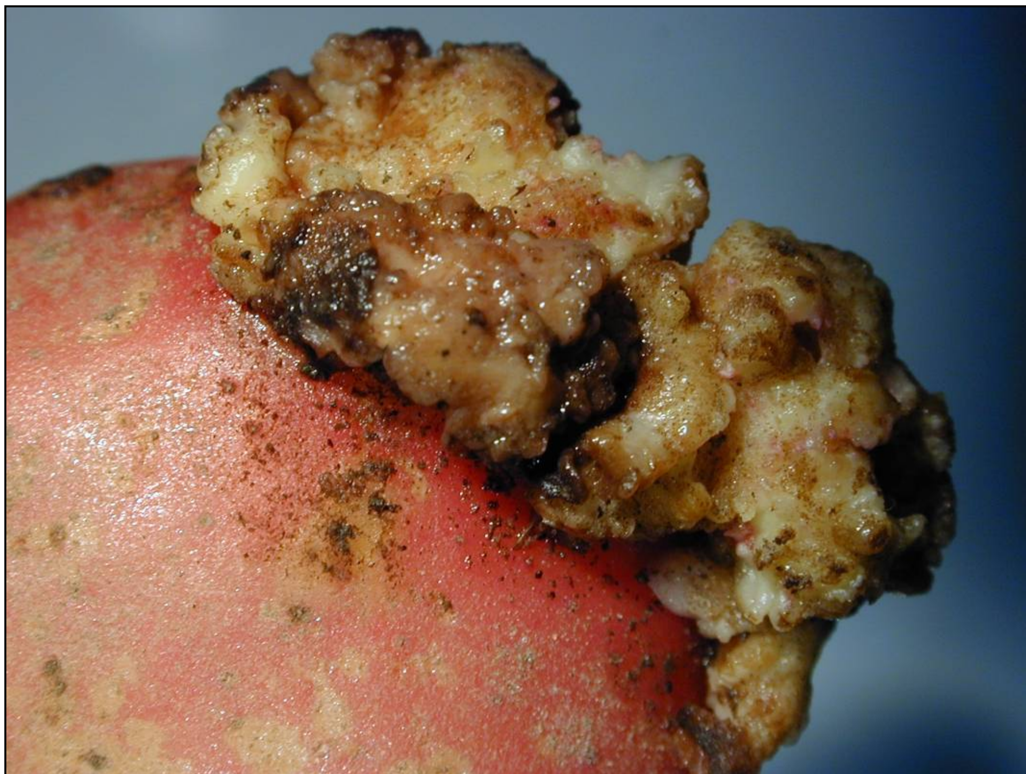
Subcommittee on Plant Health Diagnostics

National Diagnostic Protocol

Synchytrium endobioticum

The cause of potato wart



NDP 16 V2

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Department of Agriculture and Water Resources

Street Address 1: 18 Marcus Clarke Street, Canberra City ACT 2601
Street Address 2: 7 London Circuit, Canberra City ACT 2601
Postal Address: GPO Box 858, Canberra City ACT 2601
Switchboard Phone: 02 6272 3933
Web: http://www.agriculture.gov.au

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National Diagnostic Protocols (NDPs) are diagnostic protocols for the unambiguous taxonomic identification of plant pests. NDPs:

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- are consistent with ISPM No. 27 – Diagnostic Protocols for Regulated Pests
- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
- are endorsed by regulatory jurisdictions for use (either within their own facilities or when commissioning from others) in a pest incursion.

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https://www.ippc.int/core-activities/standards-setting/ispms

Process

NDPs are facilitated and endorsed by the Subcommittee on Plant Health Diagnostics (SPHD). SPHD reports to Plant Health Committee and is Australia's peak technical and policy forum for plant health diagnostics.

NDPs are developed and endorsed according to Reference Standards developed and maintained by SPHD. Current Reference Standards are available at http://plantbiosecuritydiagnostics.net.au/sphd/sphd-reference-standards/

NDPs are living documents. They are updated every 5 years or before this time if required (i.e. when new techniques become available).

Document status

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Further information

Inquiries regarding technical matters relating to this project should be sent to: sphds@agriculture.gov.au

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1 INTRODUCTION

Potato wart disease is caused by the soil-borne fungus *Synchytrium endobioticum* (Schilb.) Percival (Chytridiomycetes: Chytridiales). The principal symptom is a warty, cauliflower-like gall which develops on tubers and tuber initials. The main cultivated host affected by potato wart is potato (*Solanum tuberosum* L.), although other wild *Solanum* species are known to be infected in Mexico. In addition, a number of Solanaceous crops (including tomato) and weeds have been artificially inoculated. Experimentally, this fungus can also infect species within the genera *Nicotiana, Physalis, Capsicastrum, Datura, Duboisia, Hyoscyamus, Lycium, Nicandra* and *Schizanthus* (Hampson 1993; DEFRA 2011).

Potato wart can be destructive under favourable conditions and entire crops can be lost as a result of heavily infected potato tubers being virtually entirely converted to warts. The fungus targets meristematic tissue and infects all below ground parts of the plant except root tissue. In tubers, infection initially occurs in eye tissue and gradually spreads causing massive hypertrophy and hyperplasia, frequently involving the entire tuber. The tumour is composed of simple, large parenchyma cells crowded with starch grains and organised into a malformed and compressed foliar branch system. In near-immune potato cultivars, the warts remain superficial and scab-like, while in resistant potato cultivars the zoospore dies soon after invasion by necrotic abortion (hypersensitive reaction) of the infected tissue (Hampson and Proudfoot 1974; Pratt 1976; Hooker 1990). Very long periods of survival of the winter sporangia prevent replanting of infested fields for up to 30 years or longer (Pratt 1976).

This diagnostic manual was developed based on available published methods, and in consultation with international experts. It describes potato wart symptoms and outlines a microscopic procedure to confirm the presence of *S. endobioticum* spores in potato tissue.

2 TAXONOMIC INFORMATION

Kingdom: Fungi

Phylum: Chytridiomycota

Class: Chytridiomycetes

Order: Chytridiales

Family: Synchytriaceae

Genus: *Synchytrium*

Species: *Synchytrium endobioticum* (Schilb.) Pervical

Synonyms: *Chrysophlyctis endobioticum* Schilb. (variant spelling *C. endobiotica*), *Synchytrium solani* Massee

Common name: potato wart

3 DETECTION

The detection of *Synchytrium endobioticum* relies on symptomatology and the microscopic detection and identification of fungal spores. At the time of writing there were no rapid molecular diagnostic techniques available for the identification of this pathogen. Molecular protocols specific for the detection of potato wart have since been developed, and will need to be included once adequately validated. However the current microscopic techniques are sufficient for identification.

