National Diagnostic Protocol

Uromyces viciae-fabae

The cause of lentil rust

NDP 31 V1

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Purpose

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

- are a verified information resource for plant health diagnosticians
- 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.

Where an International Plant Protection Convention (IPPC) diagnostic protocol exists it should be used in preference to NDPs although NDPs may contain additional information to aid diagnosis. IPPC protocols are available on the IPPC website:

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

This version of the National Diagnostic Protocol (NDP) for *Uromyces viciae-fabae* is current as at the date contained in the version control box below.

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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

1.1 The Pathogen

Strains of the rust fungus *Uromyces viciae-fabae* show host specificity to legumes within the Fabeae tribe of the Fabaceae (Barilli *et al*. 2011, Conner and Bernier 1982, Emeran *et al*. 2008, Hiratsuka 1933, Laundon and Waterston 1964). Systematic analysis indicated that strains of *U. viciae-fabae* either formed monophyletic groups (Emeran *et al.* 2008) or grouped according to their host species within one clade (Barilli *et al*. 2011). The rust causes partial defoliation and under heavy infection leads to premature plant death (Laundon and Waterston 1964, Negussie and Pretorius 2012). The rust fungus completes its full lifecycle of five spore stages on one host.

Uromyces viciae-fabae is present in Australia on faba beans, but has not been recorded on lentil.

1.2 Host range

The majority of investigations have reported that *U. viciae-fabae* has host specific strains on *Vicia* (faba bean), *Lathyrus* (sweet pea), *Lens* (lentil) and *Pisum* (pea). These included studies on host inoculations and morphology (Conner and Bernier 1982, Hiratsuka 1933) and molecular analyses (Barilli *et al*. 2011, Emeran *et al*. 2008). One study on the diversity of the rust in Japan determined that host specialization did not occur (Chung *et al*. 2004), although this work was based on a small number of isolates and has not been repeated.

1.3 Available protocols

Molecular diagnostic protocols have not been developed for *U. viciae-fabae* on lentil. The study by Chung *et al*. (2004) demonstrated that the Large Subunit (LSU) region of ribosomal DNA (rDNA) was not variable enough to distinguish between isolates from different hosts. Barilli *et al.* (2011) determined the ITS region differentiated lentil rust from other strains of *U. viciae-fabae*, however this sequence was not made available on GenBank.

2 TAXONOMIC INFORMATION

Kingdom: Fungi

Phylum: Basidiomycota

Class: Pucciniomycetes

Order: Pucciniales

Family: Pucciniaceae

Genus: *Uromyces*

Species: *viciae-fabae* (Pers.) J. Schröt.

Synonyms: *Aecidium leguminosarum* (Link) Rabenh.
Caeoma appendiculatum Schltdl.
Caeoma leguminosarum Link
Capitularia fabae (Pers.) Syd.
Coeomurus fabae (Pers.) Kuntze
Nigredo fabae (Pers.) Arthur
Puccinia fabae Grev.
Puccinia fabae (Alb. & Schwein.) Link
Puccinia fallens Cooke
Puccinia globosa Grev.
Puccinia polygoni-avicularis var. fabae Alb. & Schwein.
Trichobasis fabae (Pers.) Lév.
Uredo fabae Pers.
Uredo fabae DC.
Uredo leguminosarum Rabenh.
Uredo orobi Schumach.
Uredo viciae-fabae Pers.
Uromyces fabae (Pers.) de Bary
Uromyces fabae var. *orobi* (Schumach.) Jørst.
Uromyces orobi (Schumach.) Lév.
Uromyces viciae Fuckel

Common name: Lentil rust

3 DETECTION

3.1 Plant parts affected

All aerial plant parts are affected.

3.2 Symptom description

Negussie and Pretorius (2012) thoroughly described the symptoms from all spore stages. The aecial stage occurs on the abaxial surface of leaves and pods to form white aecial cups filled with orange-yellow spores. Uredinia then develop as dark brown pustules on both surfaces of the leaves, stems and pods (Figure 1-5). Finally telia are produced from the uredinia that are black in colour. A heavy infection will result in leaf drop and premature death.

Figure 1 & 2. Uredinia of *Uromyces viciae-fabae* on adaxial leaf surface and stems, Ethiopia. Images supplied by Tadesse Negussie and Zacharias Pretorius.

