National Diagnostic Protocol for *Phakopsora euvitis*, the cause of Grapevine Leaf Rust

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This version of the National Diagnostic Protocol (NDP) for *Phakopsora euvitis* is current as at the date contained in the version control box on the front of this document.

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

The most current version of this document is available from the SPHD website:
http://plantbiosecuritydiagnostics.net.au/resource-hub/priority-pest-diagnostic-resources/

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1 Introduction

Leaf rust was first detected on grapevines in Darwin, Australia in 2001 (Weinert *et al.* 2003) and was identified as Asian grapevine leaf rust (GLR), *Phakopsora euvitis*. It was successfully eradicated by July 2007.

Phakopsora euvitis is a fungus that passes a heteroecious and macrocyclic life cycle through four different seasons. It occurs on a deciduous tree, *Melisoma myriantha* (Meliosmaceae), in late spring to early summer. It then infects *Vitis* species (Vitaceae) from summer to late autumn. Dormant teliospores are produced in autumn to overwinter in dead *Vitis* leaves. *Phakopsora euvitis* has a wide geographic distribution and can occur in regions without the alternate host *Melisoma myriantha*, which is known only from east Asia. In this case, *P. euvitis* persists on *Vitis* spp. as vegetative urediniospores without completing its life cycle.

Although spore morphology and disease symptoms of the fungus found in Darwin were characteristic of *Phakopsora euvitis*, a recent systematic study using the ITS region of ribosomal DNA showed that isolates of GLR from Australia and East Timor were different to Asian isolates (Chatasiri and Ono 2008). The Australian and adjacent East Timorese isolates of GLR are known only from urediniospores and a comparison of all spore stages could not be made with Asian GLR. A study currently in progress has indicated that *Phakopsora* found in Australia and south east Asia on grapevine may be a different species to *P. euvitis*, based on the ITS and LSU regions of ribosomal DNA.

1.1 Host range

According to the characterisation of *Phakopsora euvitis*, the vitaceous hosts of the rust are confined to the genus *Vitis* (Ono 2000). However, field collections and inoculation experiments have shown that the fungus is able to parasitise many other species of *Vitis* as well as *Ampelocissus acetosa* and *A. frutescens,* species of Vitaceae native to the northern region of Australia (Daly *et al.* 2005).

2 Taxonomic Information

Kingdom:	Fungi
Phylum:	Basidiomycota
Class:	Pucciniomycetes
Order:	Pucciniales
Family:	Phakopsoraceae
Genus:	*Phakopsora*
Species:	*Phakopsora euvitis* Y. Ono
Synonyms:	*Aecidium meliosmae-myrianthae* P. Hennings & Shirai (aecial anamorph) *Phakopsora ampelopsidis* pro parte *Physopella ampelopsidis* pro parte *Physopella vialae* (Lagerheim) Buriticá & J.F. Hennen *Physopella vitis* (Thümen) Arthur (uredinial anamorph) *Uredo vialae* Lagerheim *Uredo vitis* Thümen.

3 Detection

Currently the most reliable method for detecting GLR on vines is to sample many leaves from a number of areas within the canopy, targeting the older leaves and those with chlorotic/necrotic tissue damage. The lower surfaces of leaves should be examined for the presence of uredinia (Section 3.2, 4). If uredinia are not present, the leaves can be incubated in a sealed plastic bag at room temperature (25°C) and re-assessed after seven days. If latent infections are present, sporulation will be evident after the period of incubation.

A simple field test to detect suspected GLR is to wipe suspected pustules with an ordinary white facial tissue and look for a yellow-orange stain.

3.1 Plant parts affected

In sub-tropical and tropical regions, *P. euvitis* persists year-round via repeated production of urediniospores and infection of *Vitis* plants. The uredinial pustules most commonly appear in orange, powdery masses on the lower surface of *Vitis* leaves (Image 1). Areas of sporulation generally correspond with chlorotic and necrotic lesions on the upper surface (Images 2 and 3). As the infection progresses, yellowing of the entire leaf and premature defoliation can occur.

P. euvitis is harboured primarily by the leaves of susceptible hosts as both mycelium and uredinia. in addition the dry, powdery nature of urediniospores means they are likely to fall or be blown into fissures in the bark of the canes and trunk of a vine. The fungus may also survive as mycelium in the bud tissue of dormant vines.

Where *M. myriantha* does not occur, only the uredinia of *P. euvitis* are likely to be found, although teliospore production cannot be discounted.

3.2 Symptom description

The disease is most readily identified by the yellow to brown lesions of various shapes and sizes appearing on the upper surface of mature leaves, with corresponding yellow-orange masses of urediniospores on the lower leaf surface. Heavy infection will result in the dispersal of masses of spores when the leaf is tapped or pulled to remove it from the plant. Significant infection levels can occur on a single plant causing premature leaf drop, which may eventually lead to the weakening of the vine and impact on fruit production and quality.

The uredinial-telial stage of *P. euvitis* causes chlorotic and necrotic lesions of various shapes and sizes on the upper surface of *Vitis* leaves (Images 2 and 3). In corresponding areas on the lower surface, masses of yellow-orange coloured spores develop from tiny but densely aggregated pustules (Images 1, 4 and5). As the infection progresses entire leaf yellowing and premature defoliation can occur. In temperate areas where vines undergo a period of dormancy, telia are formed following uredinia (Image 6). Initially they are crust-like and orange-brown and can either form around the uredinia or separately from them. Eventually they become dark brown-black.

The spermogonial-aecial stage causes pale yellow, circular lesions on leaves of *M. myriantha* (Image 7). Spermogonia appear on the upper surface as small, raised orange-brown dots, later becoming black (Image 8). Dome-shaped, pale yellow-orange coloured aecia appear on the lower surface, usually opposite the spermogonia (Image 9). Aecia develop as a long tube from the lesion (Image 10). Aeciospores are released when the tip of the tube ruptures.

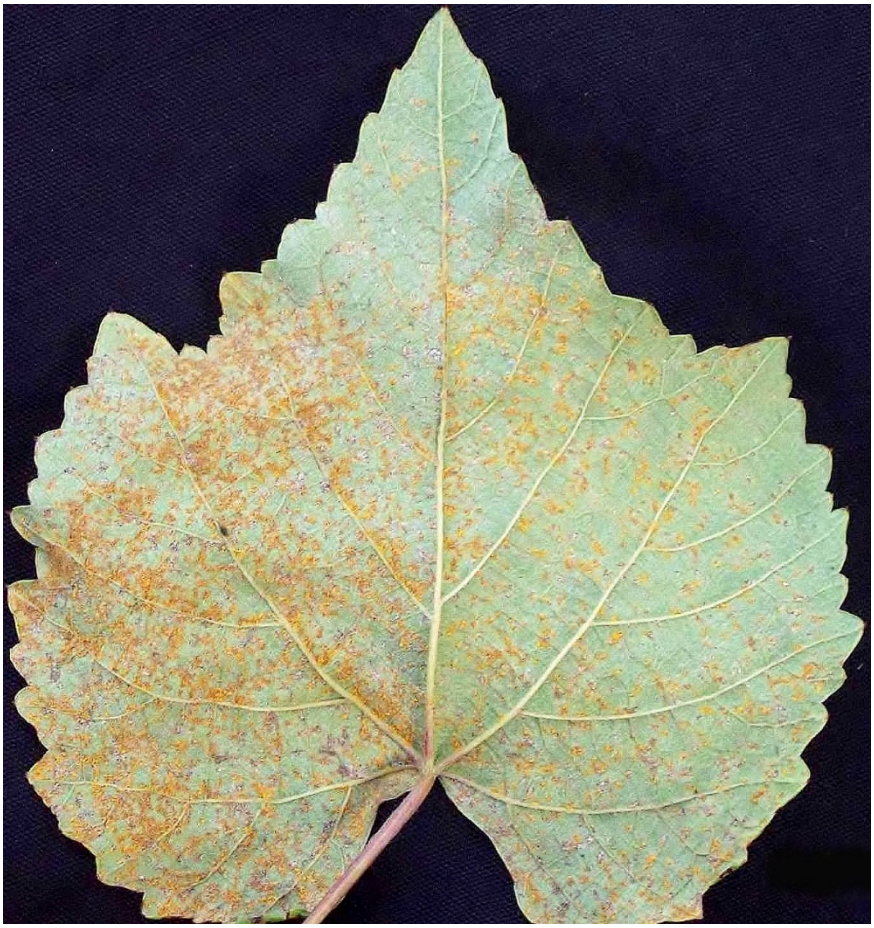

Image 1. Uredinia produced on the lower surface of a *Vitis* cultivar leaf. © NT DPIF

Image 2. Young chlorotic lesions produced on the upper surface of a *Vitis* cultivar leaf. © NT DPIF

Image 3.Older necrotic lesions produced on the upper surface of a *Vitis* cultivar leaf. © NT DPIF