S. endobioticum can be detected in soil using a wet-sieving technique (Pratt 1976; EPPO/OEPP 2003), which separates *S. endobioticum* resting sporangia from soil particles of similar size by chloroform flotation and centrifugation. Spores are then examined microscopically. For detection from soil, a tomato bioassay (Hampson and Haard 1980; Hampson 1981) can be used to detect viable spores. Stachewicz (1999) provides a method for soil sampling specifically for the detection of potato wart. Methods for soil detection are not included in this protocol.

3.1 Symptoms

Potato plants infected by *S. endobioticum* do not usually show symptoms in above ground parts, although occasionally there may be a reduction in vigour, and rarely, development of small greenish-yellow warty growths at the stem base. Normally, however, all symptoms are confined to below ground parts of the plant and no evidence of infection is seen until potato tubers are harvested (Hampson 1993; DEFRA 2011).

The fungus causes symptoms of infection on all underground potato parts except true roots, with buds on stems, stolons and tubers the main centres of infection (MPI 2009).

The most distinguishing symptom of potato wart disease is the gall, a characteristic warty, cauliflower-like swelling that may be as small as a pin or as large as a fist, which forms on developing tubers (Figure 1). The gall is soft and pulpy in texture, with a surface that is rough and corrugated, giving it the warty appearance. The galls when formed underground or in storage, are the same colour as the potato skin, however, if exposed to light, they will turn green. As the warts age they darken and decay (Hampson 1993; DEFRA 2011)

Tubers may bear more than one warty outgrowth. If infected early, potato tubers can become so distorted and spongy that they are almost unrecognisable, with entire tubers replaced by warts. In some cases, a severe infestation of the tip of the stolon where the tuber is normally formed, will cause a gall to develop instead of a tuber, subsequently destroying the potato crop by preventing tuber production (Hampson 1993; MPI 2009; DEFRA 2011).

Some symptoms of powdery scab (Figure 2) and bud proliferation (Figure 3) can be mistaken for wart disease (DEFRA 2011). However powdery scab spore balls are very different in appearance from winter sporangia of *S. endobioticum* and microscopic examination quickly reveals the spongy appearance of many spore balls made up of many small cysts. Some smut symptoms can also be confused (Figure 4) however this disease is also not in Australia.

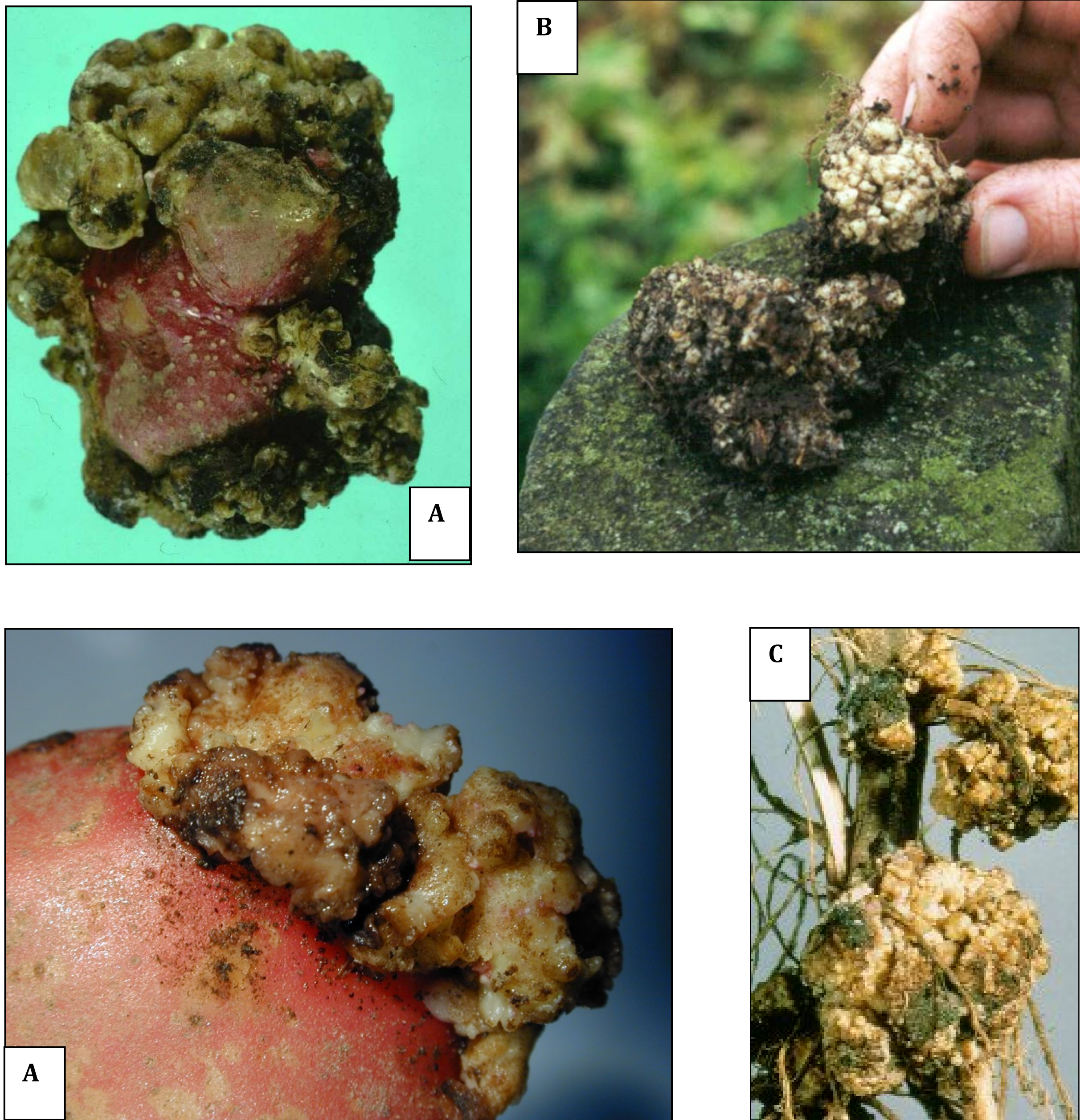

Figure 1. Warty galls on underground potato plant parts caused by *Synchytrium endobioticum* infection.

