



TWINNING PROJECT BA/12/IB/AG01

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



Phytophthora – detection and identification using classical methods

THIS PRESENTATION IS FOR ILLUSTRATIVE AND EDUCATIONAL PURPOSES ONLY

dr. Grażyna Szkuta

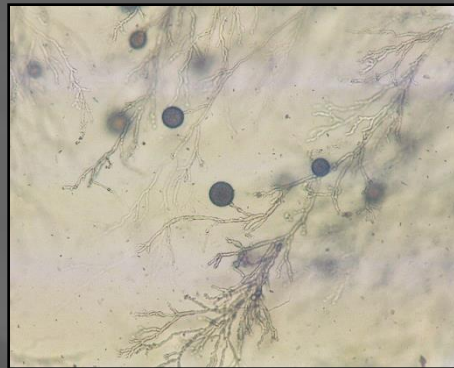
Main Inspectorate of Plant Health and Seed Inspection
Central Laboratory – Mycology Section, Toruń, Poland.

30th of November – 4th of December, 2015, Banja Luka, Bosnia & Herzegovina

ISOLATION ON AGAR MEDIA

This technique is used when disease symptoms on leaves, stems and fruits are observed.

- wash parts of plant under tap water (even one or more hours);
- then dry out the sample using a paper towel;
- cut plant material into small pieces and put them onto solid agar media (e.g. PDA, CA, V8, CMA with or without supplements e.g. PARP);
- control the presence of mycelium and sporulation under light microscope (put Petri plates up side down)
- transfer hyphae onto fresh agar media using stereomicroscope;
- incubate plates up to then obtaining a colony of *Phytophthora*



(pictures G. Szkuta)

ISOLATION ON AGAR MEDIA

P. cinnamomi, *P. nicotianae*

Desinfection 10% chlorox,
3 x water, CMA PARPH



ISOLATION ON AGAR MEDIA

P. ramorum, *P. syringae*, *P. citrophthora*, *P. citricola*

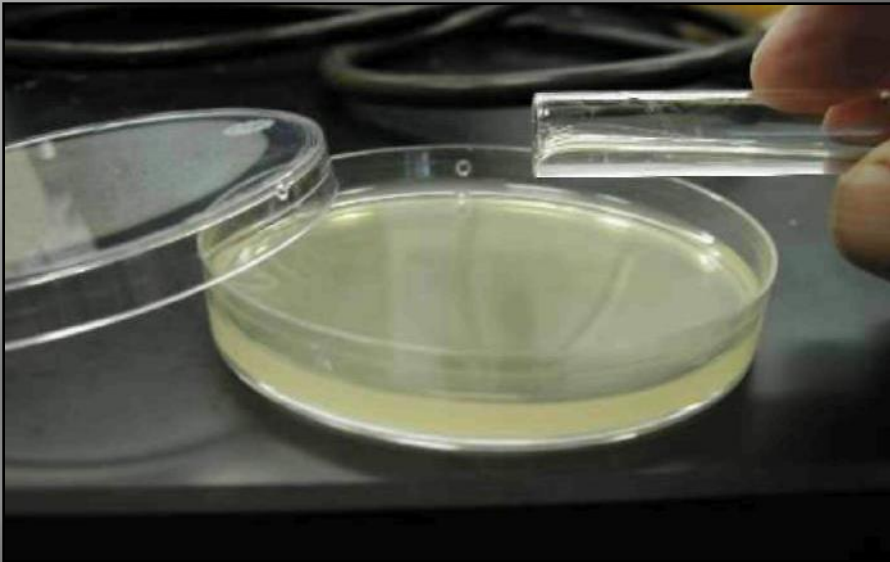


(source: <http://horticulture.ucdavis.edu>)

ISOLATION ON AGAR MEDIA

P. cinnamomi, *P. nicotianae*

0,5 g soil + 20 ml dH₂O , vortex,
and aliquot on plate with CMA
PARPH, spread with spatula

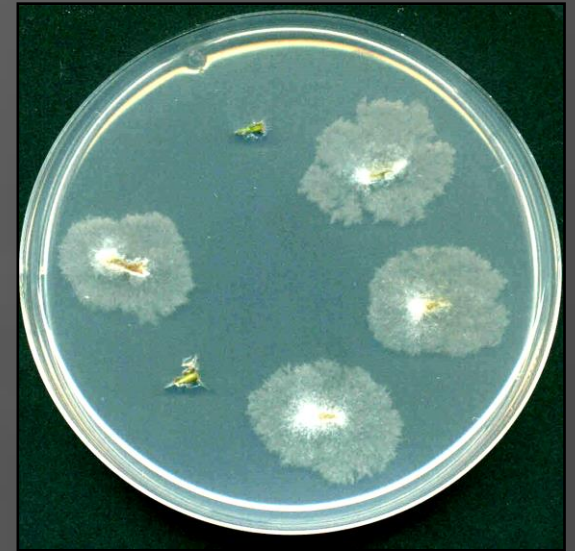
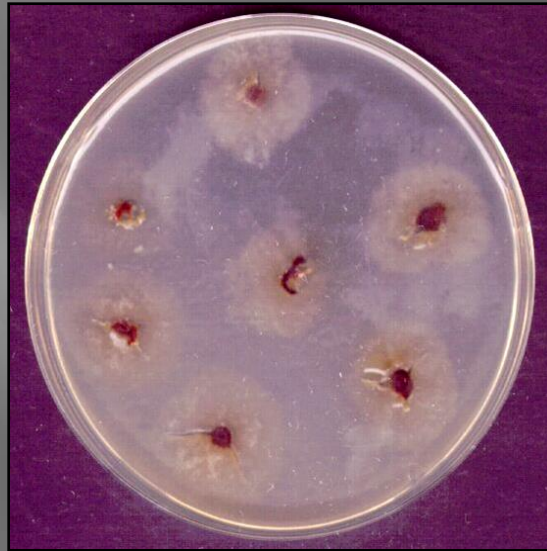


ISOLATION ON AGAR MEDIA



(pictures : G. Szkuta)