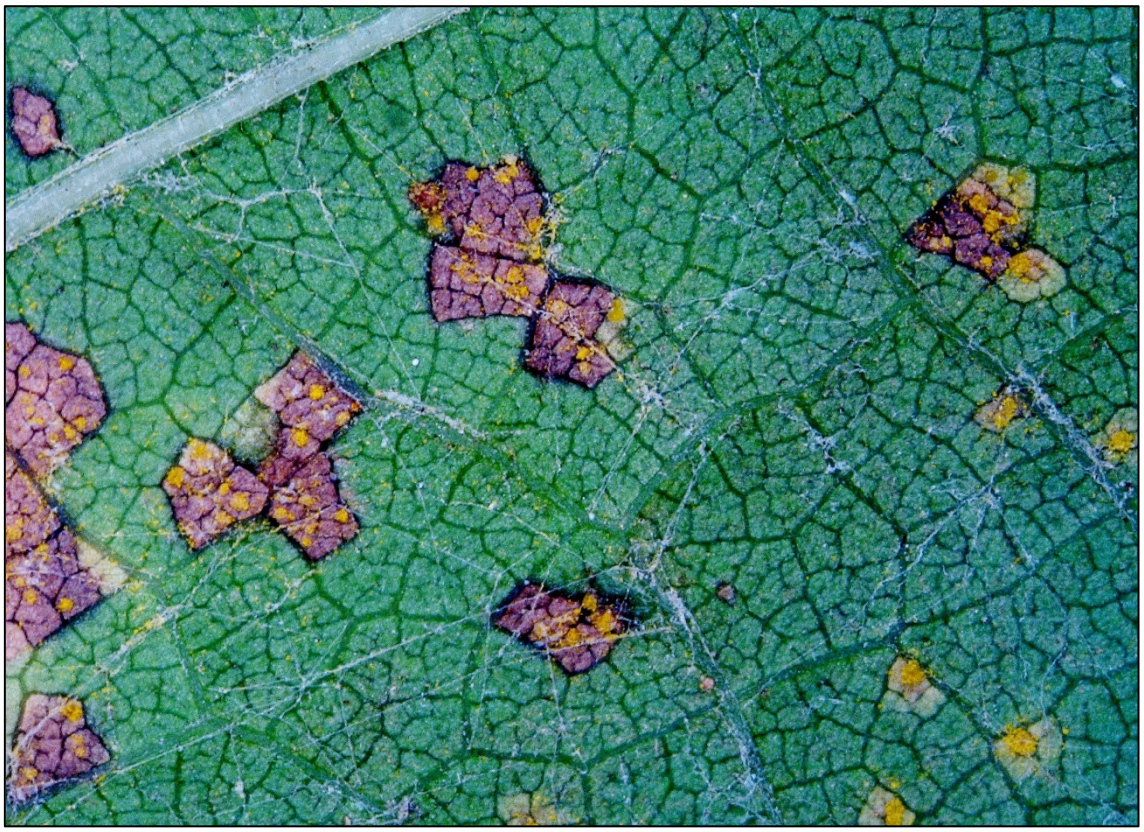

Image 4. Uredinia produced on the lower surface of a *Vitis* cultivar leaf. Uredinial infection may cause necrosis of surrounding host tissues (appearing as angular, reddish brown spots). © NT DPIF

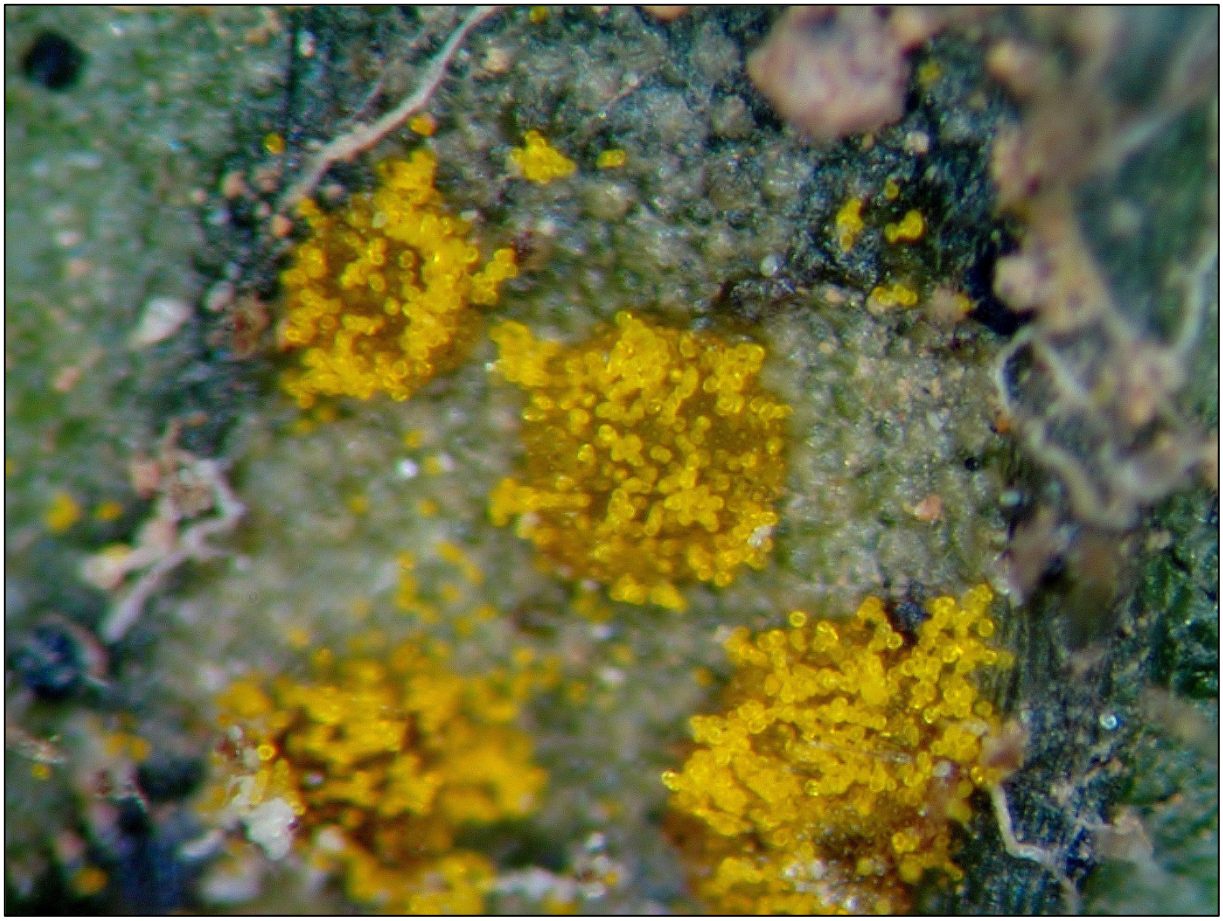

Image 5. Uredinia produced on the lower surface of a *Vitis* cultivar leaf. Yellow powdery masses of urediniospores are produced. © NT DPIF

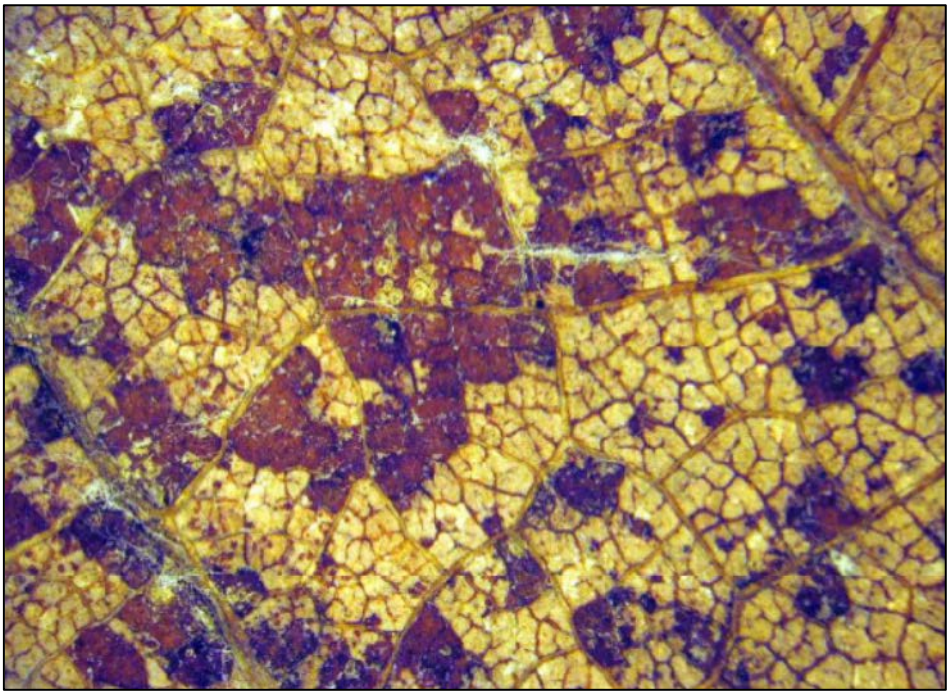

Image 6. Telia (dark brown, angular, cruster-like) produced on the lower surface of a *Vitis* cultivar leaf. © Ibaraki University

Image 7. Yellowish blotches and spots are produced after successful inoculation of basidiospores. Numerous spermogonia and aecia are produced on the infected host tissues. © Ibaraki University

