National Diagnostic Protocol

Puccinia striiformis forma specialis *hordei*

the cause of barley stripe rust

NDP 38 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 *Puccinia striiformis* f. sp. *hordei* 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

Contents

1 INTRODUCTION

Stripe rust, caused by *Puccinia striiformis* exists in several biological forms (*formae speciales*) that vary in host range between and within genera and species of the Gramineae family (Stubbs, 1985). Eriksson distinguished the wheat-attacking form of *P. striiformis* from the barley-attacking form and named them *P. striiformis* f. sp. *tritici* (*Pst*) and *P. striiformis* f. sp. *hordei* (*Psh*), respectively (Stubbs 1985). A third forma specialis, *P. striiformis* f. sp. *pseudo-hordei* (*Psph*), with barley grass as its primary host was identified in 1998 (Wellings 2007; Wellings *et al.* 2000). The three have highly similar morphology and symptoms which make them difficult to differentiate, however, their separation into distinct formae speciales has been supported by glasshouse and field data, isozyme analysis (Newton *et al.* 1985) and molecular evidence (Keiper *et al.* 2003; Spackman *et al.* 2010; Bailey *et al.* 2015).

Wheat stripe rust, caused by *Pst* was first detected in Australia in 1979, with a second incursion in 2002, and has since evolved by stepwise mutation into over 20 pathotypes (Wellings 2007). Although *Pst* has a low level of infection on barley and does not cause significant damage to barley crops, some samples of *Pst* are recovered from wild barley grass (*Hordeum* spp.) genotypes. Of more concern is *Psph* which is able to infect a number of Australian barley varieties. Most Australian barley varieties are moderately resistant or completely resistant to the disease. Some are moderately susceptible and a few are susceptible at the seedling growth stages.

Puccinia striiformis is a macrocyclic rust with uredinial and telial stages occuring during the asexual component of its life cycle on cereals and grasses. The urediniospores complete multiple asexual cycles throughout the growing season and these cycles cause the principle damage to cereal crops. The development of stripe rust is favoured by cooler temperatures. Damage to susceptible barley depends on its stage of growth relative to rust development, with infection beginning at an early plant growth stage causing the most damage.

Rust infections reduce plant vigour and root growth, increase water loss and decrease the amount of photosynthate available for grain filling, resulting in reductions in the number and weight of kernels (http://www.ipmcenters.org/cropprofiles/docs/cabarley.html; Davis and Jackson, 2002).

1.1 Primary host range

Spring barley, *Hordeum vulgare*, is the primary host for the disease. Certain races of the rust will also survive on wild barley species such as *H. jubatum* (foxtail barley) and *H. leporinum*, *H. glaucum*, *H. marinum* (barley grass) (Marshall & Sutton, 1995).

2 TAXONOMIC INFORMATION

Kingdom: Fungi
Division: Basidiomycota
Class: Pucciniomycetes
Order: Pucciniales
Family: Pucciniaceae
Genus: *Puccinia*
Species: *striiformis*

Puccinia striiformis Westend f.sp. *hordei* Erikkson & Henning

Synonyms and former names

- *Dicaeoma glumarum* (Erikss. & Henning) Arthur & Fromme
- *Puccinia glumarum* Erikss. & Henning
- *Puccinia tritici* Oerst.
- *Trichobasis glumarum* Lév.
- *Uredo glumarum* Schumach.
- *Uredo glumarum* Roberge

Common Names

Barley stripe rust, barley yellow rust, glume rust

Pathotypes

Using a set of 11 differential barley genotypes, 69 races of *Puccinia striiformis* f. sp. *hordei* have been identified as occurring in the United States (Chen, 2004). Since 1998 certain races have become predominant but because of non-race-specific resistance, selection pressure has been low and the rust population still consists of numerous races (Chen, 2004). In Europe, there has been less race diversity identified with race 24 being predominant (Stubbs, 1985; Dubin & Stubbs, 1986).