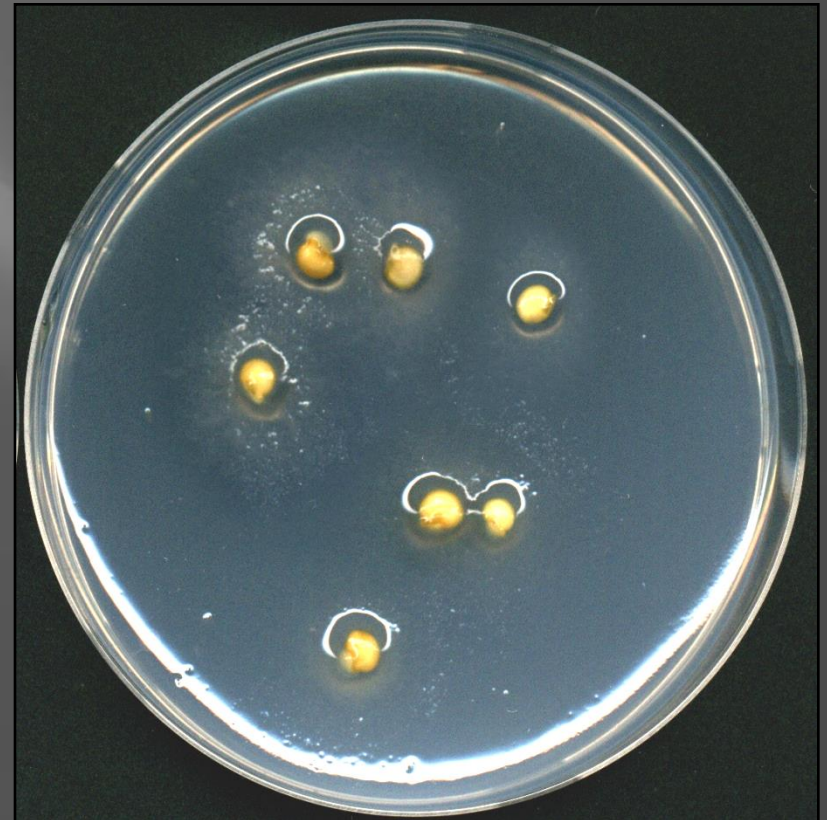
ISOLATION ON AGAR MEDIA



(pictures : G. Szkuta)