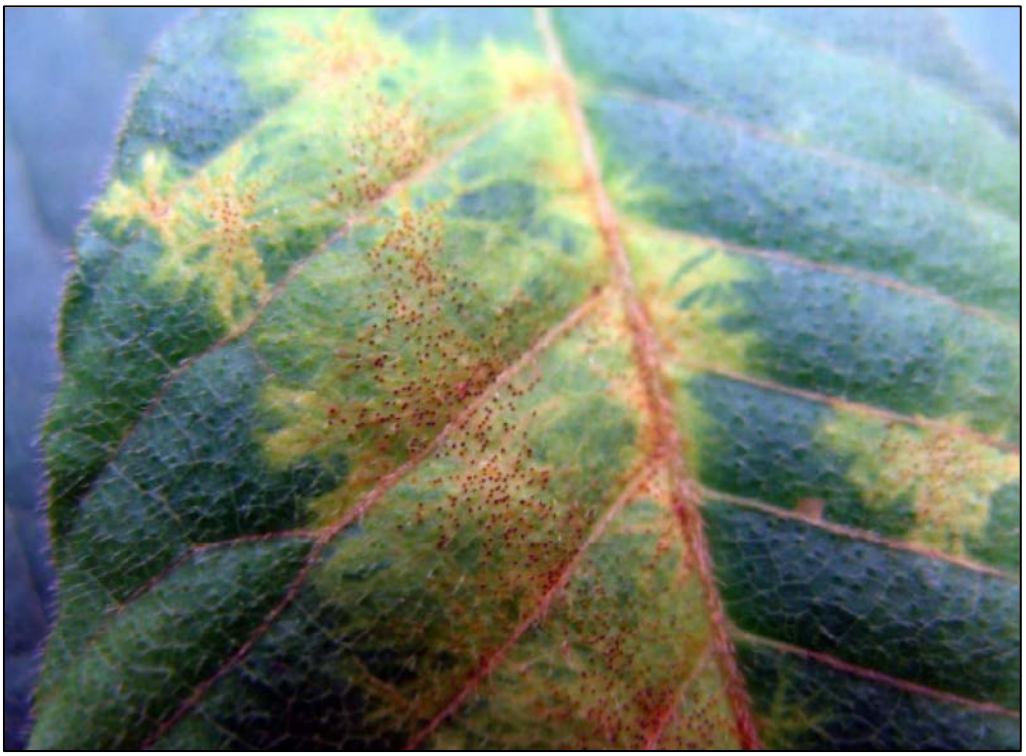

Image 8. Spermogonia produced on the upper surface of a *M. myriantha* leaf. © Ibaraki University

Image 9. Dome shaped aecia of *P. euvitis* on the lower surface of a *M. myriantha* leaf. © Ibaraki University

Image 10. Aeciospores of *P. euvitis* developing from the lower surface of a *M. myriantha* leaf. © Ibaraki University

3.2.1 Diseases causing similar symptoms

Downy mildew (*Plasmopara viticola*) of *Vitis* spp. causes lesions very similar in size and colour to GLR when mature leaves are infected (Image 11). However, it is easily distinguished by the white, “downy” growth on the lower surface of the leaves where the lesions occur (Images 12 and 13). If no sporulation is evident, it can be induced by humid incubation of the leaves in a sealed plastic bag overnight.

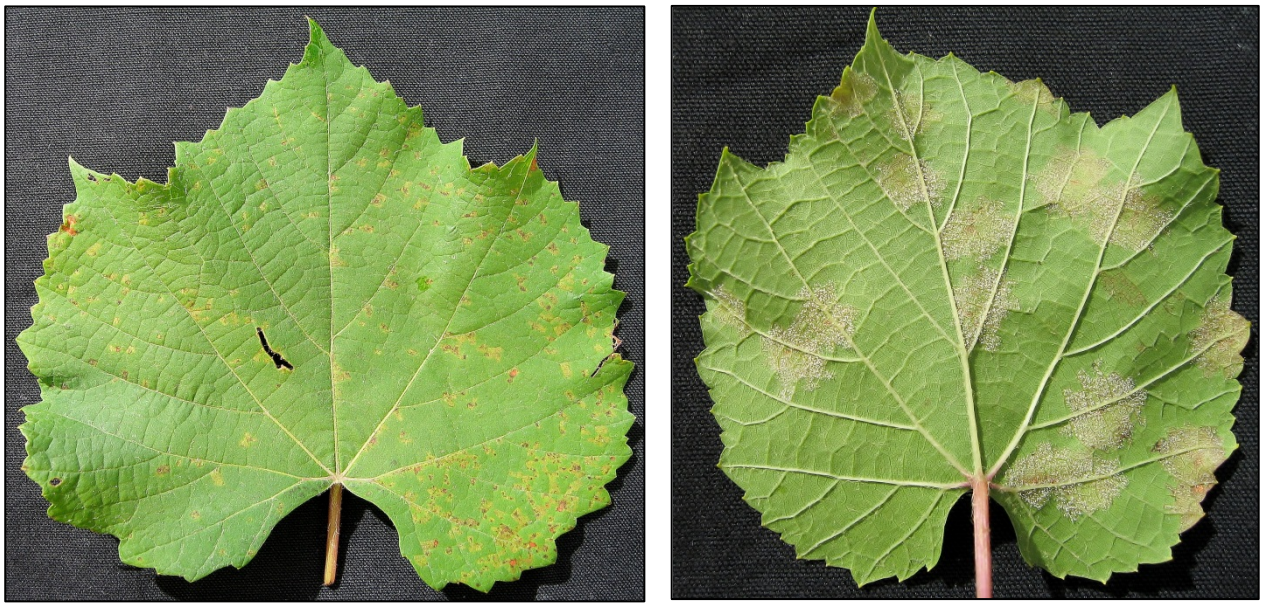

Image 11. (L) Necrotic lesions caused by *Plasmopara viticola* on the upper surface of a *Vitis* sp. leaf. © NT DPIF

Image 12 (R). Downy growth (sporulation) of *Plasmopara viticola* on the lower surface of a *Vitis* sp. leaf. © NT DPIF

Image 13. Sporulation (production of zoosporangia on branched hyphae manifest downy appearance) of *Plasmopara viticola* on the lower surface of a *Vitis* cultivar leaf. © NT DPIF

3.3 Diagnostic flow chart

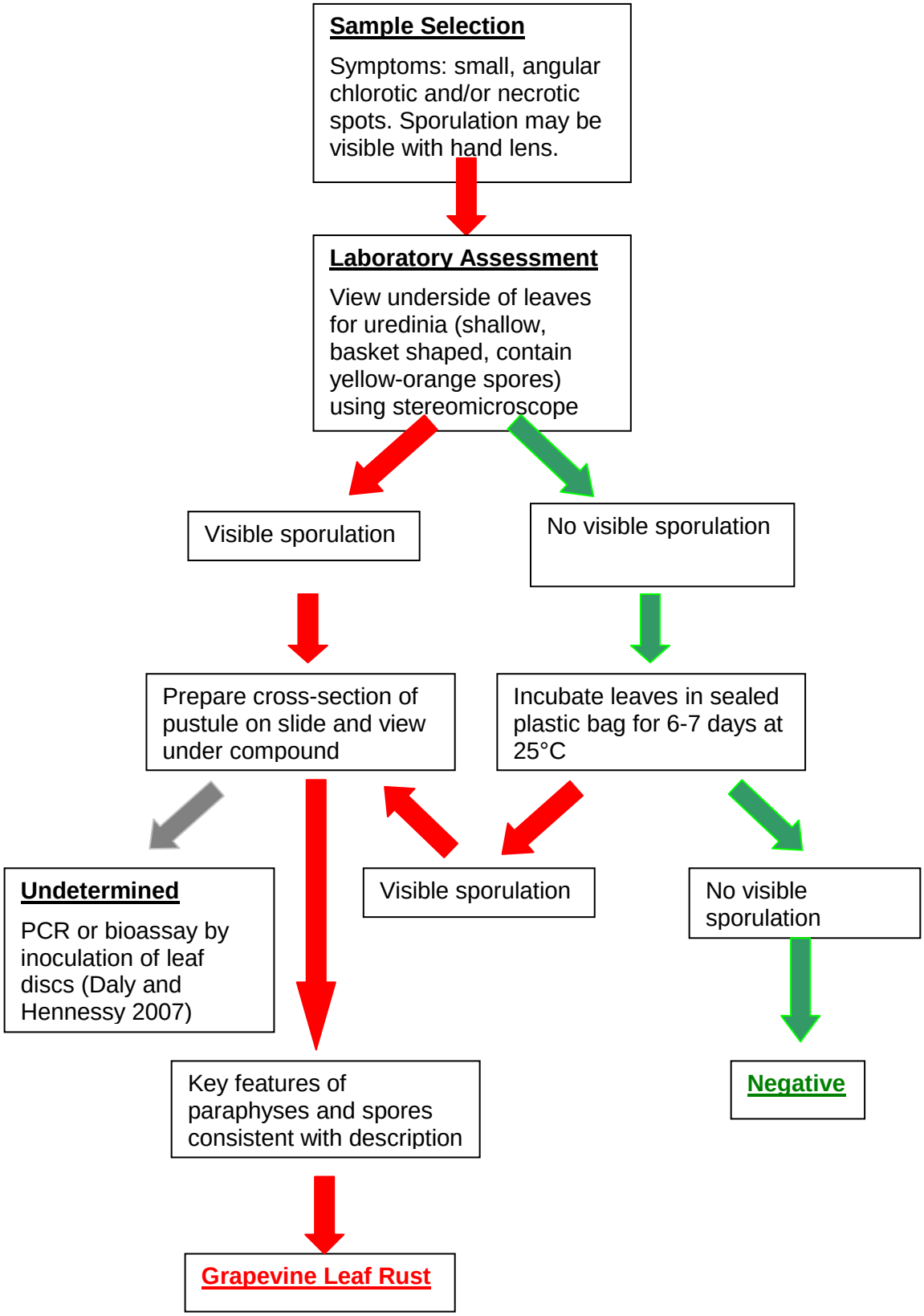

Figure 1. *Diagnostic flow chart*

4 Identification

Identification of the causal agent of GLR can be made by direct examination of morphological characteristics using light microscopy, or by molecular analysis. Morphological identification requires examination of the uredinial paraphyses and urediniospores under a compound microscope.

4.1 Morphological methods

4.1.1 Microscopic identification

Symptomatic leaves are examined under a dissecting microscope for urediniospores emerging from pustules on the abaxial surface. Any such spores are then mounted onto a slide and examined under a compound microscope.