Taxonomic description

Uredinia amphigenous on sheaths, culms and inflorescence; usually not confluent, 0·5–1 × 0·3–4 mm; with peripheral cylindrical or mostly saccate or saccate-capitate paraphyses (12–25(–30) diam.) that collapse readily; forming yellow lines of disease areas appearing as chlorotic streaks, up to 70 mm long. *Urediospores* globose to ellipsoid, (20–)25–30(–34) × 12–24(–26) µm; contents orange; wall hyaline, 1.5(–2) µm thick, with short spines, spines 1·5 µm apart, pores scattered, indistinct, 8–10(–14). *Telia* generally two- celled on abaxial leaf surface and sheaths in lines or scattered on the inflorescence, brown to black; sub-epidermal paraphyses separate spores into groups forming a compound sorus. *Teliospores* deep golden brown or chestnut-brown, clavoid, truncate or obliquely conical above, slightly constricted, attenuate below, 30–70 × (12–)16–24 µm; wall brown, smooth, 4–6(–10) µm thick at the apex; pedicel short, 15 µm long, slightly coloured. *Mesospores* sometimes present, 26–32 × 12–16 µm (Cummins 1971; Mulder & Booth 1971).

3 DETECTION

3.1 Symptoms

Distinct yellow/orange pustules are generally arranged in stripes along upper leaf surfaces (Figs 1-3).

The primary symptom of stripe rust is the appearance of yellow/orange pustules (uredinia) oriented linearly between vascular bundles of leaves in rows of varying lengths (Figs 2-4). Striping is not evident on seedling leaves or on highly susceptible adult plant leaves: the rust may cover the entire leaf blade and also extend into the leaf sheaths and grain heads (Adams, 1997; Bariana, 2004).

As plants mature the pustules turn dark and shiny as teliospores are formed. Glumes also can be infected and in-head infections of the glume can result in pinched grain and aborted florets.

Figure 1 The stripes of *Puccinia striiformis* f. sp**.** *hordei* on barley are made up of many tiny pustules arranged between the leaf veins. Photo by Jack Kelly Clark from R. M. Davis & L. F. Jackson (2002).

Figure 2 Field plot showing a susceptible infection of *Puccinia striiformis* f. sp**.** *hordei* on barley in CIMMYT, Mexico (Courtesy of Dr C. Wellings, PBI, Cobbitty).

Figure 3 Striping infection type typical of *Puccinia striiformis* f. sp**.** *hordei* infection (Courtesy of Dr M. William, CIMMYT, Mexico).

3.1.1 Confusion with endemic rusts

Stripe rust symptoms usually appear earlier in the season than other rusts because the fungus develops at lower temperatures. Stripe rust is distinguished from stem and leaf rusts based on colour, position and pattern of infection (Table 1).

Table 1 Differences in spore colour, position of infection and pustule pattern between the cereal rust diseases.

Rust	Colour	Position	Pattern
Stripe	Yellow/orange	Upper leaf surfaces. Can also infect glumes, awns and leaf sheaths	Striped pustules
Stem (Fig 5)	Dark brown	Leaves, leaf sheaths, stems and heads	Large pustules
Leaf (Fig 4)	Mid to light brown	Upper leaf surfaces and leaf sheaths	Scattered pustules

Figure 4. Barley leaf rust, *Puccinia hordei* on barley, USA (L) © Donald Groth, Louisiana State University AgCenter, USA, (R). © 2009 Merje Toome

Figure 5. Barley Stem rust.
Public Domain, https://commons.wikimedia.org/w/index.php?curid=1214935

Magnification of spores also allows *P. striiformis* to be distinguished from *P. graminis* (stem rust) and *P. triticina* (leaf rust). Figure 6 shows the difference between the 3 spore types at 400x magnification.