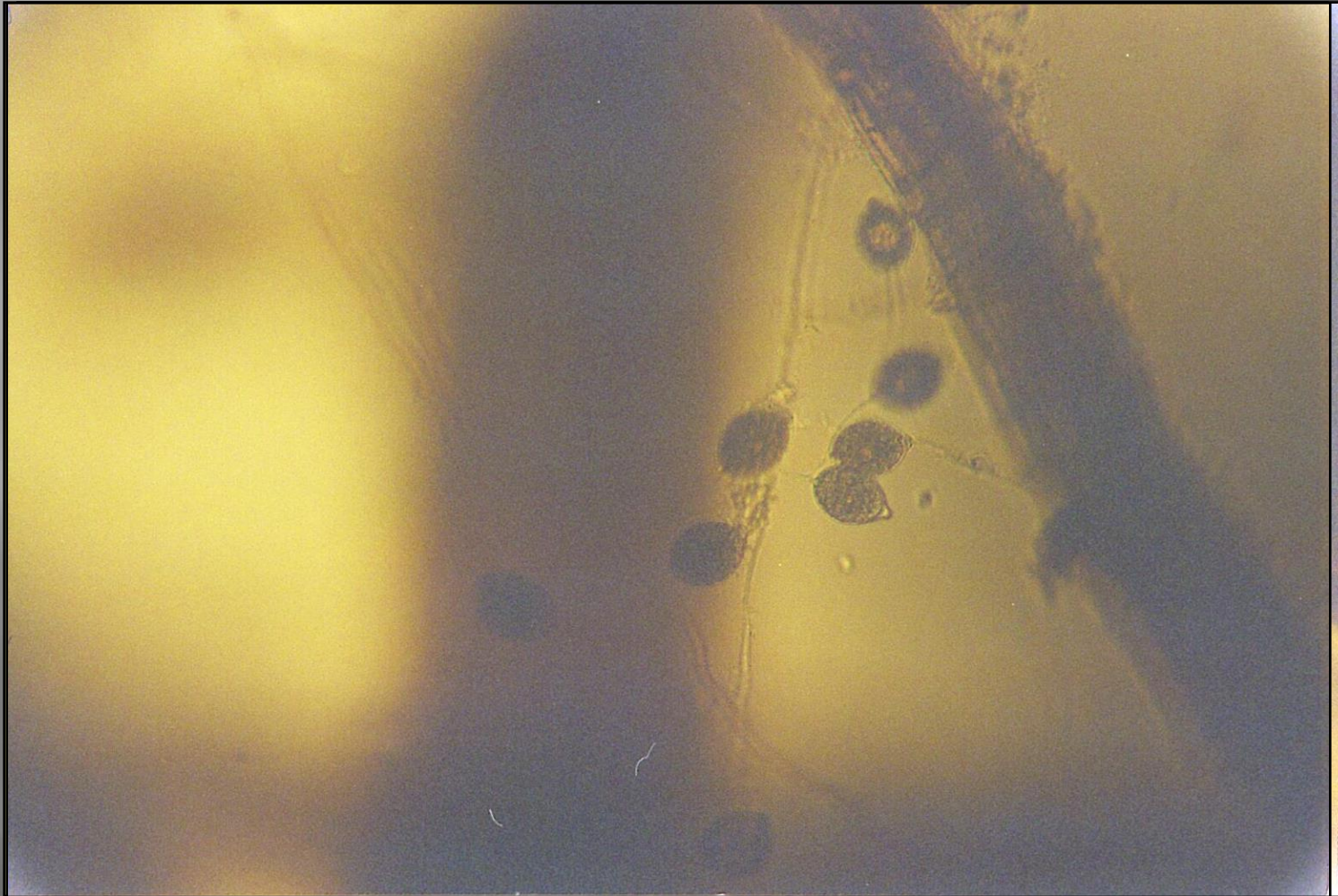
IDENTIFICATION

Stimulation of vegetative sporulation



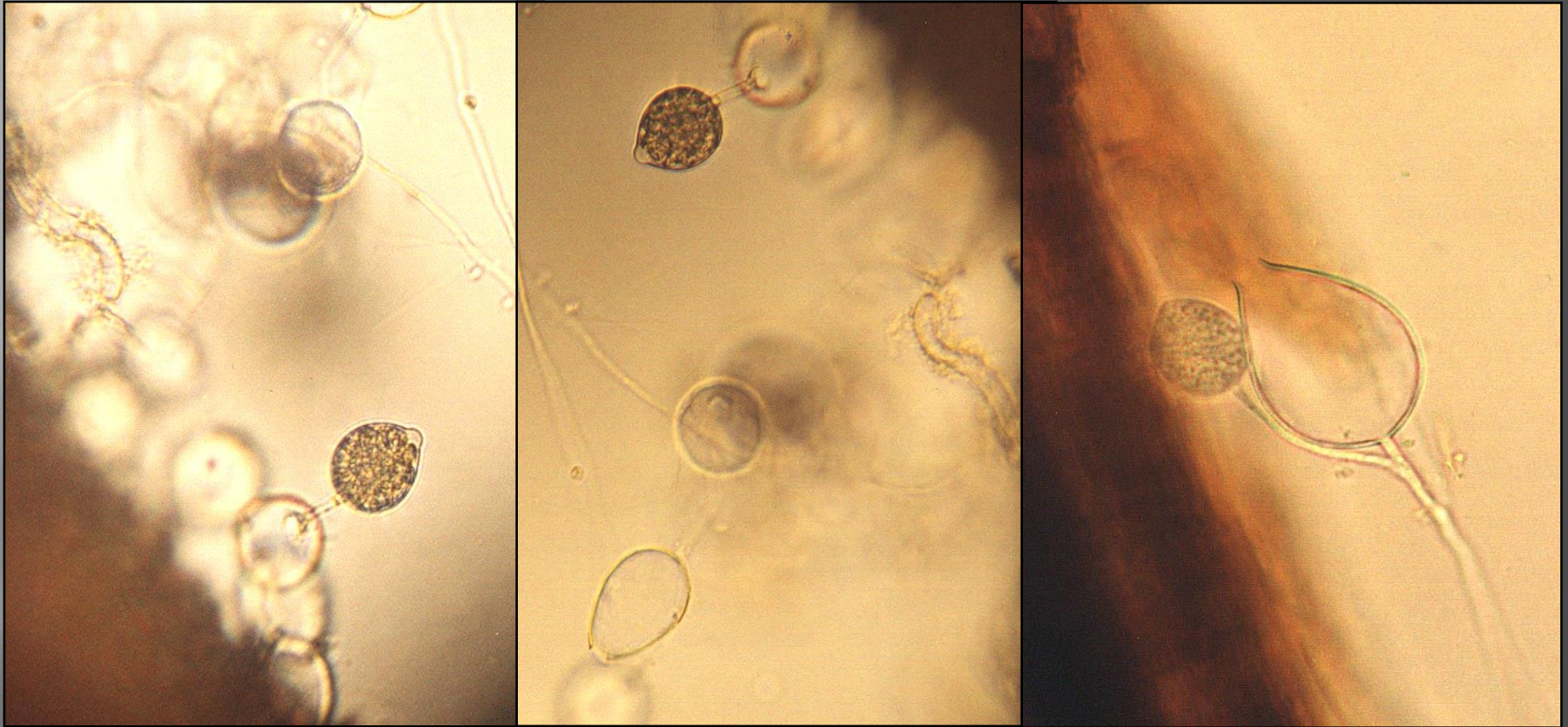
(pictures : G. Szkuta)

SPORULATION



(pictures : G. Szkuta)

SPORULATION



Raspberry roots – papillate sporangia in water

DETECTION USING BAITING TEST

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.

SOME *PHYTOPHTHORA* AND THEIR BAITS

SPECIES	BAIT
<i>P. cactorum</i>	fruits (apple, pear, strawberry), seedlings of sunflower
<i>P. cinnamomi</i>	Fruits (apple, pear, avocado), leaves of pineapple, avocado, eukaliptus, seedlings of lupine, needles of pinus
<i>P. citricola</i>	needles of pinus
<i>P. megasperma</i>	fruits (apple, pear), needles of pinus seedlings of lupine
<i>P. cambivora</i>	needles of pinus fruits (apple)
<i>P. lateralis</i>	Parts of tissues of <i>Ch. lawsoniana</i> ;