P. euvitis urediniospores are yellow-orange, 15-29 x 10-18 µm in size with echinulate walls. Spermogonia are formed in clusters on both surfaces of the *Meliosma myriantha* leaf (Image 8). They are formed under the cuticle on the epidermal-cell layer and are conical or hemispherical, 90-135 µm wide and 60-80 µm high (Image 14).

Aecia are formed mostly on the abaxial surface of the leaf opposite the spermogonia (Image 9). They are surrounded by a well-developed peridium (Image 15) and become long and columnar under an experimental condition (Image 10). Under a natural condition, the aecia most frequently appear cupulate. The tip of the peridium ruptures to release aeciospores. Aeciospores are formed in chains from the basal sporogenous layer in the aecia (Image 15). They are subglobose or broadly ellipsoid, often angular and 15-20 x 12-16 µm. The wall is evenly thin (ca 1 µm) at the sides and thickened (-4 µm) at the apex (Image 16), colourless and minutely and evenly verrucose.

Uredinia are formed mostly on the abaxial surface of the leaf. They are minute and either scattered or aggregated in small groups (Images 3, 4 and 5). They are formed subepidermally, soon becoming erumpent with densely surrounding paraphyses (Image 17). The paraphyses are cylindrical to weakly incurved, evenly thin-walled or dorsally thick-walled (1.5-4.0 µm) and 30-75 µm high (Image 18). Urediniospores are obovoid, obovoid-ellipsoid or oblong-ellipsoid and 15-29 x 10-18 µm (Image 19). The wall is evenly thick (ca 1.5 µm) and uniformly echinulate (Image 20). Usually six germ pores are scattered on the wall, or rarely four germ pores are located on the equatorial zone (Image 21). Telia are formed almost strictly on the abaxial surface of the leaf, either associated with uredinia or separately. They are crustose, brown to blackish brown, often confluent, subepidermal and applanate (Image 6). Teliospores are more or less regularly arranged in 3-5 layers, oblong to oblong-ellipsoid and 13-32 x 7-13 µm (Images 22 and 23). The wall is evenly thin and pale brown at the top layer of a spore but slightly thickened and brownish in the teliospores of the uppermost layer. When overwintered teliospores germinate, yellowish basidia and basiospores with a fluffy appearance are produced (Image 24). Basidiospores are thin-walled, reniform and 8.2-11.4 x 5.0-8.0 µm (Image 25).

This fungus seems to be highly host-specific, being restricted to the genera *Vitis*, *Meliosma* (Ono 2000) and *Ampelocissus* (Daly *et al* 2005). Thus, correct identification of the host aids the identification of this species. On *Vitis, Phakopsora euvitis* forms cylindrical or weakly incurved uredinial paraphyses with a moderately thickened dorsal wall (1.5-4 µm) and a thin ventral wall urediniospores usually with six scattered germ pores in each and kidney-shaped basidiospores. This fungus is easily distinguished by these characters from the morphologically related *P. ampelopsidis* on *Ampelopsis* and *P. vitis* on *Parthenocissus*. *P. uva*, the American grapevine leaf rust fungus, can be distinguished from *P. euvitis* by the apically thickened urediniospore-wall and basally inflated uredinial paraphyses.

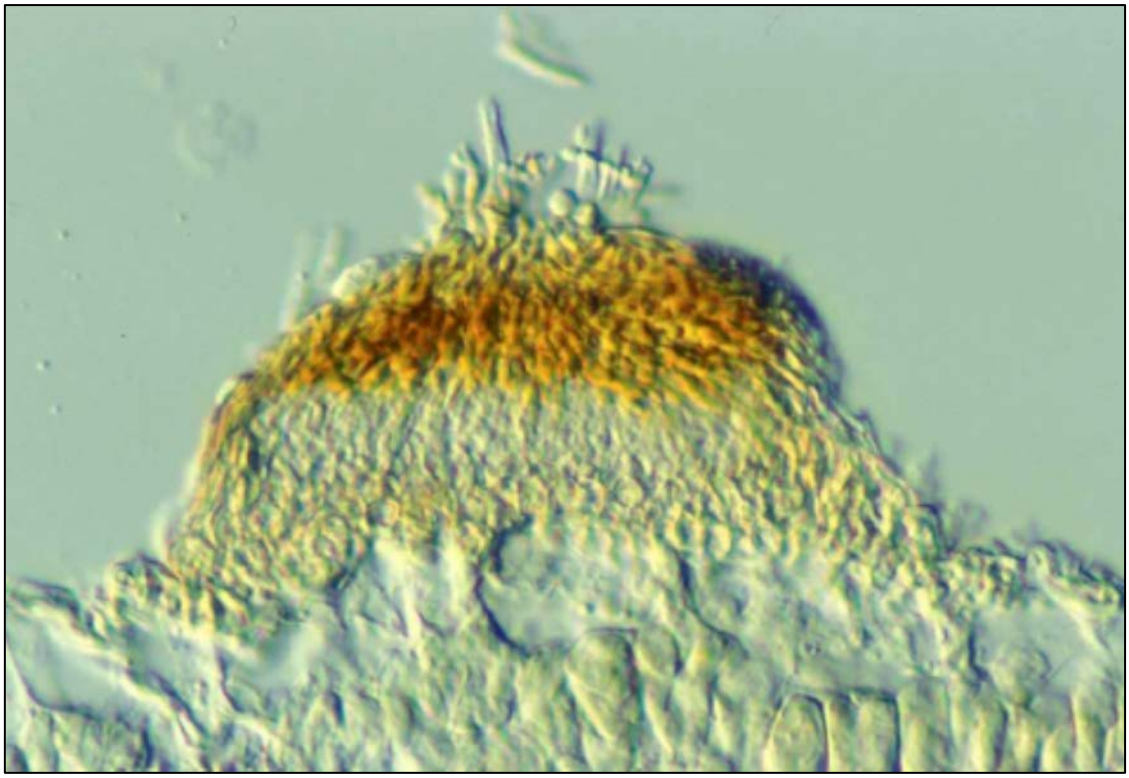

Image 14. A vertical section of a spermogonium formed under a cuticular layer on the host epidermis. The spermogonial appear conical or hemispherical. © Ibaraki University

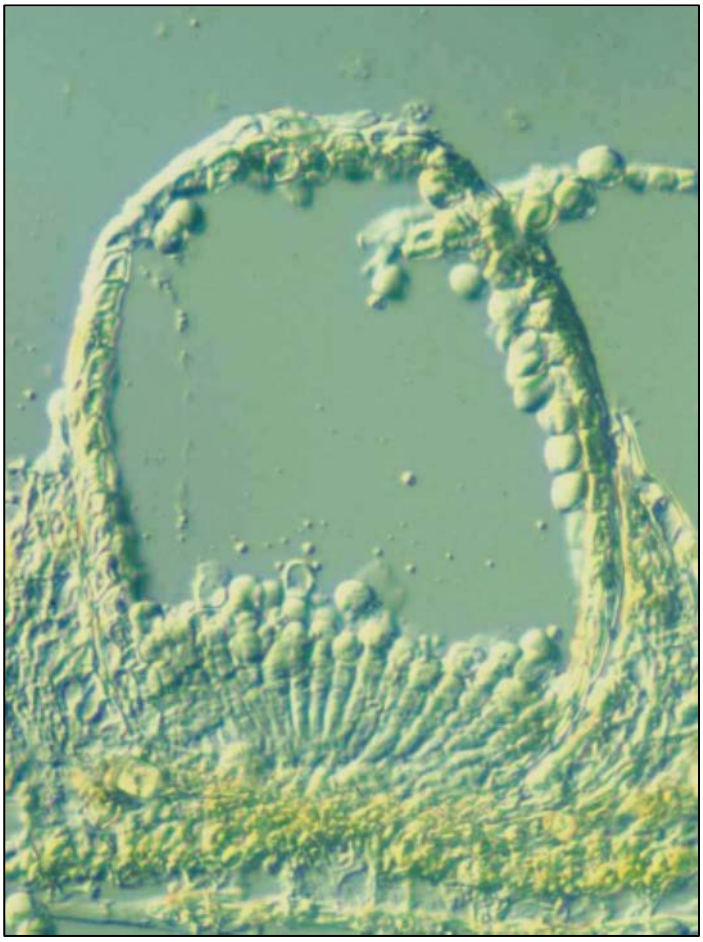

Image 15. A vertical section of an aecium developing subepidermally and rupturing the host epidermis as it mature. The mature aecium ruptures a peridial layer at the tip, from which aeciospores are released. Aeciospores are produced in chains from the basal sporogenous cell in the peridiate aecium. © Ibaraki University