Sources. A: © Plant Health & Environment Laboratory, Ministry for Primary Industries, New Zealand. B: © Peter Wilkins, AsureQuality, New Zealand. C: © Department for Environment, Food and Rural Affairs, UK.

Figure 2. Powdery scab (© SARDI)

Figure 3. Sprout proliferation on tuber (© SARDI)

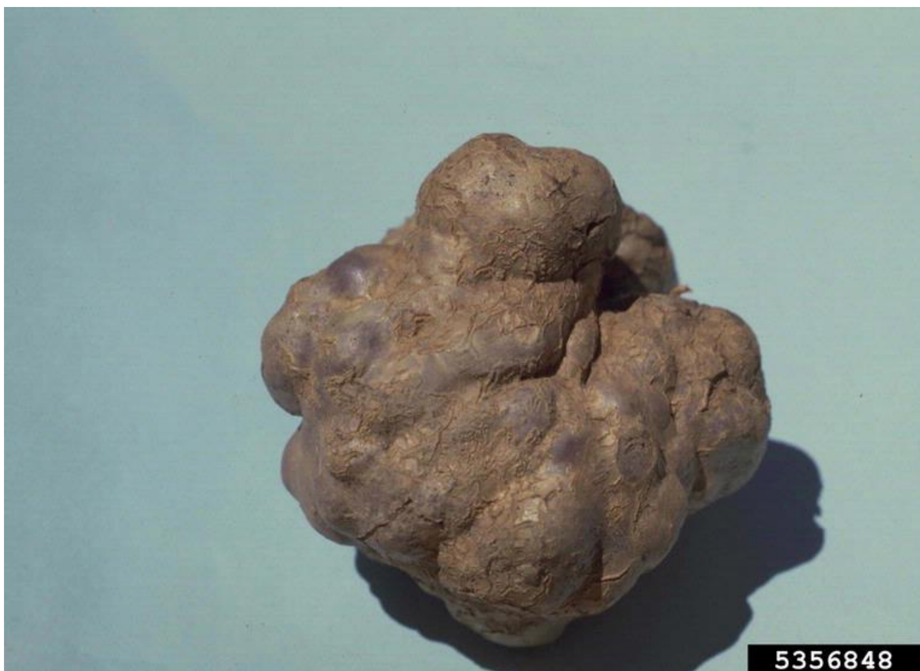

Figure 4. Potato tuber in Bolivia showing symptoms of infection with potato smut (*Thecaphora solani*). © William M. Brown Jr., Bugwood.org

4 IDENTIFICATION

4.1 Microscopic examination for sporangia

Potato tubers can be examined directly by mounting a small portion of tissue from a warty growth in lactic acid on a microscope slide.

Equipment required

- Scalpel blades
- Dissecting microscope
- Compound microscope (interference contrast optics preferred)
- Glass microscope slides and coverslips
- Small flame such as a cigarette lighter of spirit lamp (optional)
- Lactic acid ($C_3H_6O_3$) ~98%

Method

1. Using a scalpel blade make two incisions into the warty outgrowth, as thin as possible (less than 1 mm thick) and 3 mm long
2. Remove the thin section of potato tissue and place on a glass microscope slide
3. Add a droplet of lactic acid large enough to wet the entire section of potato tissue
4. Place a coverslip over the potato tissue
5. Examine the prepared slide with a compound microscope using at least the X40 objective. If sections are too thick or if it is difficult to visualise the sporangium morphology adequately, prepare another section, place in lactic acid and tease out with a pair of fine needles under the dissecting microscope, before adding a coverslip
6. Optional: heat the microscope slide with the hand-held flame to remove any bubbles in the lactic acid

4.2 Morphology

1. Compare structures seen with those illustrated in Section 4.2.1
2. Winter sporangia (survival structure - see Appendix) are thick-walled and golden brown and embedded in the wart tissue, each sporangium filling its host cell almost completely. The thick outer wall is furrowed, irregularly thickened and bears prominent ridges which give the otherwise subglobose sporangium an angular appearance in median view. Winter sporangia measure 25 – 75µm (mean 50µm) in diameter
3. The prominent ridges on the outer wall are diagnostic for *S. endobioticum* and serve to distinguish winter sporangia of this fungus from any other structures or organisms that might be encountered in potato warts or soil
4. Summer sporangia (see Appendix) are of similar size to winter sporangia but are transparent and thin-walled and are unlikely to be found in mature warts. They lack the characteristic ridged wall of winter sporangia and their morphology is not diagnostic for potato wart.
5. Other species of *Synchytrium* may be encountered in potato soils but are unlikely to be found in potato wart tissue. These other species lack the characteristic ridges on the winter sporangia.
6. Powdery scab (caused by *Spongospora subterranea*) causes irregular scabby outgrowths on

potato tubers, but microscopic examination of scab contents reveals ovoid, irregular or elongate, spongy spore balls composed of multiple, closely single spores (Fig. 15).

7. Potato smut (caused by *Thecaphora solani)* also causes warty swellings on potato tubers, but the wart tissue contains black spores when examined microscopically (Fig 16). Potato smut is absent from Australia.

4.2.1 Images to aid identification of *Synchytrium endobioticum* sporangia

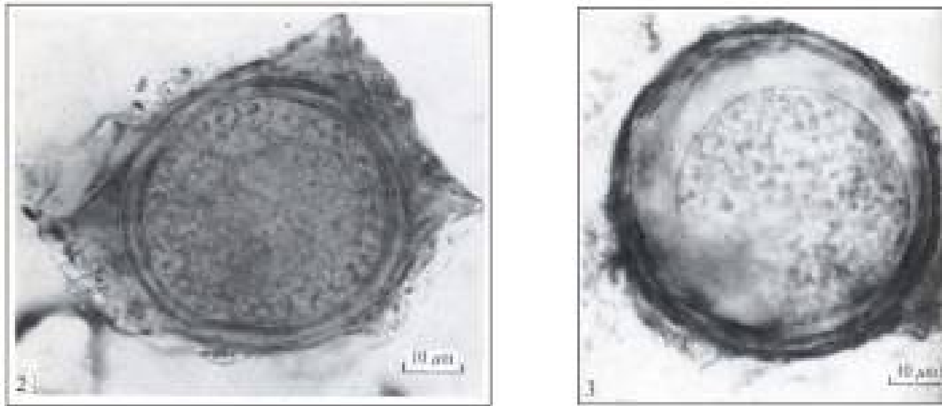

Figure 5. Live *S. endobioticum* sporangium: (L) resting and (R) with contents rounding off (Pratt 1976)