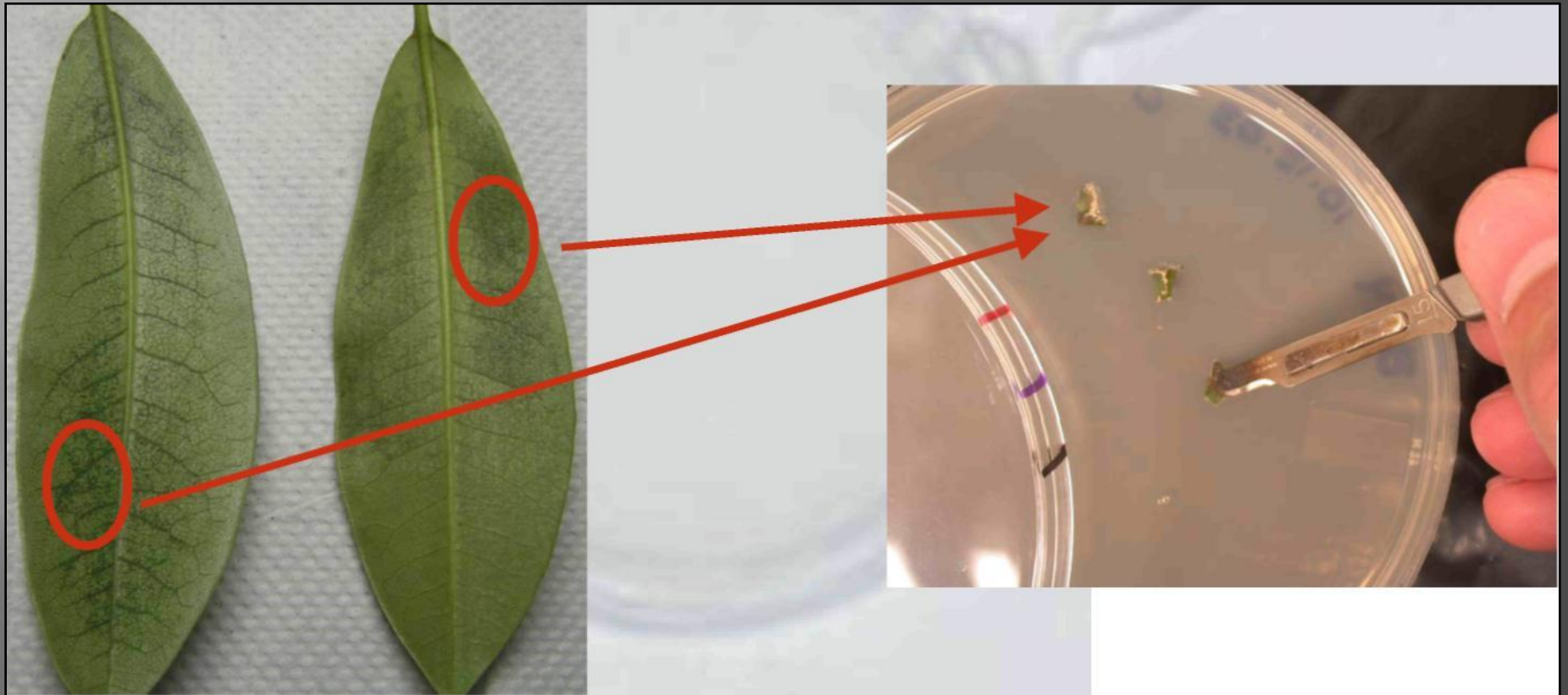
SOME *PHYTOPHTHORA* AND THEIR BAITS



More often used:

- Apples
- *Rhododendron* leaves
- Oak leaves

ISOLATION AFTER BAITING TEST



(source: <http://horticulture.ucdavis.edu>)

IDENTIFICATION

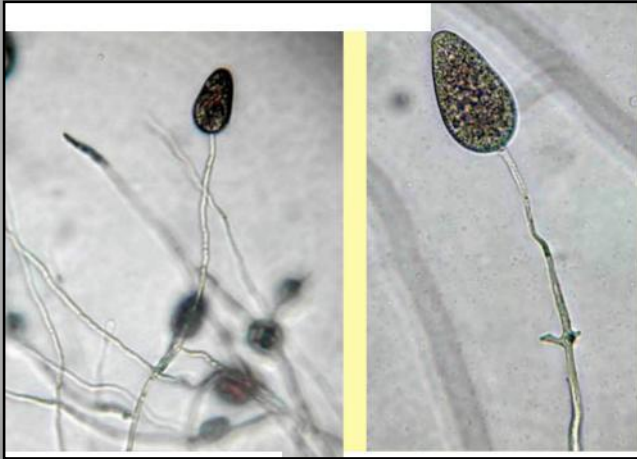
Mycelium and hyphal swellings



(pictures: G. Szkuta)

IDENTIFICATION

Morphology of sporangiophores according to Gloria Abad (USDA)



Unbranched *P. erythroseptica* i *P. kelmania*



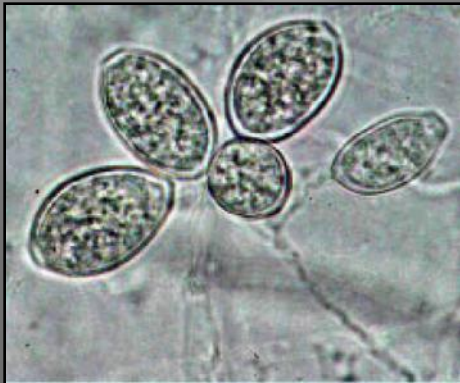
Branched *P. sp.*



Internal proliferation



Long symposium *P. ramorum*



Botryose clumps *P. botryosa*



Umbela type *P. capsici*



Simple symposium *P. kernoviae*

(pictures: G. Abad)

IDENTIFICATION

Sporangia - type of papilla



IDENTIFICATION

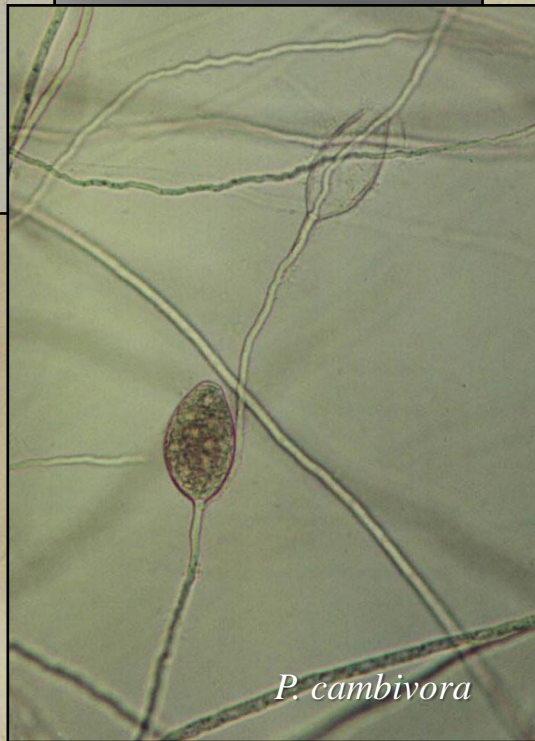
Germinating zoospores of *Phytophthora cryptogea*



(picture: G. Szkuta)