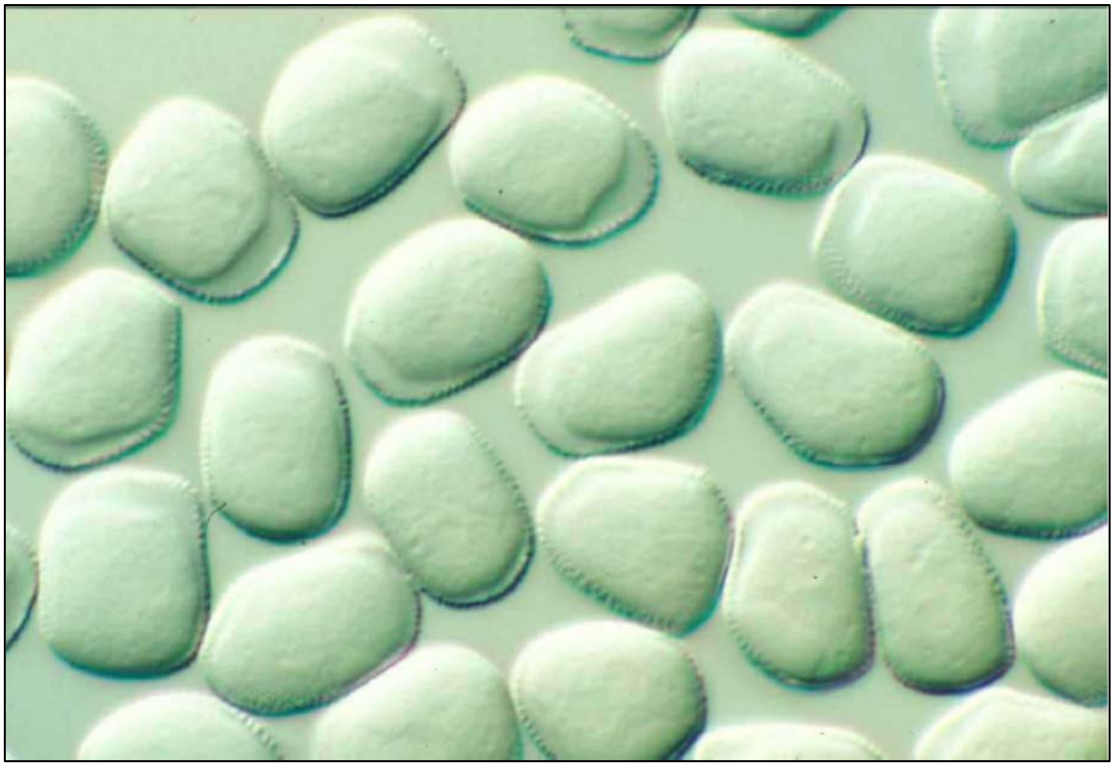

Image 16. Mature aeciospores. Note the apical wall thickening. © Ibaraki University

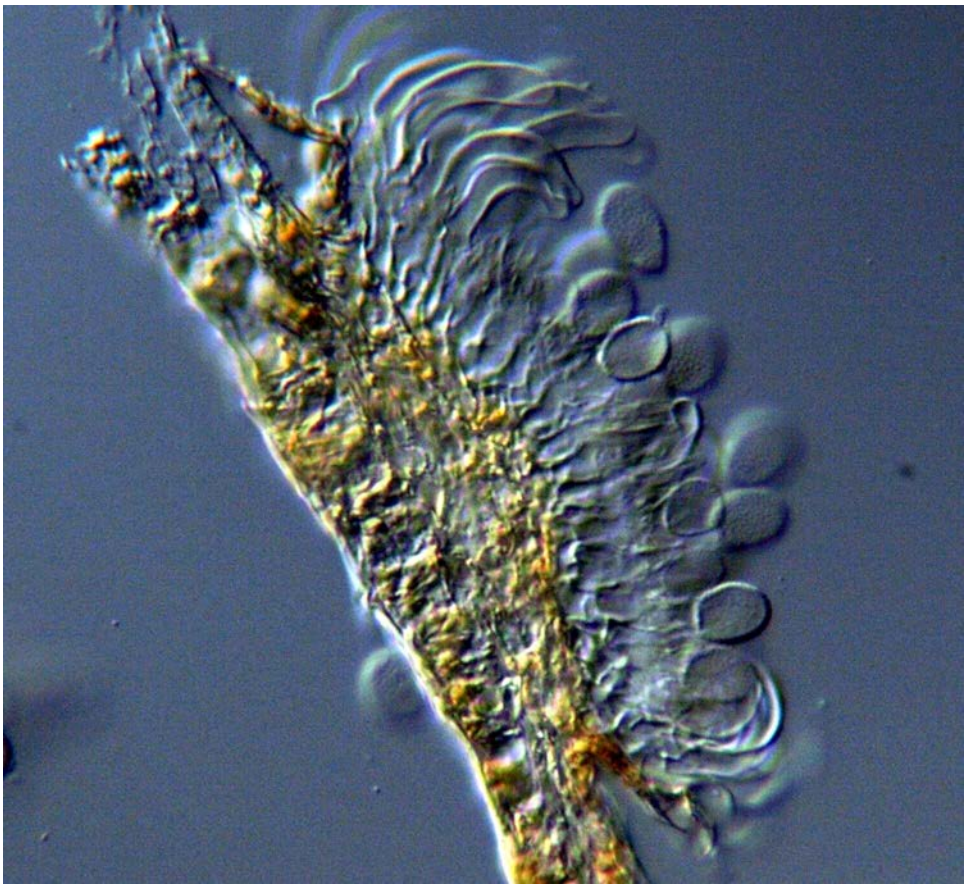

Image 17. A vertical section of a uredinium. Note the densely paraphysate uedinium and pedicellate urediniospores produced in the central part of the uredinium. © NT DPIF

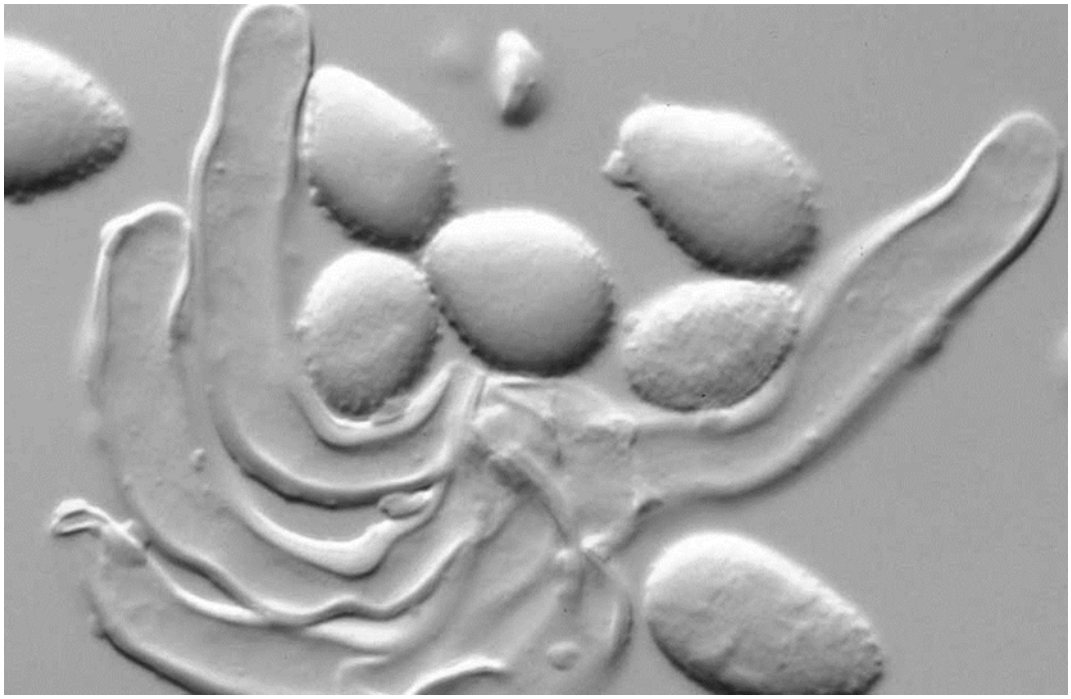

Image 18. Basally united incurved paraphyses. The dorsal wall is slightly thicker than the ventral wall. © Ibaraki University

Image 19. Mature urediniospores focused at a medial section. The spores are various in shape, but mostly oblong-ellipsoid or obovoid. © Ibaraki University

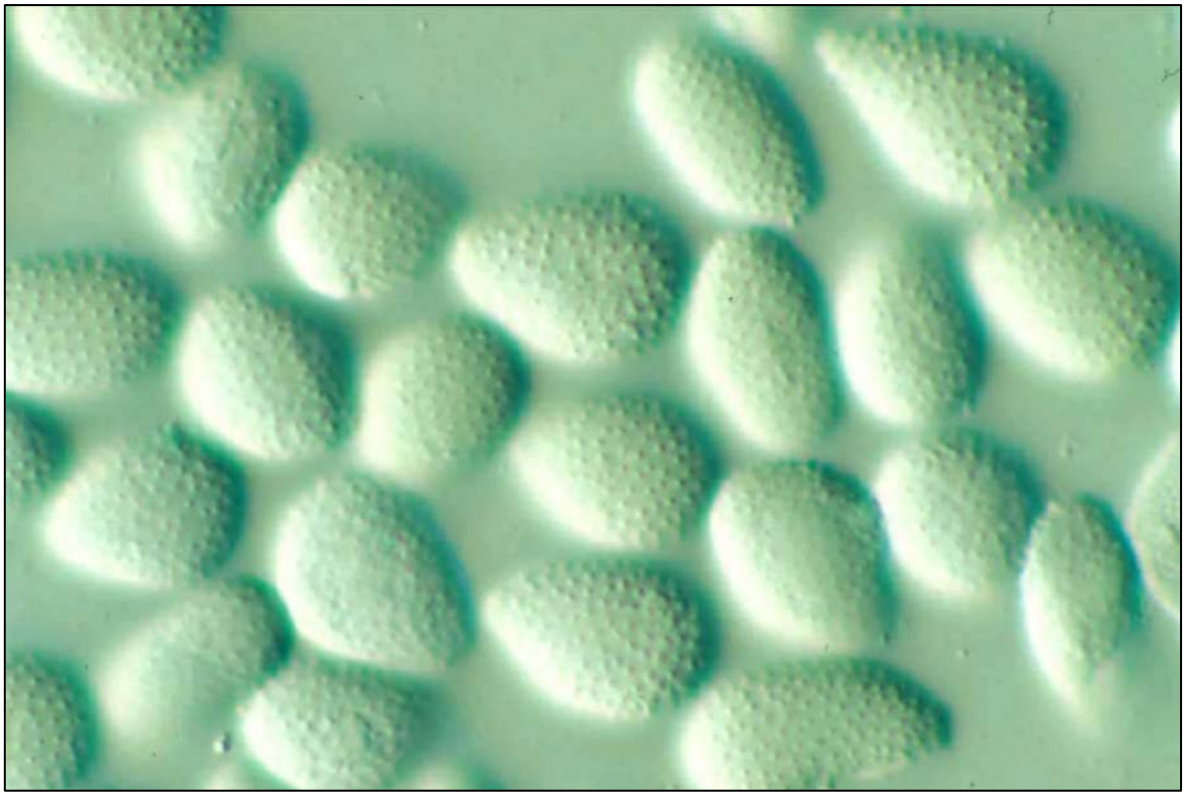

Image 20. Mature urediniospores focused at a tangential section. The wall appears completely echinulate. © Ibaraki University