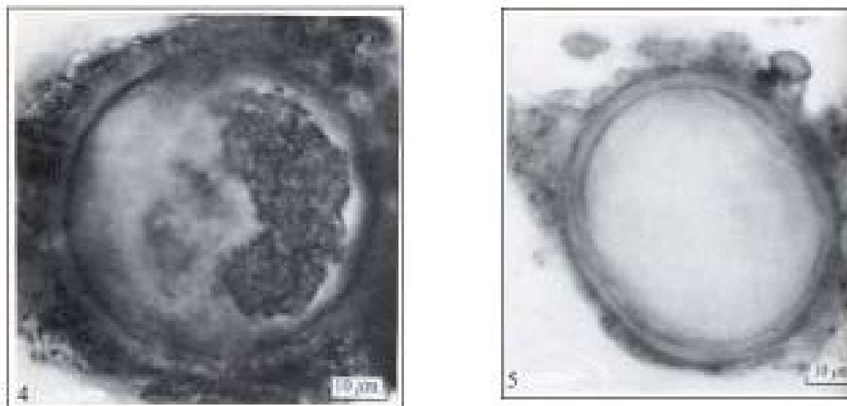

Figure 6. Dead *S. endobioticum* sporangium: (L) showing plasmolysis of contents and (R) without contents (Pratt 1976)

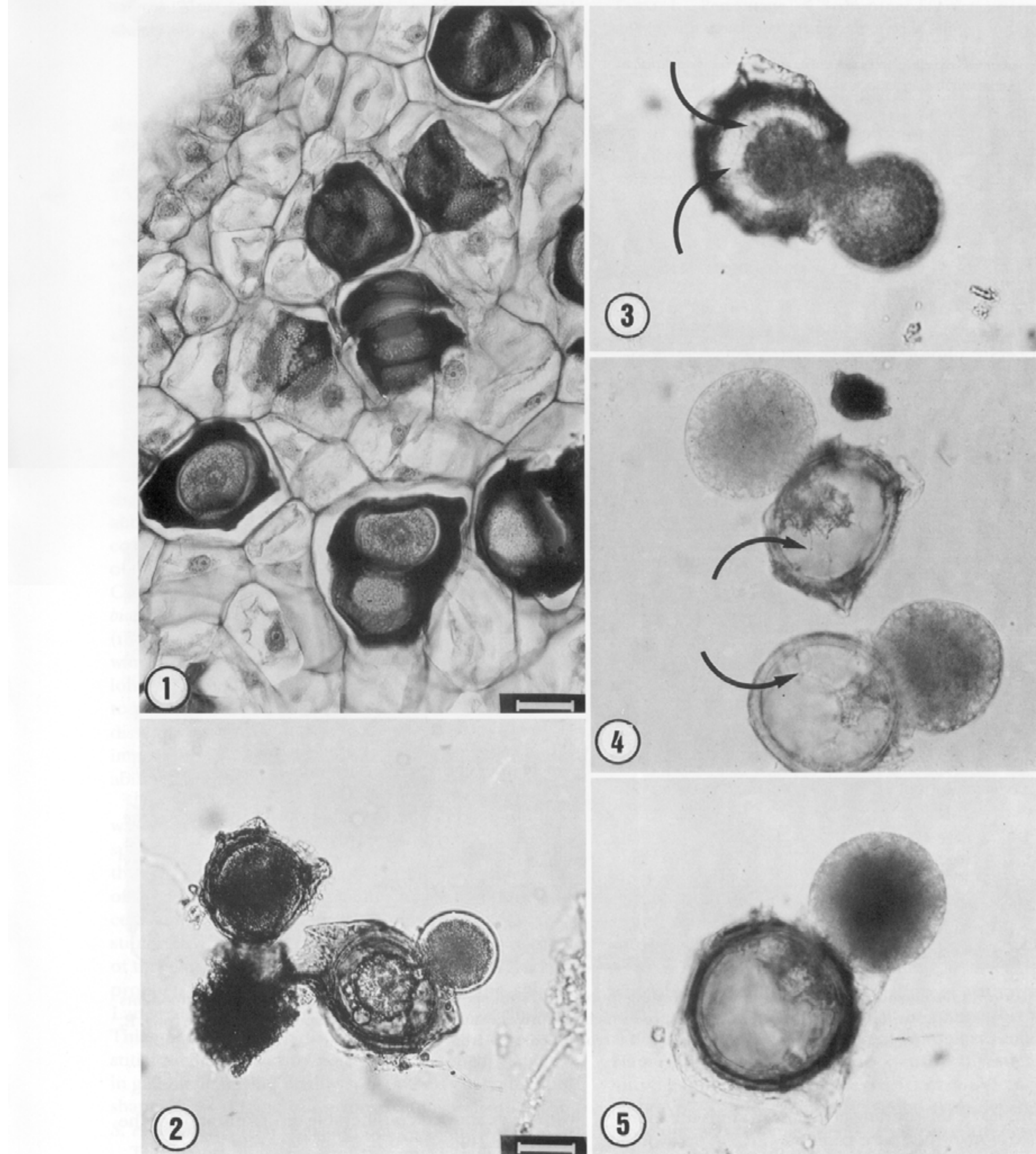

Figure 7. Various stages of *Synchytrium endobioticum* sporangia, as published in Hampson et al. (1994). **1.** Resting spores of *S. endobioticum* in potato gall tissue (bar = 25µm). The tissue was stained with Fleming's triple stain: although some shrinkage took place, the spore walls conform to the surrounding cell walls. **2-5** Germinating resting spores of *S. endobioticum* (bar = 20µm). **2**. Early stage with content in process of flowing into the extruded vesicle. **3**. Similar stage, showing attachments to wall (arrow). **4.** Mid stage. The connection to the mesospore wall are clear (arrows). **5.** End stage. The filled vesicle darkens with time.