Figure 3. Uredinial infection by *Uromyces viciae-fabae* on adaxial leaf surface of partially resistant cultivar of lentil, Ethiopia. Image supplied by Tadesse Negussie and Zacharias Pretorius.

Figure 4. Uredinia of *Uromyces viciae-fabae* on adaxial leaf surface, Ethiopia. Image by Tadesse Negussie and Zacharias Pretorius.

Figure 5. Severe uredinial infection of stems and leaves by *Uromyces viciae-fabae*, Ethiopia. Image by Tadesse Negussie and Zacharias Pretorius.

3.3 Diseases causing similar symptoms

Uromyces viciae-fabae is the common cause of rust on lentil. Some species of rust on other host genera in the Fabaceae have indistinguishable symptoms from *U. viciae-fabae* on lentil, for example, strains of *U. viciae-fabae* on *Cicer arietinum* (chickpea), *Lathyrus* spp., *Lens* spp., *Pisum sativum* (pea), and *Vicia* spp. (Barilli *et al*. 2011). The leguminous genera *Lotus*, *Medicago*, *Pisum*, *Trifolium* and *Vigna*, are host to *Uromyces anthyllidis*, *U. striatus*, *U. pisi-sativi*, *U. trifolii-repentis* and *U. vignae*, respectively. These five species of *Uromyces* are similar in morphology to *U. viciae-fabae*, and all are present in Australia and many can be identified on the Rust Fungi of Australia Lucid Key (available: http://collections.daff.qld.gov.au/web/key/rustfungi/Media/Html/browse.html) (Shivas et al. 2014).

4 IDENTIFICATION

Identification of the lentil strain of *U. viciae-fabae* is based on host identity together with the morphology of teliospores and urediniospores. A specific molecular test has not been developed for the lentil strain of *U. viciae-fabae*, although sequences for many other strains of *U. viciae-fabae* are available for comparison on GenBank.

4.1 Morphological methods

The morphology of the lentil strain of *U. viciae-fabae* is considered identical to other strains of the pathogen. Identification of the host plant is important for the correct determination of the lentil strain. Common species of Fabaceae can be identified using this interactive key: https://gobotany.newenglandwild.org/dkey/fabaceae/.

4.1.1 4.1.1 Microscopic identification

The urediniospores and teliospores can be removed from the leaf or stem surface with scalpel or forceps and mounted on a microscope slide in water or lactic acid. The slide should be heated and then examined with 100x oil immersion objective to visualise surface ornamentation and spore size.

Spermogonia mostly abaxial, amphigenous in small groups associated with aecia. **Aecia** mostly abaxial, predominantly along veins, surrounding spermogonia or scattered, peridium cupulate, white, 0.3–0.4 µm diam. **Aeciospores** 18–26 × 15–21 µm, broadly ellipsoid, hyaline, finely verruculose, wall 1–1.5 µm thick. **Uredinia** amphigenous, yellowish brown, 0.5 mm diam. **Urediniospores** are 22–32 × 17–25 µm, broadly ellipsoid, uniformly echinulate, with 3–5 germ pores equatorial or scattered, and a light golden brown wall 1–2.5 µm thick (Figure 6). **Telia** adaxial or amphigenous, exposed, blackish brown, compact, 1–2 mm diam. **Teliospores** are ellipsoidal, obovoidal or cylindrical, with a rounded or sub-acute apex; 24–40 × 17–26 µm; wall chestnut-brown, smooth, 1–3 µm thick at the sides and 5–12 µm at the apex; pedicels brownish, up to 100 µm long (Figure 7) (from Negussie and Pretorius 2012).