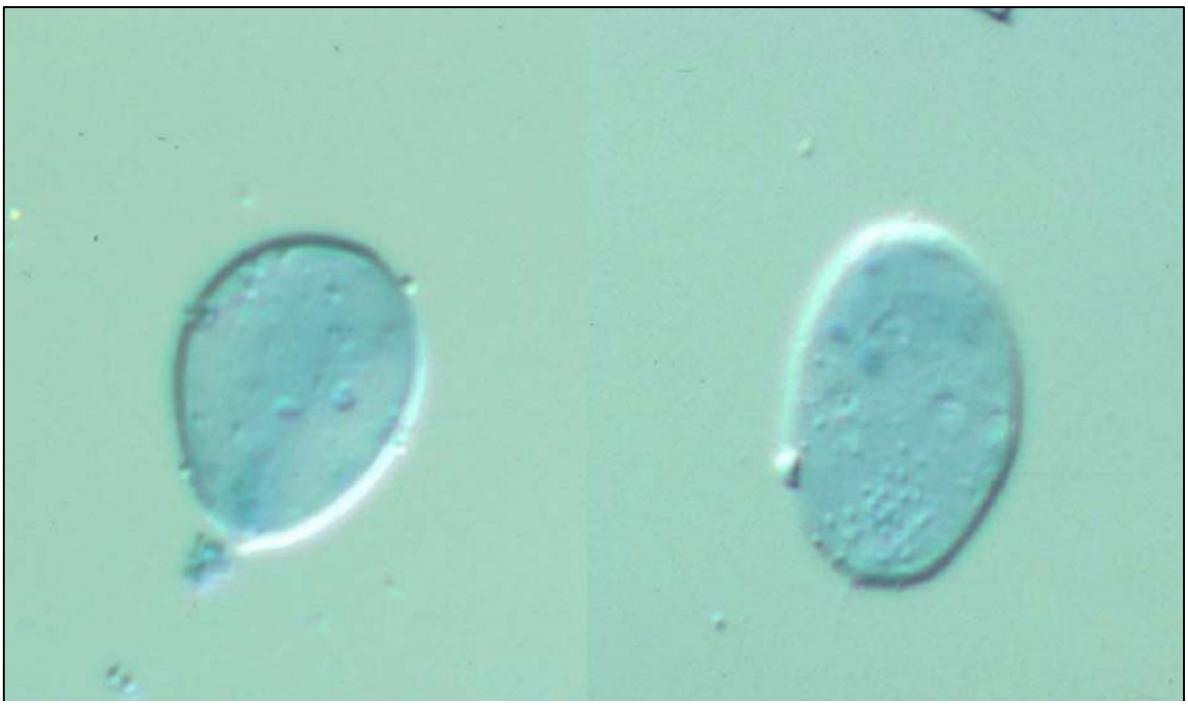

Image 21. Urediniospore germ-pores are faint. Six germ pores are distributed on the wall, or four germ pores are distributed on the equatorial zone. © Ibaraki University

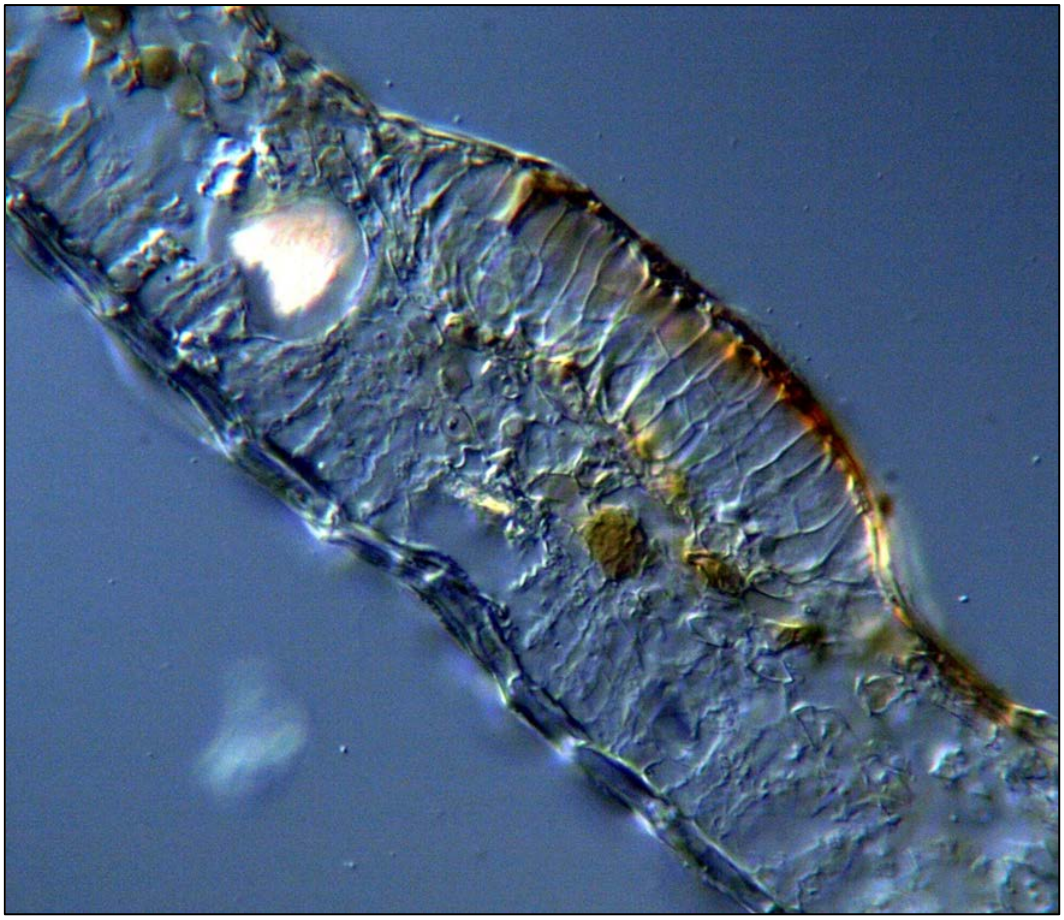

Image 22. A vertical section of a telium produced in the host mesophyll. © NT DPIF

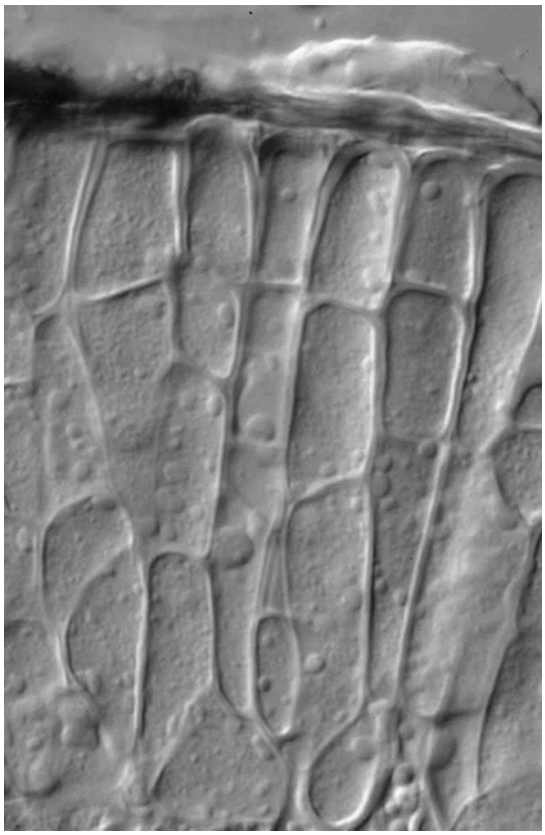

Image 23. A vertical section of a telium. Teliospores are arranged more or less regularly in a few layers. © Ibaraki University