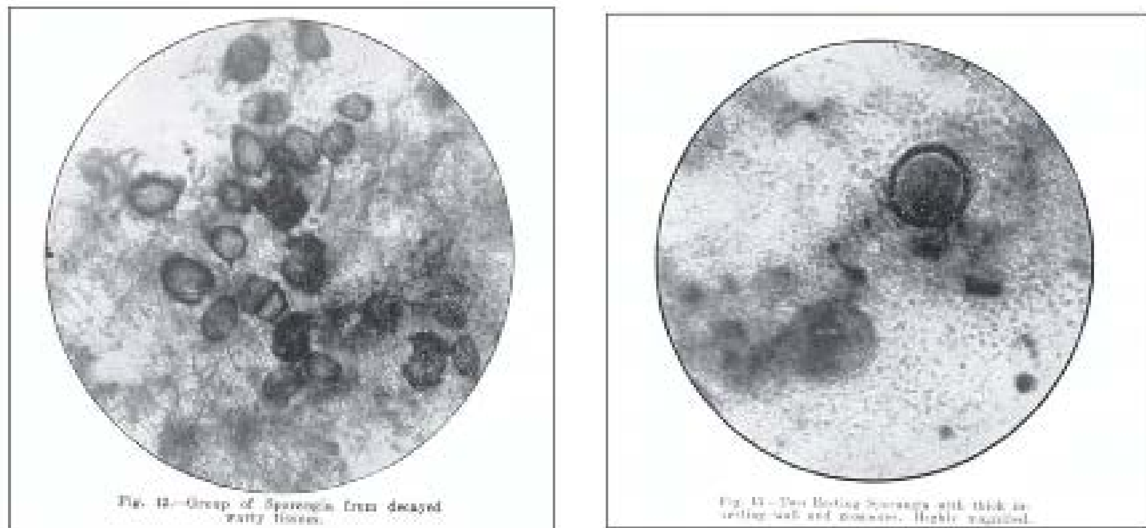

Figure 8. (L) Group of *S. endobioticum* sporangia isolated from decayed warty tissue. (R) Two *S. endobioticum* resting sporangia (Hedworth Foulkes 1910)

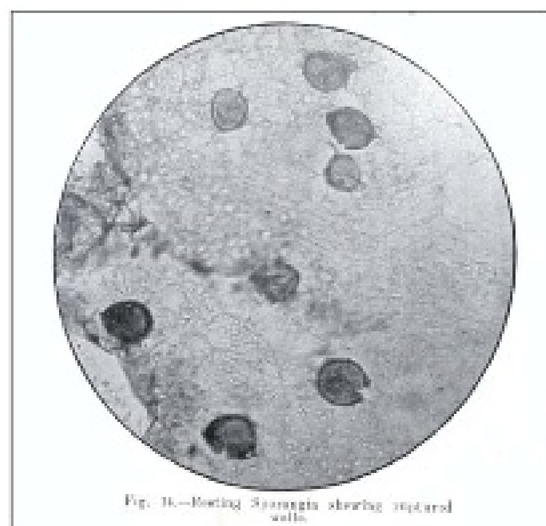

Figure 9. *Synchytrium endobioticum* resting sporangia with ruptured cell walls (Hedworth Foulkes 1910)

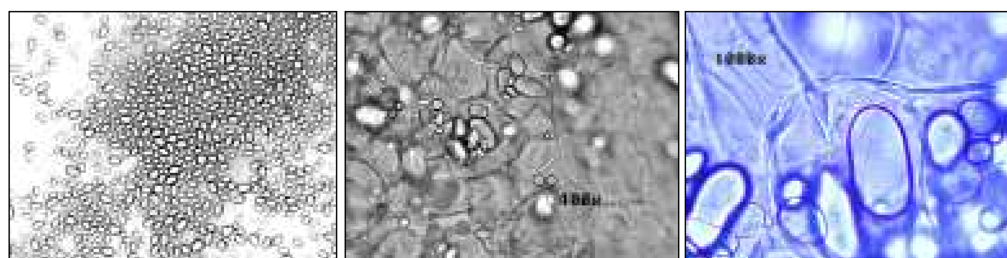

Figure 10. Starch grains in potato tissue, at 100, 400 and 1000 times magnification (left to right) (Source: http://botit.botany.wisc.edu/images/130/Plant_Cell/starch_grains)

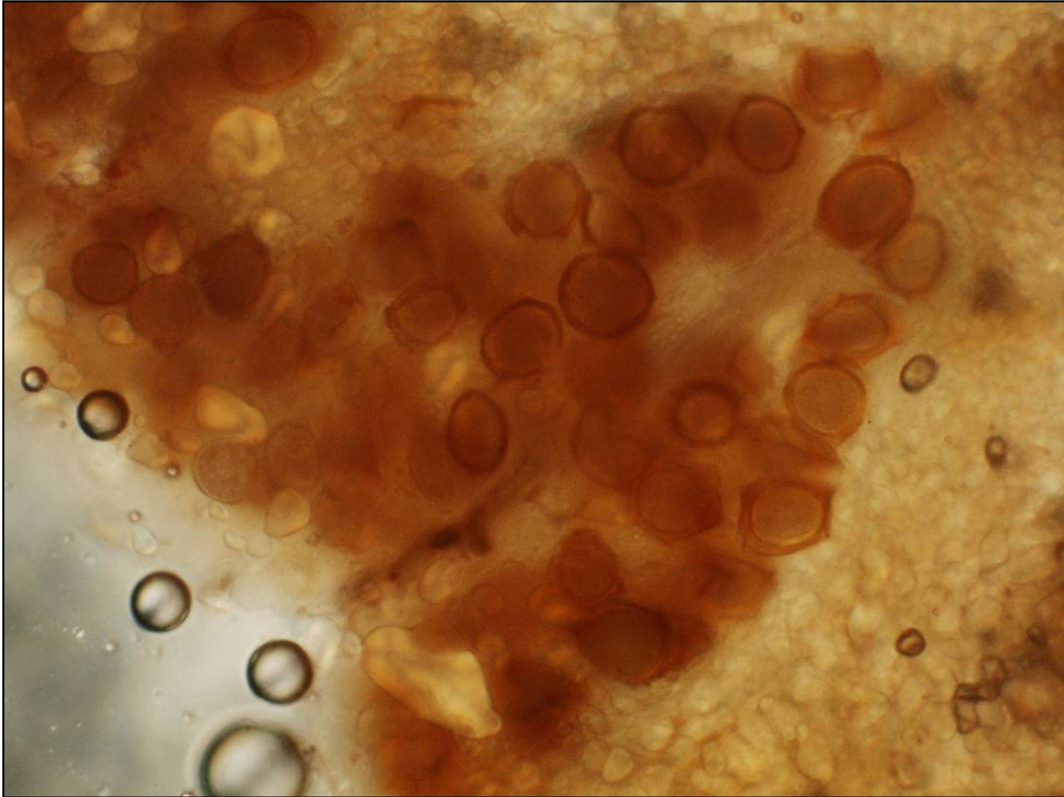

Figure 11. *S. endobioticum* resting spores at 20X (© IDCR-PHEL Ministry for Primary Industries, New Zealand).