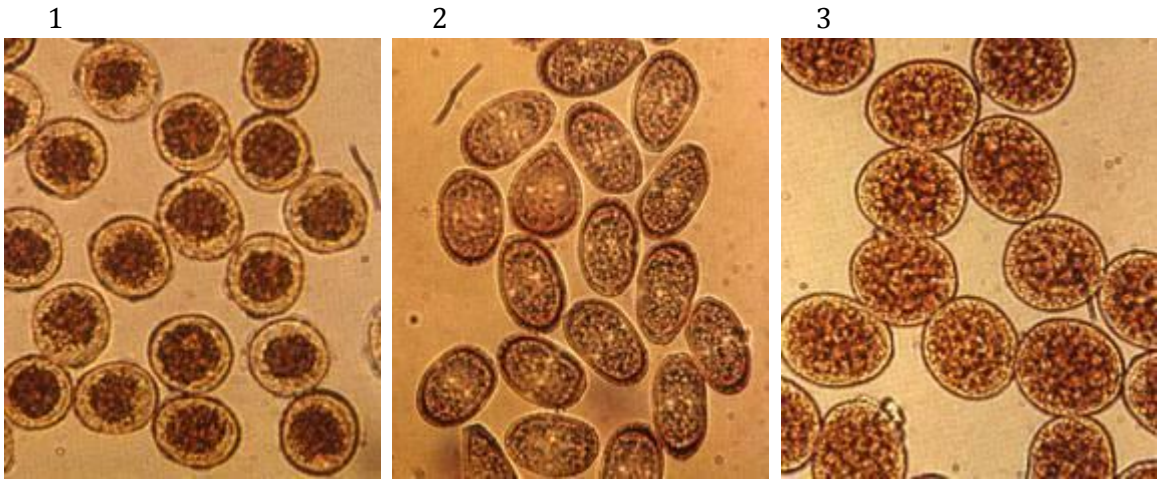

Figure 6 400x magnification of urediospores from 1. *P. triticina* (leaf rust) 2. *P. graminis* (stem rust) and *P. striiformis* (stripe rust). (M Spackman)

3.2 Sampling

It is important that rusted plant material be stored and transported in paper packaging and never in plastic. A good sample will have sufficient rust lesions to allow DNA extraction as well as an inoculation directly onto a differential set of cultivars. In the case of stripe rust, a 10 cm length of leaf that was completely covered by rust pustules is required. See Figure 7 for an example of the minimum leaf area infected that represents a good sample. It is important that the rust sample is sporulating. When a leaf is wiped with a clean white cloth, a yellow dust-like smear should result. Samples of leaves and stems must be dry. Leaves may be folded from top to bottom, so that the rust pustules are protect during transport.

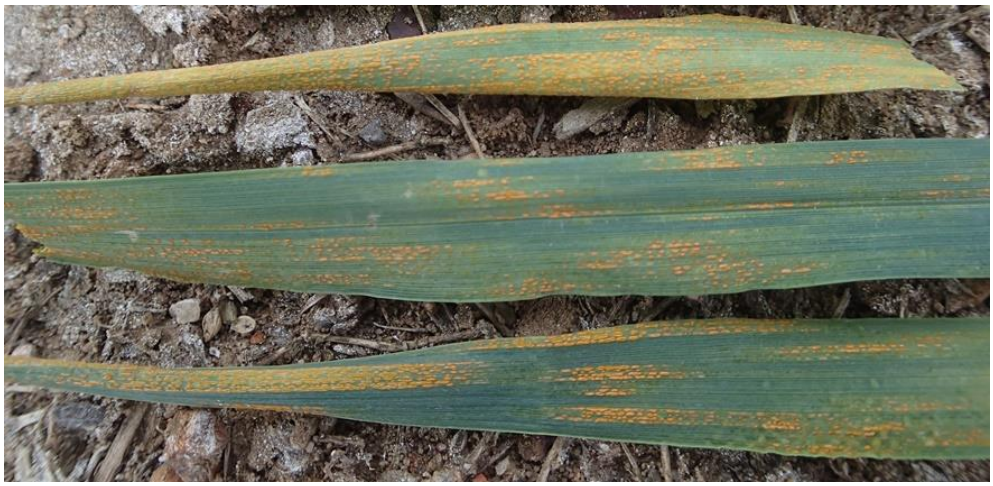

Figure 7. Example of leaf sample required for inoculation and DNA extraction. (Stripe rust on wheat, © W Cuddy)

4 IDENTIFICATION

Direct examination of symptoms and fungal morphology is sufficient to confirm the diagnosis of *P. striiformis*. However this method is not capable of distinguishing *formae speciales*.