IDENTIFICATION

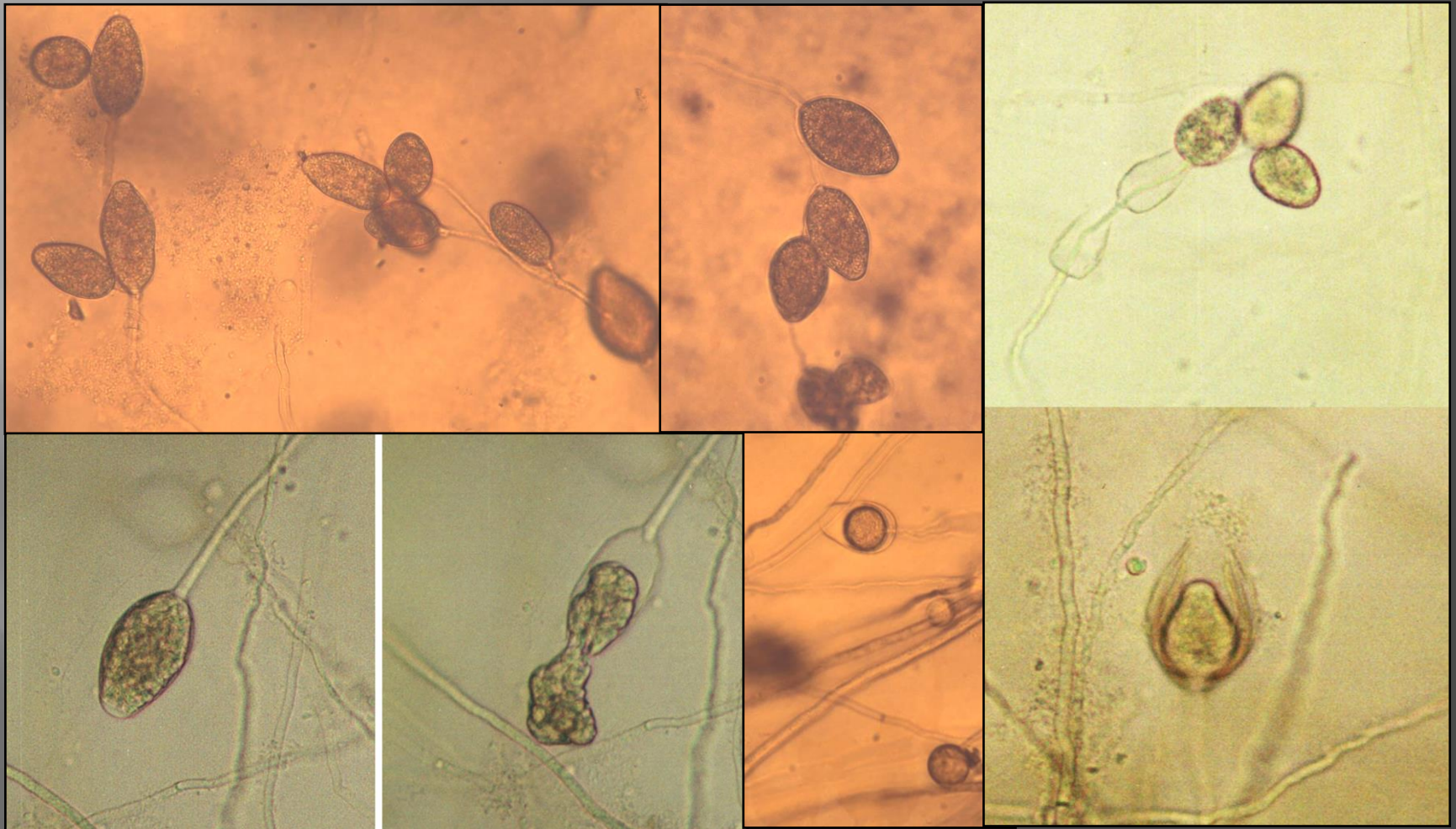
Nonpapillate sporangia



(pictures : G. Szkuta)

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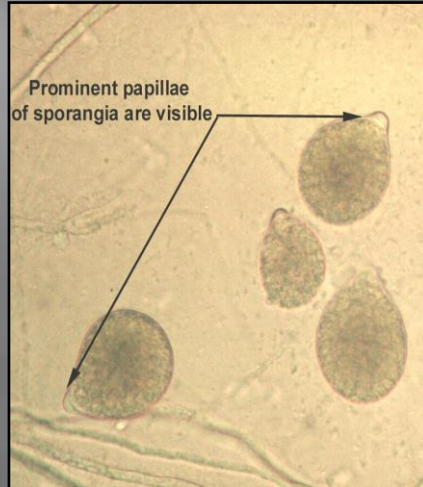
Nonpapillate sporangia of *Phytophthora cambivora* and *P. alni*



(pictures : G. Szkuta)

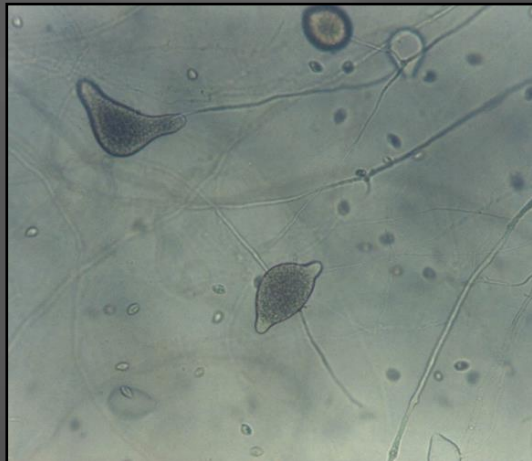
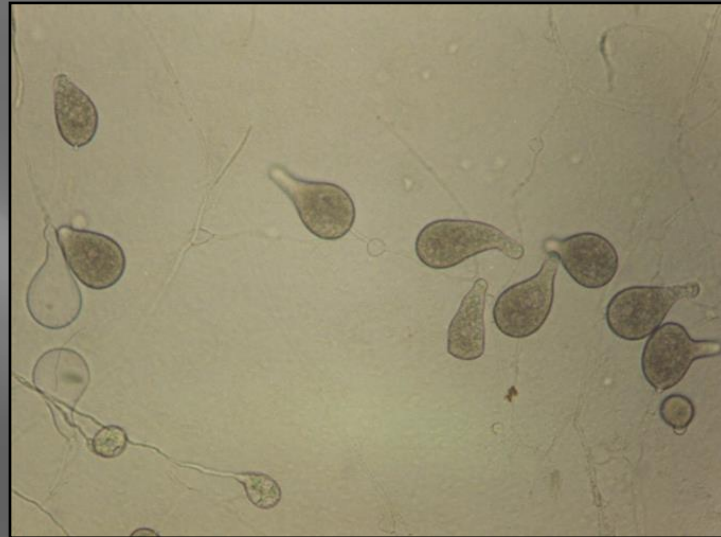
IDENTIFICATION