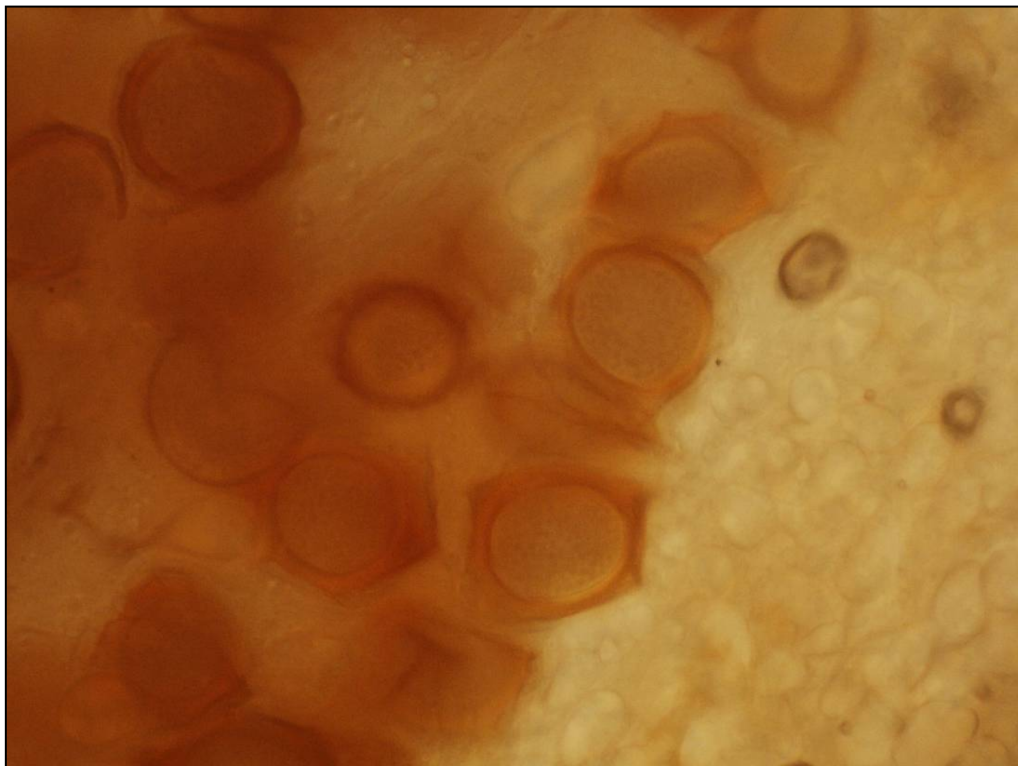

Figure 12. *S. endobioticum* resting spores at 40X (© IDCR-PHEL Ministry for Primary Industries, New Zealand).

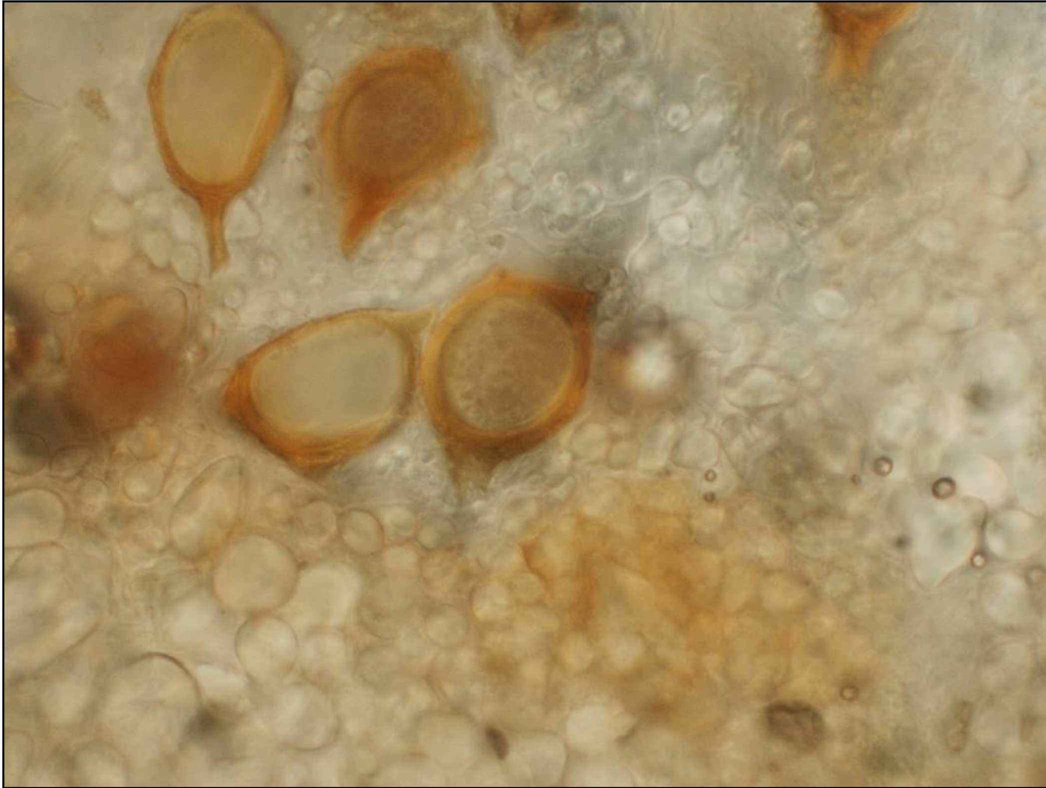

Figure 13. *S. endobioticum* individual resting spores (© IDCR-PHEL Ministry for Primary Industries, New Zealand)