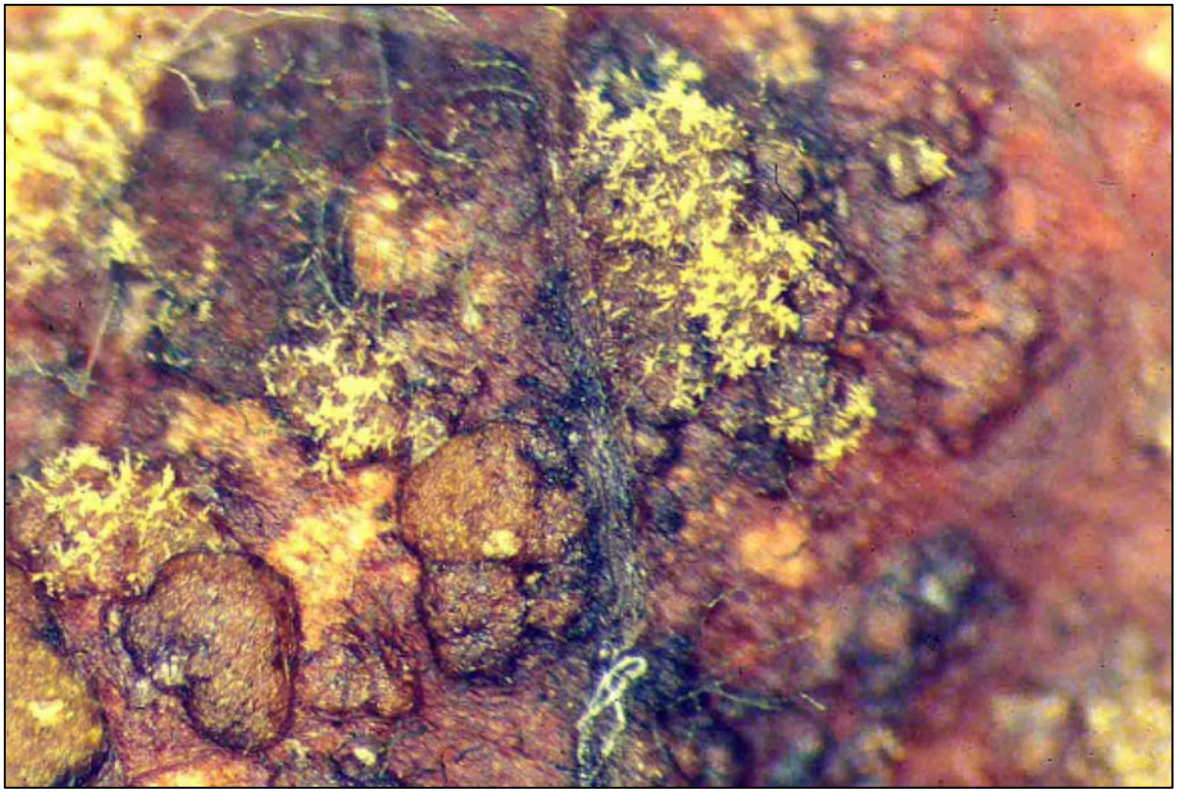

Image 24. Basidia and basidiospores produced on overwintered telia. © Ibaraki University

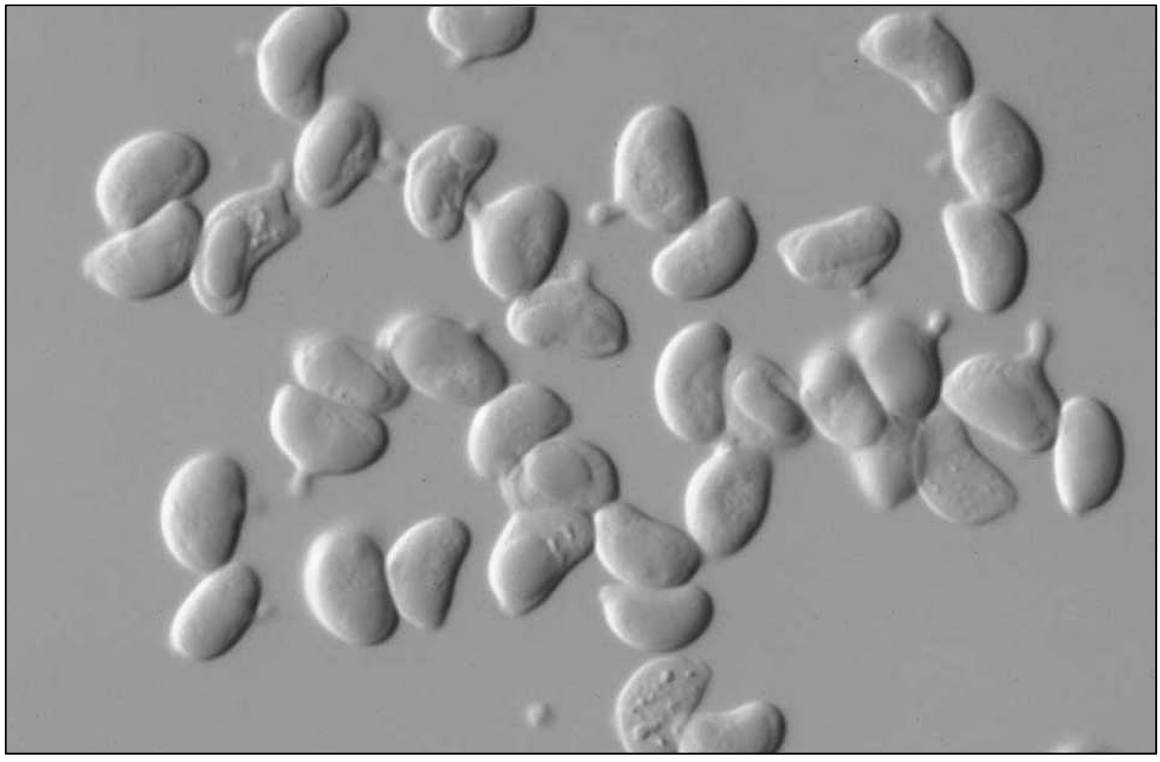

Image 25. Kidney-shaped basidiospores. © Ibaraki University

4.2 Molecular methods

PCR tests will not work properly if plant material is present. They should be done on fungal material only.

4.2.1 Molecular barcoding of *Phakopsora euvitis*

Amplified copies of the ITS or LSU regions of rDNA can be sequenced and compared to known sequences on GenBank for identification of rust fungi (see numbers section 4.2.1.4). The LSU region of rust fungi is more easily sequenced than the ITS region, as the ITS region may contain indels that inhibit direct sequencing. The ITS2-LSU region can be amplified with primers *Rust 2INV* and *LR7*. In some cases, if a product is not amplified, a nested reaction can be performed using the primers *LROR* and *LR6*. In the case of *Phakopsora euvitis*, sequences of the ITS region are required to determine whether the pathogen is Asian grapevine leaf rust or south-east Asian grapevine leaf rust (i.e. the population from Australia and East Timor).

4.2.1.1 Equipment

- Thermocycler
- Taq polymerase and PCR components
- Micropipettes and aerosol resistant tips
- Disposable gloves (powder free)
- Gel electrophoresis apparatus

Primers

ITS primers:
ITS1F: 5'- CTTGGTCATTTAGAGGAAGTAA -3' (Gardes and Bruns, 1993)
ITS4: 5'- TCCTCCGCTTATTGATATGC -3' (White et al., 1990)

LSU primers:
Rust 2INV: 5'- GATGAAGAACACAGTGAAA -3' (Aime, 2006)
LR7: 5'- TACTACCACCAAGATCT -3' (Vilgalys and Hester, 1990)
LROR: 5'- ACCCGCTGAACTTAAGC -3' (Vilgalys and Hester, 1990)
LR6: 5'- CGCCAGTTTCTGCTTACC -3' (Vilgalys and Hester, 1990)

4.2.1.2 DNA Extraction

Any standard fungal DNA extraction protocol can be used for rust fungi. However, the protocol of the MoBio Microbial DNA Extraction Kit is recommended.

At least five rust pustules are excised from a leaf using fine forceps or a scalpel and placed into extraction buffer. The kit protocol is then followed to completion and DNA is stored at -20°C.

4.2.1.3 DNA Amplification

1. Prepare PCR cocktail on ice in a sterile Eppendorf tube

For each 25 µl sample the cocktail will contain:

PCR buffer (10x)	2.500 µl (final concentration 1x)
$MgCl_2$ (50 Mm)	0.750 µl (final concentration 1.5mM)
dNTPs (10 mM)	0.500 µl (final concentration 200µM)
Forward primer (10 µM)	0.250 µl (final concentration 0.2µM)
Reverse primer (10 µM)	0.250 µl (final concentration 0.2µM)
Taq	0.200 µl (final concentration 1%)
H_2O	20.550 µl

To prepare the cocktail, the above volumes are multiplied by the number of samples and added to a single tube. 24 µl aliquots are then made into 0.2 mL tubes.

2. Add 1 µl of DNA template to 24 µl of PCR cocktail
3. Run PCR

ITS reaction
ITS PCR conditions

Dentauration	94° for 4 min	x 1 cycle
Denaturation	94° for 30 sec	
Annealing	50° for 45 sec	x 35 cycles
Extension	72° for 1.5 min	
Final extension	72° for 7 min	x 1 cycle

LSU reaction
First cycle conditions

First reaction with primers *Rust 2INV* and *LR7*

Dentauration	94° for 4 min	x 1 cycle
Denaturation	94° for 30 sec	
Annealing	57° for 45 sec	x 45 cycles
Extension	72° for 1.5 min	
Final extension	72° for 7 min	x 1 cycle

Upon completion of this reaction, 1 µl of PCR product is added to 99 µl of sterile H_2O. 1 µl of the dilution is then used as the template for the next reaction at step 2.