Papillate sporangia



IDENTIFICATION

Semipapillate sporangia



IDENTIFICATION

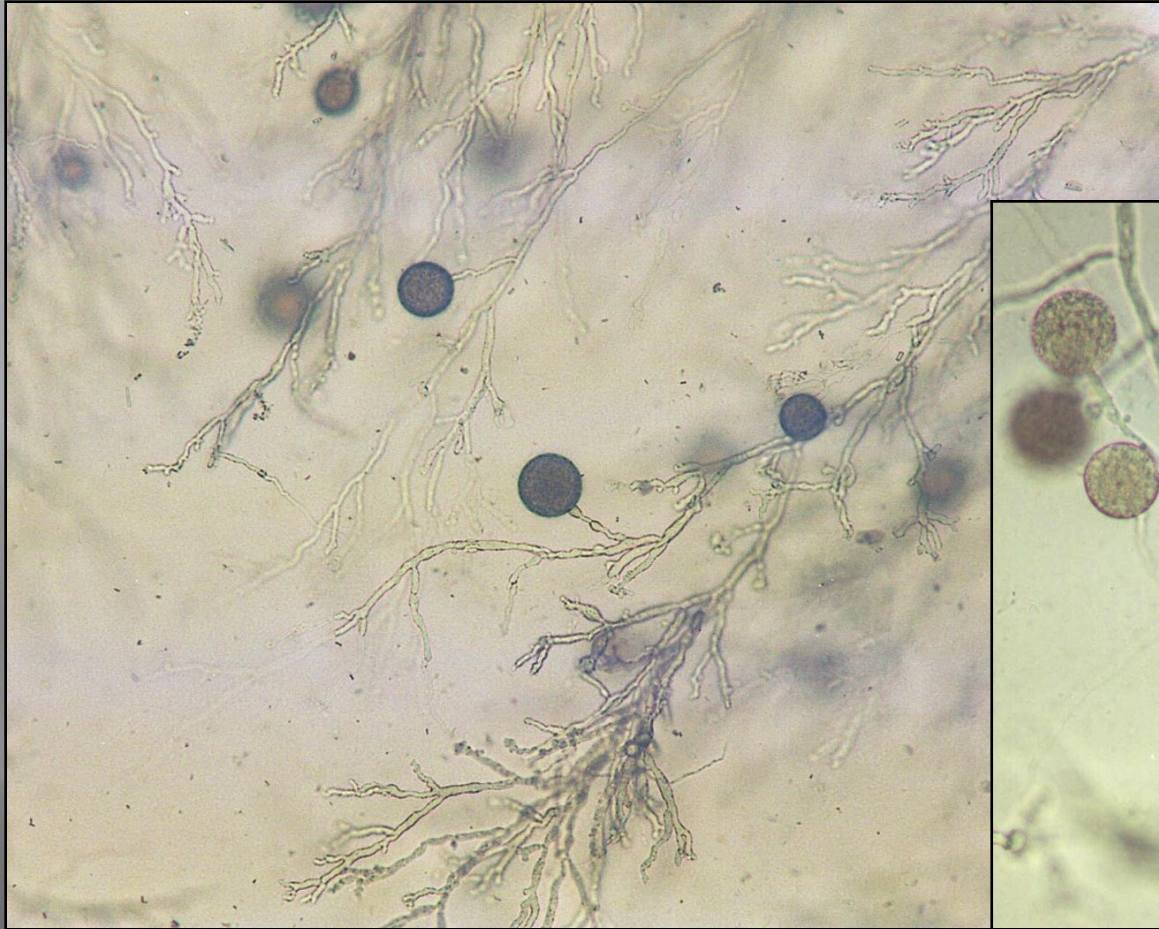
Oogonia, antheridia, oospores



(pictures G. Szkuta)

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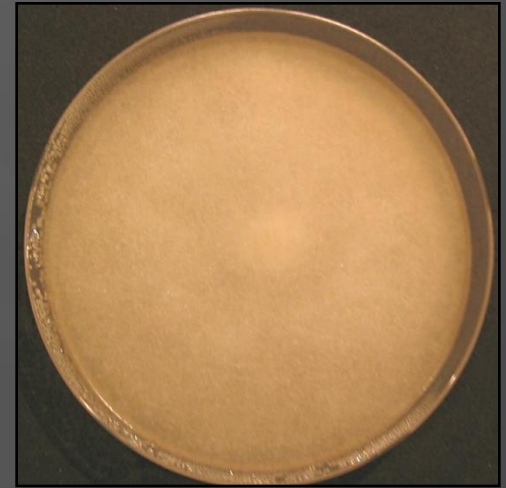
Chlamydozooids



(pictures G. Szkuta)

IDENTIFICATION

Patterns of growth on agar media



(pictures : G. Szkuta)

IDENTIFICATION ACCORDING TO GRACE WATERHOUSE

Group I

Sporangia papillate; pore narrow 5-7 μm ; form on solid agar media, usually caducous. Homothallic species, antheridia paragynous.

e.g. *P. cactorum*, *P. cladestina*, *P. idaei*, *P. pseudotsugae*

Group II

Sporangia papillate; pore narrow 5-7 μm . Heterothallic species, antheridia amphigynous.

e.g. *P. capsici*, *P. citrophthora*, *P. nicotianae*, *P. palmivora*

Group III

Sporangia semipapillate, persistent or caducous. Homothallic species, antheridia paragynous.

e.g. *P. citricola*, *P. porri*, *P. primulae*, *P. syringae*

IDENTIFICATION ACCORDING TO GRACE WATERHOUSE

Group IV

Sporangia semipapillate, usually caducous.

Heterothallic species, antheridia amphigynous

e.g. *P. hibernalis*, *P. ilicis*, *P. infestans*, *P. phaseoli*, *P. ramorum*

Group V

Sporangia nonpapillate, pore wide 12 μm ; persistent with internal proliferation. Rare form on solid agar media. Homothallic species, antheridia paragynous .

