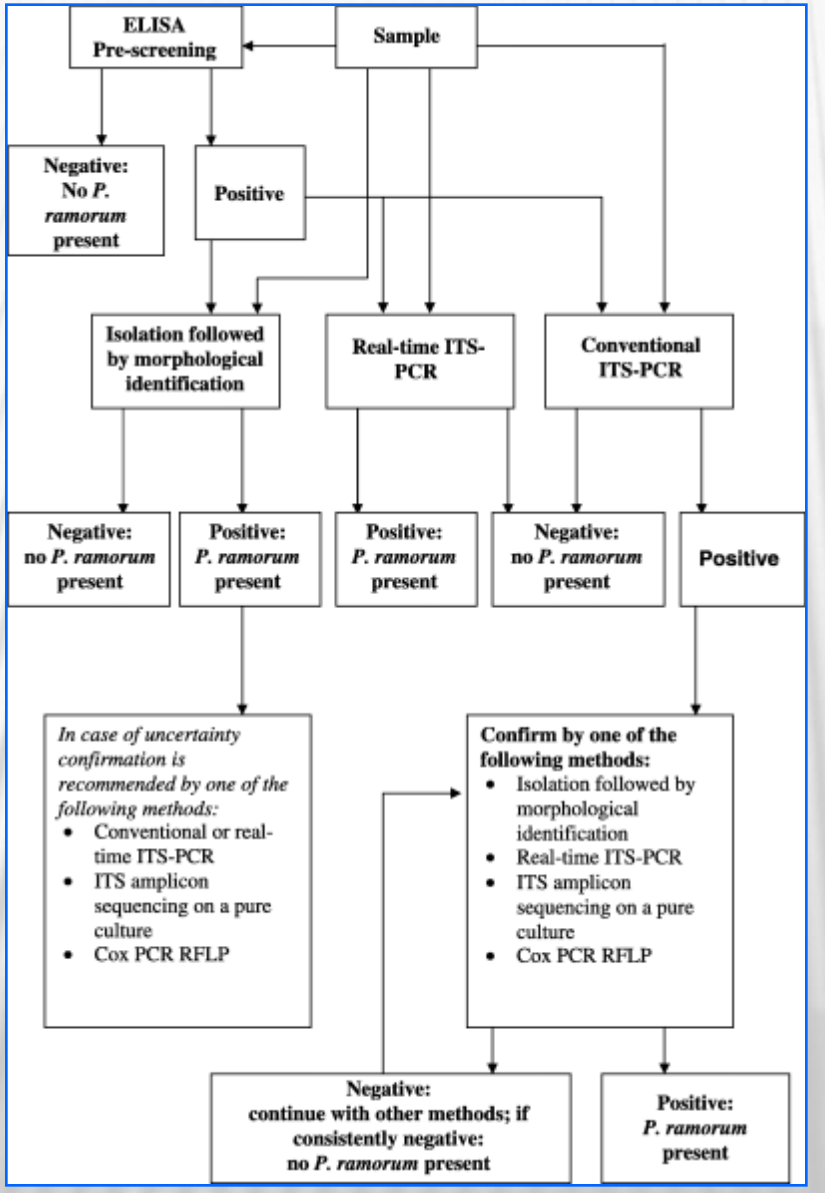
(source: http://www.eppo.int)

PHYTOPHTHORA KERNOVIAE– EPPO DETECTION SCHEME



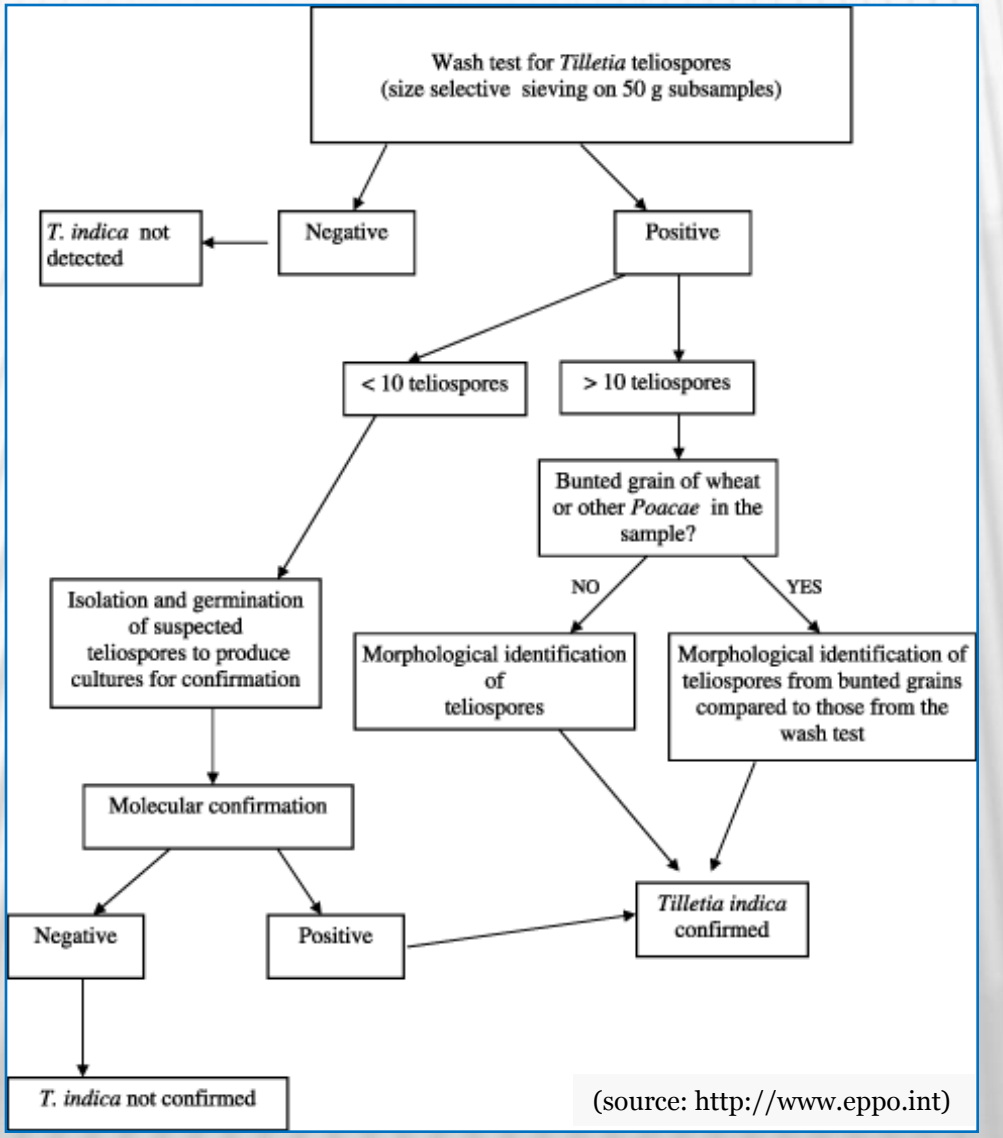
(source: http://www.eppo.int)

PHYTOPHTHORA RAMORUM– EPPO DETECTION SCHEME



(source: http://www.eppo.int)

TILLETIA INDICA– EPPO DETECTION SCHEME



(source: http://www.eppo.int)