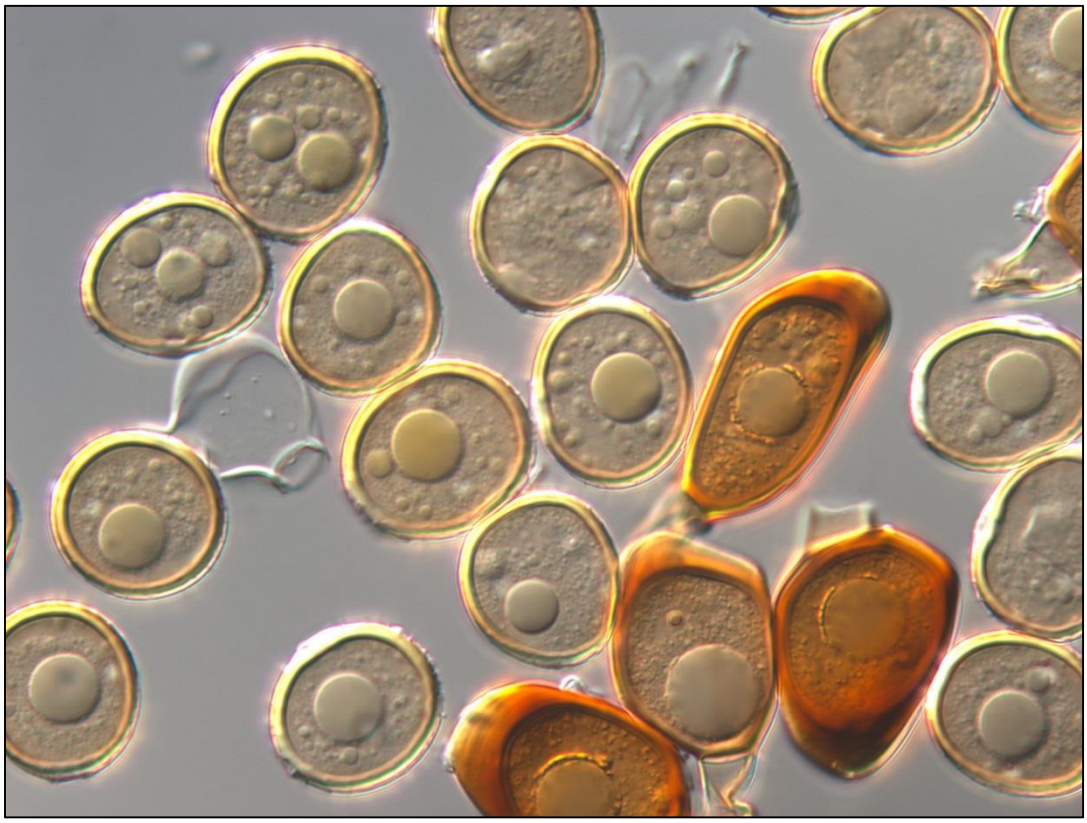

Figure 6. Urediniospores and teliospores of *Uromyces viciae-fabae* on *Vicia sativa*, Tasmania (BRIP 60149). Image by Alistair McTaggart.