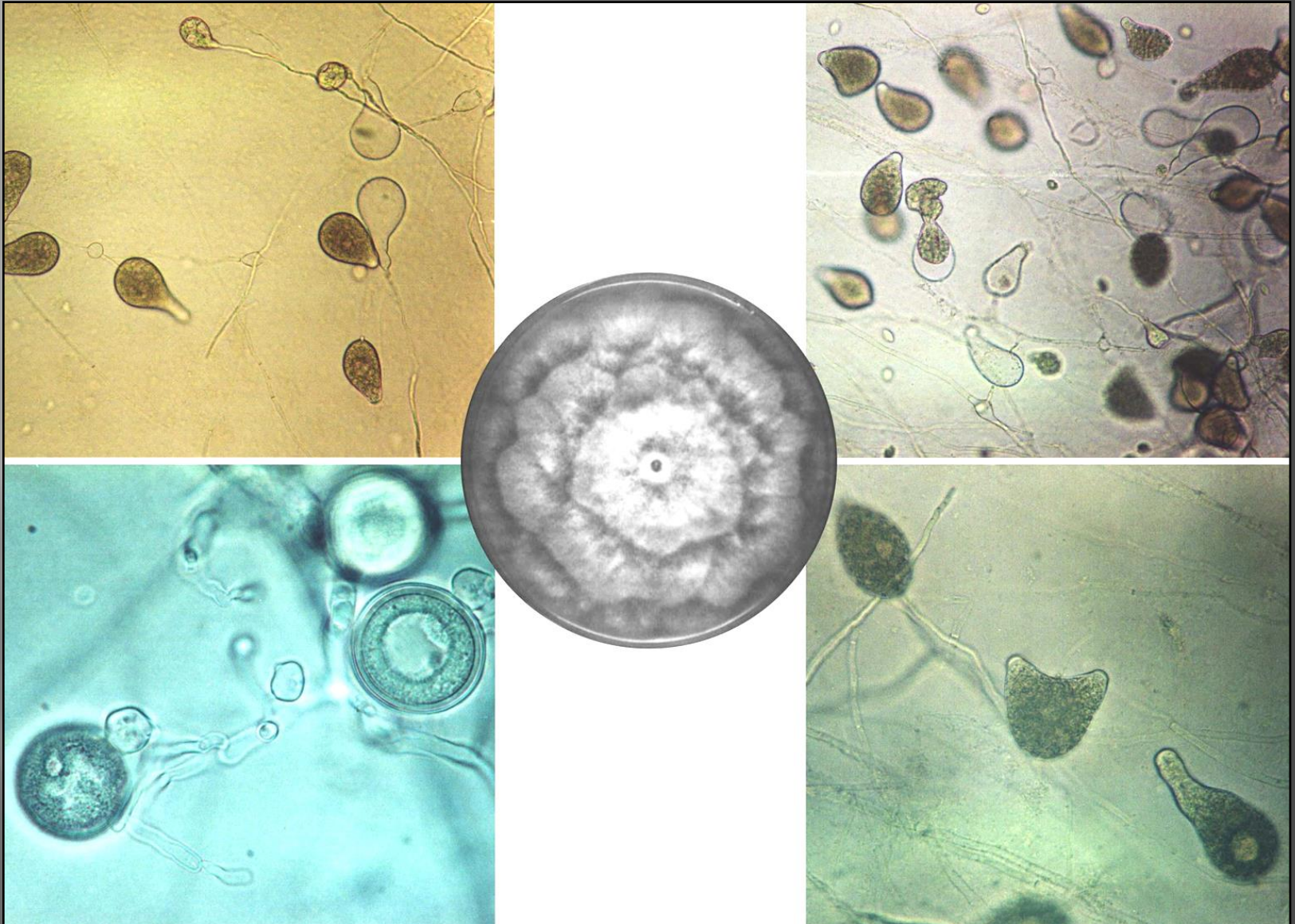
e.g. *P. fragariae*, *P. lateralis*, *P. megasperma*, *P. insolita*

Group VI

Sporangia nonpapillate, pore wide 12 μm ; periantheridia amphiginous sistent with internal and external proliferation. Heterothallic species , anteridia amphigynous .

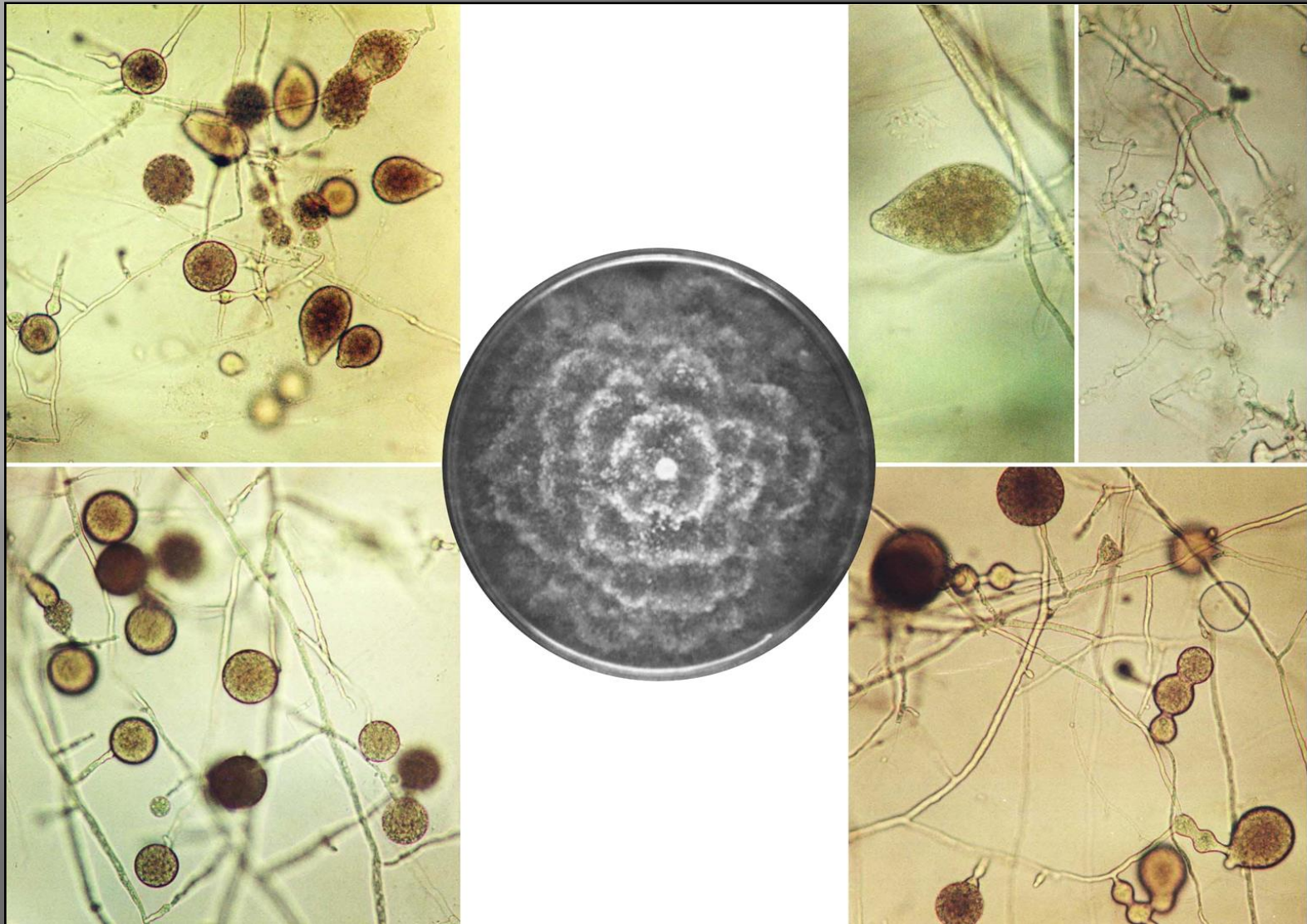
e.g. *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*,
P. erythroseptica