PCR is the primary test to be used in the identification of *Psh* infections. The test uses specific diagnostic molecular markers to distinguish barley stripe rust from wheat and barley grass stripe rusts in infected plant material.

Identification using differential cultivars is reliable but slow, and is the current test used for all rust infections. It can distinguish *formae speciales* and pathotypes within *formae speciales*. Note that pathotype determination, which cannot be performed with PCR tests, is important in order to predict the potential impact to the barley industry.

Thus the PCR test is used as the primary diagnostic test, and the biological test on differentials is used as the confirmatory test although both can be commenced simultaneously.

Note: *All the SSR markers in the protocol have only been tested against Psh samples from the USA. Their usefulness for determining the presence of Psh from Europe and potentially South America has not been determined.*

4.1 Morphological characteristics

The urediniospores are yellow to orange in colour, spherical, binucleate, echinulate and 28–34 µm in diameter (Singh et al., 2002). Infection hyphae have 4 nuclei (Line, 2002).

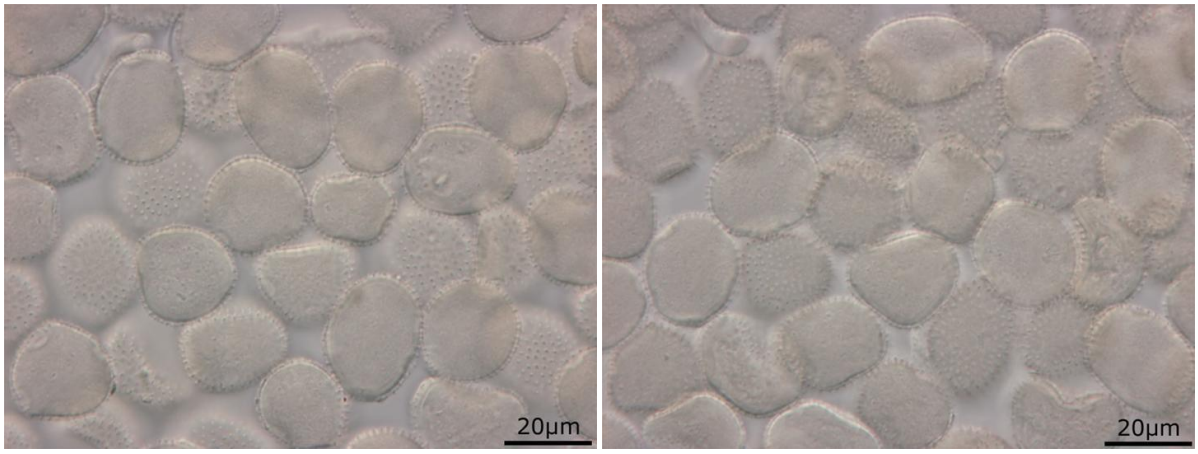

Figure 7. Urediniospores of *Puccinia striiformis* f. sp. *hordei.* © A.R. McTaggart Department of Primary Industries and Fisheries http://www.padil.gov.au/pests-and-diseases/pest/main/136639#

Telia, containing teliospores, may form as shiny black stripes on infected leaves, leaf sheaths and on glumes as adult plants mature, especially as temperatures increase towards the end of the season.

4.2 PCR test for detection of *P.s. hordei*.

Preliminary examination of the suspect plant sample will determine if the causal pathogen is *P. striiformis*. Characteristics of the colour, position and pattern of infection will be assessed in the preliminary examination (see Table 1).

The PCR test will distinguish *P. striiformis* from other common barley attacking foliar pathogens and identify the *forma specialis* present. The test has been verified to correctly identify the pathogen when DNA is extracted from 0.5 cm^2 of sporulating leaf material.