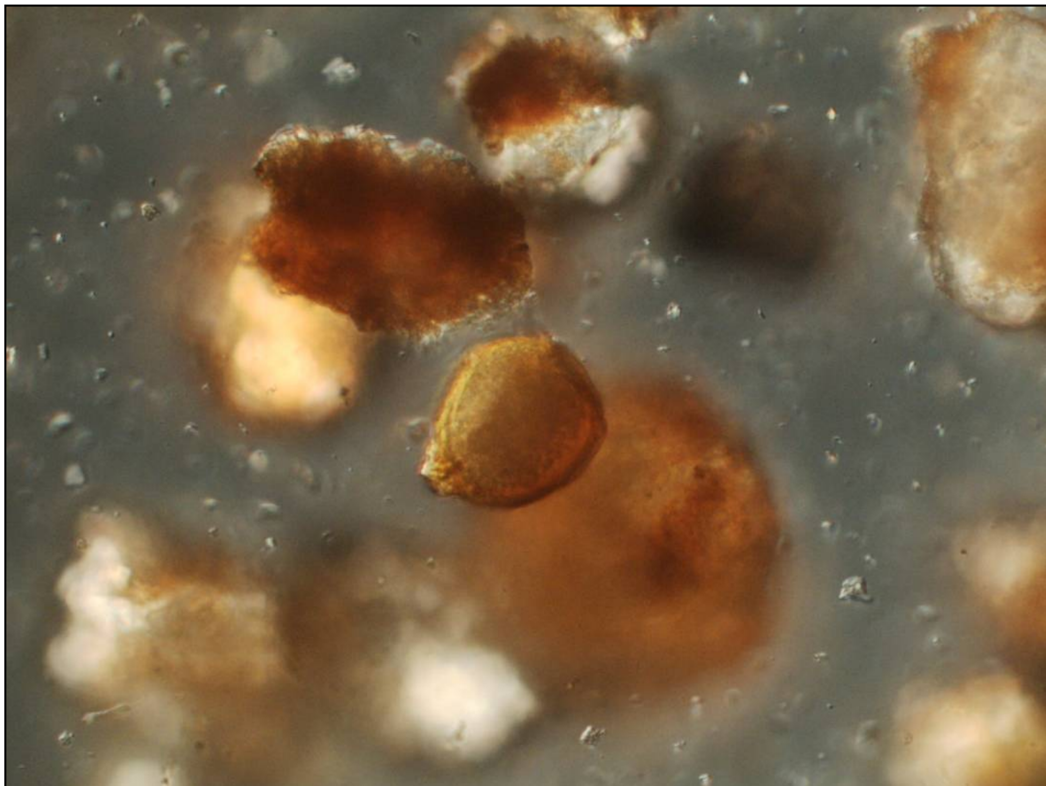

Figure 14. A single *S. endobioticum* resting spore in sol particles at 40X (© IDCR-PHEL Ministry for Primary Industries, New Zealand)

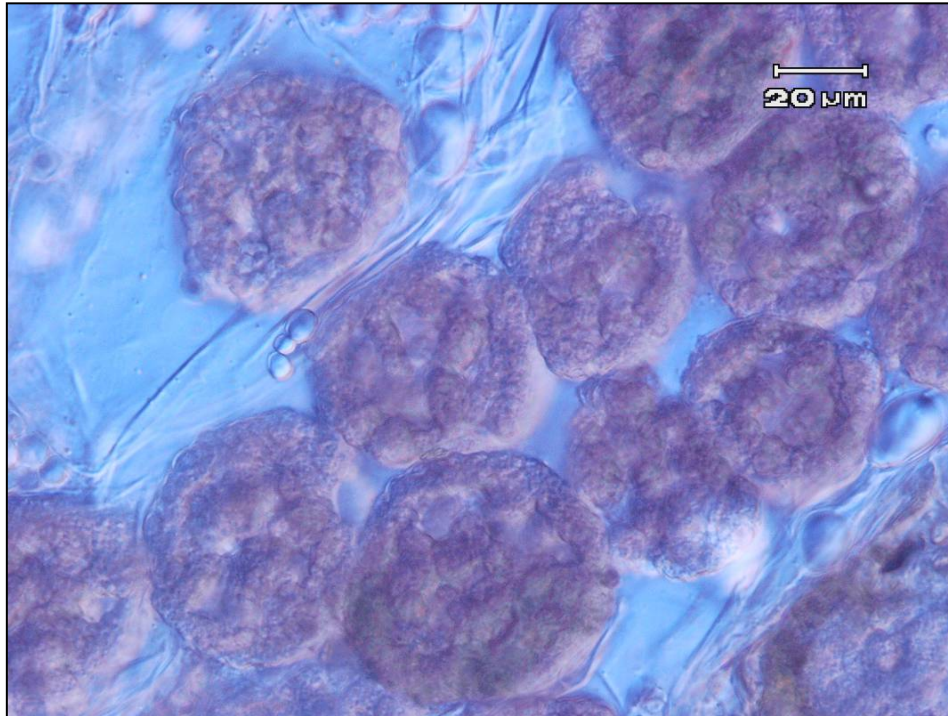

Figure 15. *Spongospora subterranean* spore balls at 40X (© IDCR-PHEL Ministry for Primary Industries, New Zealand)