IDENTIFICATION OF *PHYTOPHTHORA CITRICOLA*



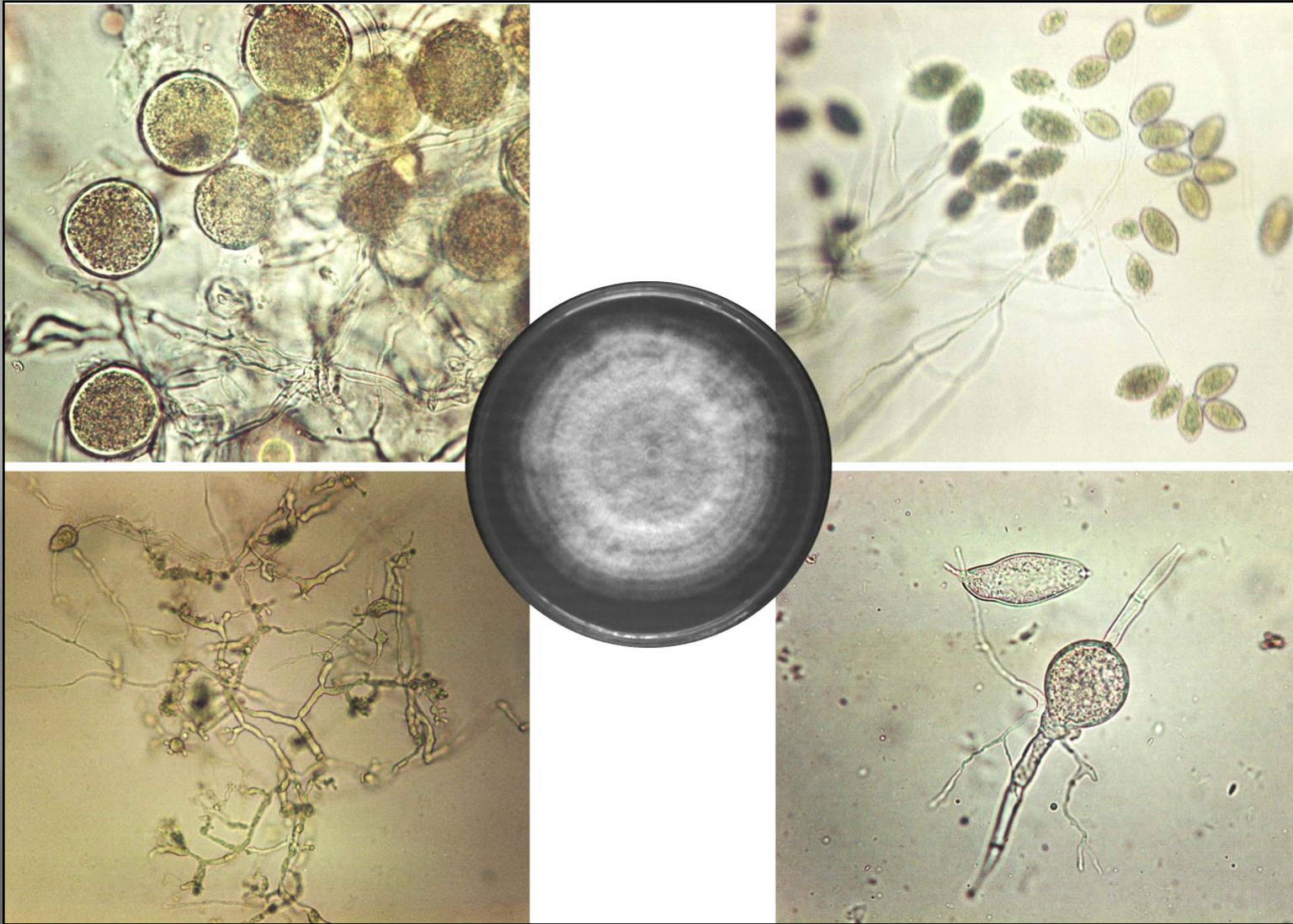
(pictures : G. Szkuta)

IDENTIFICATION OF *PHYTOPHTHORA CINNAMOMI*



(pictures: G. Szkuta)

IDENTIFICATION OF *PHYTOPHTHORA RAMORUM*



(pictures : G. Szkuta)