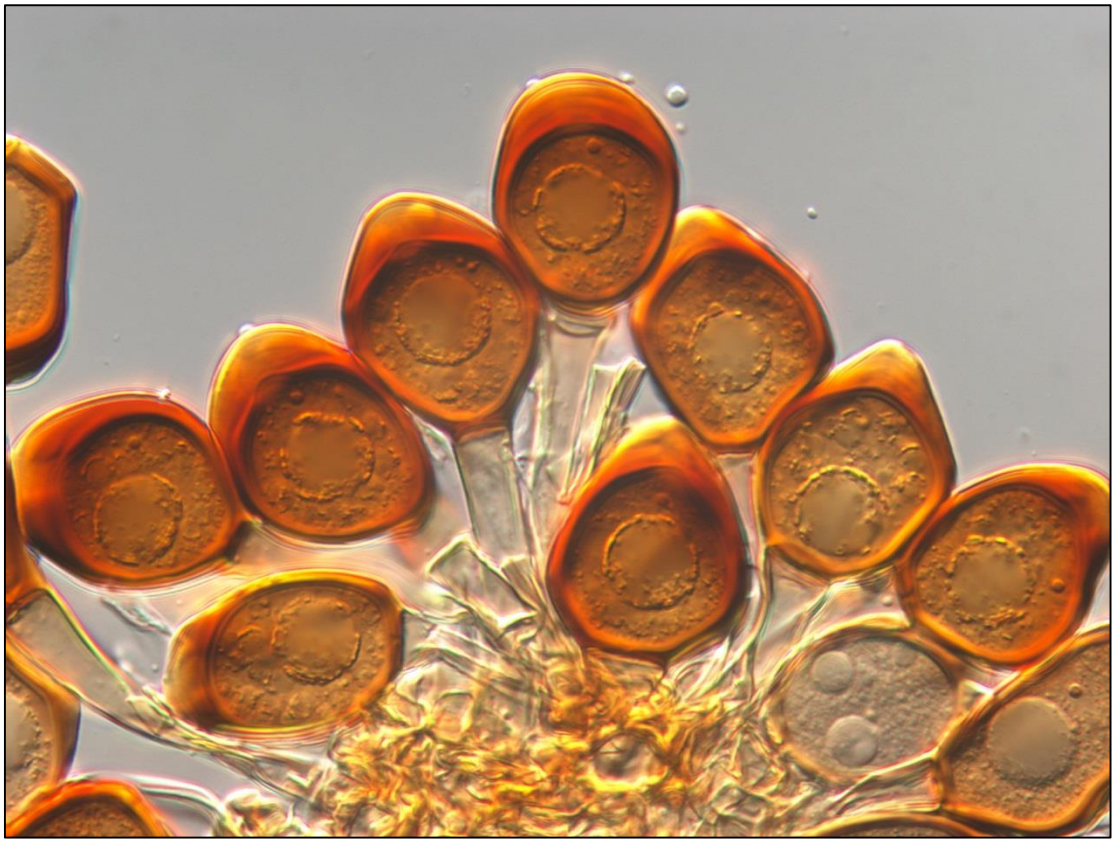

Figure 7. Teliospores of *U. viciae-fabae* on *Vicia sativa*, Tasmania (BRIP 60149). Image by Alistair McTaggart.

4.2 Molecular methods

4.2.1 Molecular barcoding of *U. viciae-fabae*

Amplified copies of the Large Subunit (LSU) region of rDNA can be sequenced and compared to known sequences on GenBank for identification of rust fungi. The LSU region is more easily sequenced than the ITS region for rust fungi, as the ITS may contain indels that inhibit direct sequencing. The ITS2-LSU region can be amplified with primers *Rust 2INV* and *LR7*. In cases when a product is not amplified or is of low concentration, a nested reaction can be performed using the primers *LR0R* and *LR6*. In the case of *U. viciae-fabae*, sequences of the ITS region are unavailable for comparison with the lentil strain.

Equipment and reagents

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

Primers (LSU primers):

Rust 2INV: 5'- GATGAAGAACACAGTGAAA -3' (Aime, 2006)
LR7: 5'- TACTACCACCAAGATCT -3' (Vilgalys and Hester, 1990)
LR0R: 5'- ACCCGCTGAACTTAAGC -3' (Vilgalys and Hester, 1990)
LR6: 5'- CGCCAGTTCTGCTTACC -3' (Vilgalys and Hester, 1990)

DNA Extraction

Any standard fungal DNA extraction protocol can be used for rust fungi. A recommended protocol for DNA extraction from rust fungi is the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA)..

Between 5 and 20 rust sori 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.

DNA amplification protocol

1. Prepare PCR cocktail on ice in a sterile microcentrifuge tube. This reaction can be performed with any polymerase enzyme according to the manufacturer conditions.

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, multiply the above volumes by the number of samples and add 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

Cycle conditions:

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

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

4. Run a 5 µL aliquot of this reaction on a 1–1.5% agarose gel to confirm successful amplification. A ~1200 base pair product should be expected for the reaction with *Rust 2INV* and *LR7*. If a product is observed proceed to step 6. If weak or no product is observed continue with the following reaction:

Dilute 1 µL of PCR product in 99 µL of sterile H_2O. 1 µL of this dilution is then used as template for the next reaction with primers *LR0R* and *LR6* with the protocol from step 1.

Nested reaction with *LR0R* and *LR6*

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

5. Run a 5 µL aliquot of the nested reaction on a 1–1.5% agarose gel to confirm successful amplification. A ~1000 base pair product should be expected for the nested reaction with *LR0R* and *LR6*.
6. Successful PCR product should be sequenced. An example of a third party sequencing company is Macrogen, Korea. The directions for sample submission of the third party should be followed.
7. Sequences should be determined using chromatograms from both primers. A comparison of the sequence should be made with sequences of *U. viciae-fabae* on GenBank using a nucleotide BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A 99-100% sequence identity to any of the following *U. viciae-fabae* LSU sequences AB115592-AB115611, AY745695, KJ716343 indicates the specimen is *U. viciae-fabae* and the host must be identified to confirm it is the lentil strain. High sequence identity to HQ317516 *U. phaesoli*, which likely is a misapplication of this name on *Pisum*, should also be considered a positive identification of *U. viciae-fabae*.

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The protocol was reviewed and verified by Dr Merje Toome, MPI, New Zealand.

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