Nested reaction with *LROR* and *LR6*

Dentauration	94° for 2 min	x 1 cycle
Denaturation	94° for 30 sec	
Annealing	59° for 30 sec	x 45 cycles
Extension	72° for 1.5 min	
Final extension	72° for 7 min	x 1 cycle

4. Run a 5 µl aliquot of the first and nested reactions on a 1-1.5% agarose gel to confirm successful amplification. A ~1200 base pair product should be expected for the first reaction with *Rust 2INV* and *LR7*. A ~1000 base pair product should be expected for the nested reaction with *LROR* and *LR6*.
5. Successful PCR product should be sent to a third party for sequencing. The recommended sequencing company is Macrogen, Korea. The directions for sample submission of the third party should be followed.
6. Sequences should be determined using chromatograms from both primers. A comparison of the sequence should be made with sequences of *P. euvitis* on GenBank using a nucleotide BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

4.2.1.4 Genbank numbers

High identity (99-100%) of the ITS and LSU regions to the following GenBank numbers indicate the pathogen is *Phakopsora euvitis s. lat.*

ITS	LSU
AB354778	AB354744
AB354779	AB354745
AB354780	AB354746
AB354781	AB354747
AB354782	AB354748
AB354783	AB354749
AB354784	AB354750
AB354785	AB354751
AB354786	AB354752
AB354787	AB354753
AB354788	
AB354789	

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The protocol was reviewed by Alistair McTaggart.

7 References

Chatasiri S, Ono Y (2008) Phylogeny and taxonomy of the Asian grapevine leaf rust fungus, *Phakopsora euvitis*, and its allies. *Mycoscience* 49, 66-74

Daly AM, Hennessy CR and Schultz GC (2005) New host record for the grapevine leaf rust fungus, *Phakopsora euvitis*. *Australasian Plant Pathology* 34, 415-416

Ono Y (2000) Taxonomy of the *Phakopsora ampelopsidis* species complex on Vitaceous hosts in Asia including a new species, *P. euvitis*. *Mycologia* 92, 154-173

Ono Y and Imazu M (2001) Variation in the D1/D2 region of nuclear large subunit ribosomal DNA in *Phakopsora ampelopsidis, P. euvitis* and *P. vitis* (Uredinales). *The Bulletin of the Faculty of Education, Ibaraki University* 50, 21-26

Weinert MP, Shivas RG, Pitkethley RN, Daly AM (2003) First record of grapevine leaf rust in the Northern Territory, Australia. Australasian Plant Pathology 32: 117-118.

7.1 Other useful references

Hennessy CR, Daly AM and Hearnden MN (2007) Assessment of grapevine cultivars for resistance to *Phakopsora euvitis. Australasian Plant Pathology* 36, 313-317

Mio LLM; Novaes QS and Alves E (2006) Methodologies processed of some rusts fungi by scanning electron microscopy. *Summa Phytopathologica* 32(3), 267-273

8 Appendix: Life cycle

In the temperate areas, as vines move into their dormant phase, teliospores may develop, enabling the pathogen to overwinter on leaf litter. However, basidiospores, which form from surviving teliospores in the following summer, will be unable to infect *Vitis* plants and so will not be able to establish a new population of *P. euvitis*. Basidiospores can only infect the alternate host, *M. myriantha* (and possibly its allies). Despite this, a population of *P. euvitis* may survive as mycelium in the infected host tissues and urediniospores produced on the infected plant, which retain some canopy throughout seasons. Although urediniospores are only short-lived and highly subject to desiccation and UV degradation, a small number may survive on *Vitis* plants uninfected for a certain period of time, while grapevine experiences dormancy, and become able to cause a new infection in the following season. The chance of this occurring however appears very remote (Hennessy CR, Daly AM and Hearnden MN 2007).

Disease may not be evident early on, depending on the mode of introduction, but urediniospores are highly infectious and, if left unchecked, can spread and multiply rapidly. For these reasons, the incidence of disease in an infested vineyard is likely to reach a high level within a short period of time depending on the degree of exposure to inoculum, influenced by mechanical practices that contribute to spread and climatic conditions that contribute both host and fungal growth. Disease severity is likely to reach high levels towards harvest after the vegetative growth of vines has significantly slowed. As an example of the potential for rapid disease spread, under laboratory conditions, the latent period for urediniospore infection was found to be 6-8 days at temperatures between 20°C and 30°C. If a multiplier of 1.7 is used (i.e. a single sporulating pustule produces 1.7 new pustules per generation), as developed for stem end rust of grass, then on a six day cycle (one generation) *P. euvitis* reaches 1000 pustules after 12 weeks and more than 14,000 pustules after 16 weeks. Given that a single pustule of *P. euvitis* under ideal conditions has been shown to produce up to 300 spores this estimate may be conservative, but none the less indicates how rapidly disease might build up within a vine canopy and the potential for spread.

P. euvitis is a heteroecious rust fungus, i.e, it typically completes its life cycle on two dissimilar hosts: *Vitis* genus and a non vitaceous species - *Meliosma myriantha*. This type of life cycle occurs only under cool, temperate conditions in the presence of the alternate host involves several spore forms including:

Spermagonia are formed on *M. myriantha*.

- This leads to the production of aeciospores (still on *M. myriantha*) which then disperse through the air to infect *Vitis* spp.
- Infection of *Vitis* spp. leads to the production of urediniospores, which cycle endlessly throughout summer on *Vitis* spp.
- At the end of the season, late autumn, teliospores are produced. These overwinter on the dead leaves of *Vitis* spp.
- In spring these teliospores germinate, which results in the production of basidiospores.
- These basidiospores can only infect *M. myriantha*, which commences this typical lifecycle all over again.

M. myriantha is not recorded as being present in Australia in any national herbarium collection or an ornamental plant. There are no native *Meliosma* species known in Australia. Therefore asexual reproduction by urediniospores is the only way for *P. euvitis* to survive in Australia. In the NT, only this one spore form, the urediniospore, has been observed (Weinert et al. 2003, AM Daly & CR Hennessy, 2007 pers. comm.).

In tropical weather conditions where grapevines do not have a dormant 'over-wintering' phase without leaves, but produce leaves all year round, *P. euvitis* is able to persist on *Vitis* spp.. with a simplified “without alternate host" life cycle. However, this can be regarded as an atypical life cycle. As rust infection occurs only on the leaves, with the limited survival of the urediniospores (10

days), a continuous source of inoculum is required for the long-term survival of the rust under these conditions.

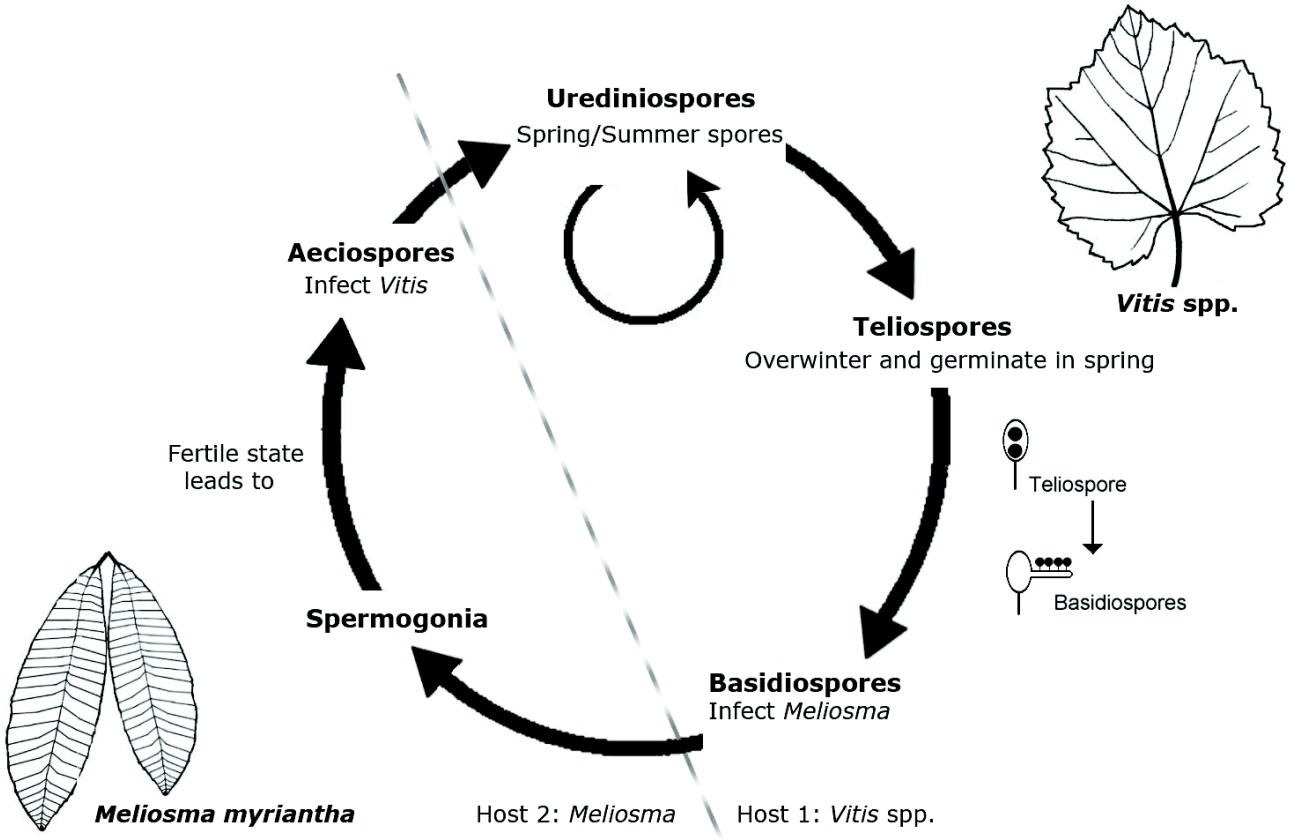

Figure 2. Phakopsora euvitis lifecycle (Source: C. R. Hennessy, NT Government, DPIFM 2006)