Note: *All the SSR markers in the protocol have only been tested against Psh samples from the USA. Their usefulness for determining the presence of Psh from Europe and potentially South America has not been determined.*

4.2.1 DNA Extraction

General items required

1. Samples - infected/suspect plant tissue
2. 2–20 µl pipettes, 20–200 µl pipettes, 200–1000 µl pipettes, and sterile tips
3. Balance (that weighs to at least two decimal places) and weigh boats.
4. Disposable gloves
5. Microcentrifuge
6. 1.5 ml and 2 ml sterile microcentrifuge tubes
7. 65°C and 20°C incubators
8. Fume hood
9. Sterile distilled water

DNA extraction method (adapted from Bailey *et al*. 2015):

1. Collect infected leaf tissue from the field and dry leaf material for 2–3 days in tubes over dehydrated silica gel (Alternatively snap freeze 0.5 cm^2 segments in 1.5 ml microcentrifuge tubes with liquid nitrogen).
2. This review extracted DNA from dried leaf material
3. A 0.5 cm^2 piece of dried sporulating leaf tissue was cut up and put into a 2 mL Lysing Matrix C tube with the manufacturer-supplied RNAse and DNAse free beads (MP Biomedicals).
4. Tubes were agitated in a FastPrep (MP Biomedicals) at 6 m s^{-1} for 45 s and then tubes were put on ice.
5. 1 mL of 2× CTAB Extraction Buffer was added to each tube, mixed by inversion and then placed back on ice for 2 min.
6. Tubes were agitated in the Fastprep for a further 30 s before being put back on ice for 1 min.
7. Tubes were transferred to a hot water bath at 65°C and were incubated for 30 min with mixing by gentle inversion every 10 min.
8. The tubes were centrifuged at 12,000 × *g* for 2 minutes and then the supernatant was transferred to fresh tubes.
9. One volume of cold (4°C) phenol-chloroform-isoamyl alcohol solution was added to each tube before they were mixed with gentle inversion and centrifuged at 14,000 × *g* for 30 min.
10. The top phase of supernatant was transferred to new tubes to which 1 volume of cold (4°C) chloroform-isoamyl alcohol solution was added. Tubes were mixed gently by inversion before being centrifuged at 14,000 × *g* for 10 min.

11. The top phase of supernatant was again transferred to new tubes and 0.1 volume of cold (-20°C) 3M sodium acetate and 1 volume of cold (-20°C) isopropanol were added. Tubes were mixed by gentle inversion and stored at -20°C for 3 h.
12. Tubes were centrifuged at 14,000 × *g* for 30 min. The supernatant was discarded before 1 mL of cold (4°C) 70% v/v ethanol was added to the tube gently. The tubes were mixed gently by inversion, centrifuged at 14,000 × *g* for 10 min.
13. The supernatant was removed and a second rinse with 1 mL cold (4°C) 70% v/v ethanol was repeated. Again, tubes were centrifuged at 14,000 × *g* for 10 min. The supernatant was discarded and the DNA pellet was left to air dry.
14. Once the pellet was completely dry, the DNA was resuspended in 100 µL of TE buffer.

4.2.2 PCR method

Items required

1. 0-2 µl, 2-20 µl, 20-200 µl, and 200-1000 µl pipettes and sterile tips
2. 0.2 ml sterile PCR tubes
3. Microcentrifuge
4. Disposable gloves
5. Cooler racks
6. Thermocycler
7. DNA Molecular Weight markers (Hyperladder IV, Bioline®)
8. Gel electrophoresis tanks and rigs
9. Power pack
10. UV transilluminator with camera

Primers

For specific, sensitive amplification of *P. striiformis* DNA use the following specific simple sequence repeat (SSR) primers for analysis by agarose gel electrophoresis.

Primer	Sequence	Annealing temperature (°C)
RJ18f	5' CTGCCCATGCTCTTCGTC 3'	50
RJ18r	5' GATGAAGTGGGTGCTGCTG 3'	
ERJ24f	5' TTGCTGAGTAGTTTGCGGTGAG 3'	50
ERJ24r	5' CTCAAGCCCATCCTCCAACC 3'	
SUNIPst10-06f	5' TGCGGCATTAGCGTCTCTTCGT 3'	56
SUNIPst10-06r	5' TTCGCTTTCGTTCTCCATTGTC 3'	
SUNIPst09-48f	5' AGCACCCCAACAATCATCACAT 3'	60
SUNIPst09-48r	5' GGCCGAGGGTGAGTTTGGTTGA 3'	

PCR controls

1. Positive control, ie. a DNA extract from barley tissue infected with *P. striiformis*
2. Uninfected plant control, ie. a DNA extract from uninfected barley tissue.
3. No template control, ie. an aliquot of the PCR Master Mix minus DNA template.