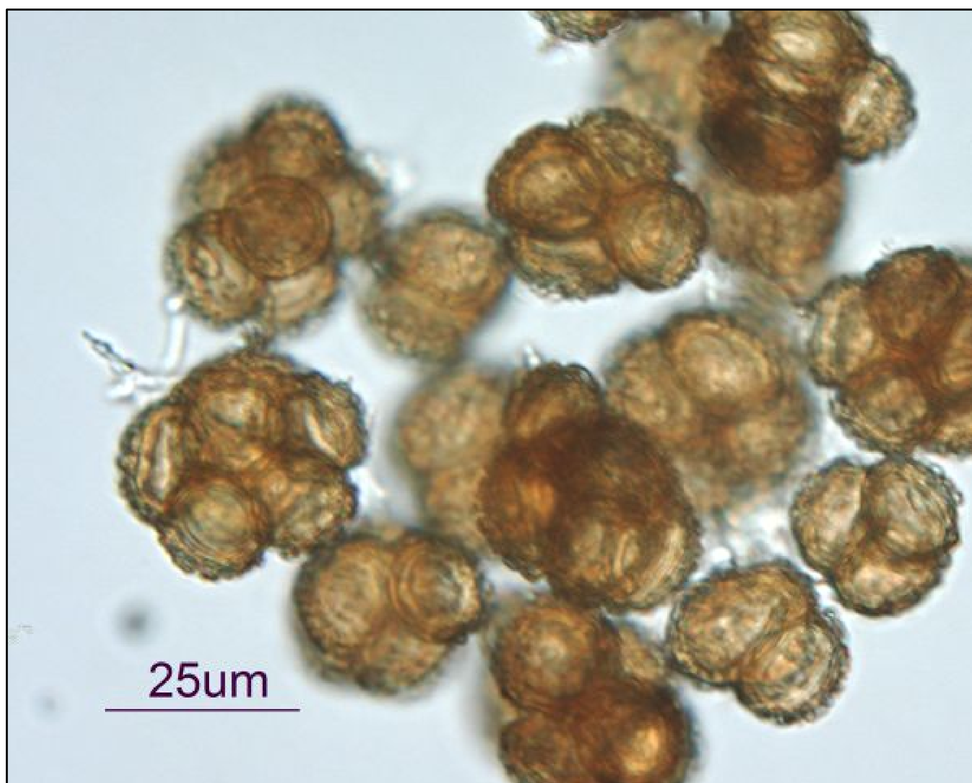

Figure 16. *Thecaphora solani* Spore balls from potato tuber, 400X. - BPI 179335. Chalkley, D. Systematic Mycology and Microbiology Laboratory, ARS, USDA.

5 CONTACTS FOR FURTHER INFORMATION

AUSTRALIA	**AUSTRALIA**
James Cunnington DAWR james.cunnington@agriculture.gov.au	Dolf De Boer Victorian Department of Primary Industries dolf.deboer@ecodev.vic.gov.au
	THE NETHERLANDS
	Peter Bonants Plant Research International B.V. Business Unit Biointeractions & Plant Health PO BOX 16 6700 AA Wageningen The Netherlands Phone: 31 317 476213 peter.bonants@wur.nl

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6 ACKNOWLEDGEMENTS

This diagnostic manual was originally compiled by:

Bonny Rowles-van Rijswijk, Dr James Cunnington, Dr Joanne Luck
Department of Primary Industries, Knoxfield
Private Mail Bag 15
Ferntree Gully Delivery Centre
Victoria 3156, Australia

Reviewed and edited by Ian Pascoe, Consulting Mycologist, pascoeig@bigpond.net.au

Procedures verified by Ministry for Primary Industries (previously MAF Biosecurity New Zealand).

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7.1 Related Websites

Australasia

http://www.biosecurity.govt.nz/pests/potato-wart

North America

http://www.inspection.gc.ca/english/plaveg/pestrava/synend/tech/synende.shtml

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8 APPENDICES

8.1 Life Cycle

Synchytrium endobioticum belongs to the fungal phylum Chytridiomycota, a large, primitive group of fungi regarded as basal to the true fungi. The fungus is an obligate parasite that depends entirely on its host, *Solanum tuberosum* for development and reproduction. Its simple, coenocytic thalli do not develop hyphae but develop into sporangia containing large numbers of flagellate zoospores.

As *S. endobioticum* does not produce hyphae, infection is initiated by spherical haploid zoospores 1.5-3.0 µm in diameter, which penetrate epidermal cells of meristematic tissues of growing points, buds, stolon tips, or young leaf primordia of the potato tuber and lower stem. The invaded and surrounding cells enlarge, and rapid cell division following infection from either zygotes or haploid zoospores causes an increase in meristematic tissue, providing additional infection courts. This can occur relatively quickly, as zoospores encyst and penetrate epidermal cells of susceptible tissue approximately 2 hours after formation. The zoospores swell into prosori, and then develop into sori. The prosori are oval, aseptate, smooth, thick walled, light golden brown, 40-50 µm in diameter, and usually lie at the bottom of the infected plant cell. There may be up to 4 prosori per infected plant cell. Contents escape the prosorus through the prosorus outer wall to form an ovoid, flattened or spherical haploid sorus of sporangia with each sorus containing 1-9 hyaline, thin-walled summer sporangia which quickly release zoospores to initiate new infection sites (Hampson and Proudfoot 1974; Hooker 1990; Walker 1983).

Winter sporangia (resting spores or meiosporangia) are the characteristic survival and dispersal spores of the fungus and are released from decaying warts (Fig 2). Under normal environmental conditions, the hyperplasic tissue quickly senesces and the disease passes through the "black wart" or "black scab" stage. The sub-epidermal tumour tissue is packed with winter sporangia and as the wart decays, sporangia are released into the surrounding soil. They are golden brown, spheroidal, measuring 35-80 µm in diameter. The thick sporangium wall is prominently ridged, and this morphology is diagnostic for *Synchytrium endobioticum.* These winter sporangia can survive between potato crop rotations for very long periods of time and can remain viable for at least 30 years. Techniques have been developed to test soils for presence of winter sporangia and to determine numbers of viable sporangia in old infested soils (Pratt 1976; EPPO/OEPP 2003).

After release from the decaying wart tissue, sporangia germinate to release zoospores into free water (Hampson and Proudfoot 1974; Hampson 1993). "Germination of both resting [winter] and soral [summer] sporangia occurs in water, and there is an indispensable minimum of water for the distribution of the motile cells. If the soil-moisture content does not at any time reach saturation, germination is prevented, but if it is constantly near saturation, infection is repressed, probably through the reaction on the host. The most favourable condition is periodic flooding, followed by drainage and aeration. Infection may occur, if the temperature is favourable, in soil that is wet at insufficient intervals to afford a normal crop" (Weiss 1925). After studying disease peaks, Hampson (1979) found that the fungus could control its own germination rate, to some extent, with dormancy varying from a few months to thirty years or more. This long period of infectiveness creates one of the major problems in controlling potato wart disease (Hampson and Proudfoot 1974). The second major problem in controlling potato wart disease is that by natural mutation, adaptation and hybridisation,

the fungus readily changes its pathogenic properties to develop races favoured by the local environmental conditions (Hooker 1990; Matskiv et al. 1998).

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