PCR reagents

Reagents	1 x reaction
10x PCR buffer (Bioline)	1.5 µL
dNTPs (2 mM) (Bioline)	1.5 µL
$MgCl_2$ (25 mM) (Bioline)	0.9 µL
F Primer (10 ng μL^{-1})	0.9 µL
R Primer (10 ng μL^{-1})	0.9 µL
Taq polymerase (Bioline BioTaq)	0.15 µL
DNA (10 ng μL^{-1})	0.9 µL
H_2O	7.15 µL

PCR Program

Temperature	Time	Cycle #
95	5 min	1
95 Annealing temperature (see Primers section) 72	30 s 30 s 30 s	35
72	7 min	1
4	Hold	1

Electrophoresis

1. Add 2ml loading dye to PCR reaction.
2. Run 5ml PCR reaction on 3% agarose gel containing a suitable staining dye, in 1x TBE at 140 volts for 3 h or until light blue dye has migrated ¾ the length of the gel. Use 5µL hyperladderIV as size ladder. (*N.B. the review used gels containing GelRed. Any suitable DNA staining method whether it be ethidium bromide, GelRed or SYBR Green should provide sufficient diagnostic resolution*).
3. Visualise on UV transilluminator.

4.2.3 PCR Results

RJ18

- RJ18 amplifies a single band from *Psh* (362bp). Double banding patterns are observed in *Psph* (362bp and 335bp) and *Pst* (362bp and 341bp).
- Host tissue does not amplify similar bands.
- Overlap of band sizes with yellow leaf spot was observed by Merrin Spackman.

Note: For the marker RJ18 to be useful in a diagnostic test the infection must not be a mixture of *formae speciales.*

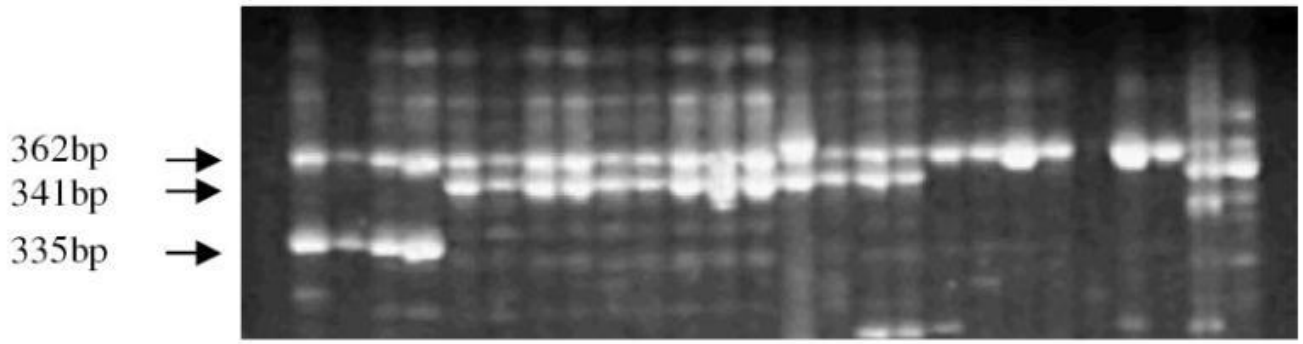

Figure 8. Amplification products using SSR primers RJ18. Lanes 1-4 - *P.s. pseudo-hordei* (*Psph*). Lanes 5-17 - *P.s. tritici* (*Pst*). Lanes 18-24 - *P.s. hordei* (*Psh)*. Lanes 25-26 - *P.s. poae* (*Psp*) (bluegrass stripe rust, US origin)

RJ24

- RJ24 amplifies 2 bands from *Psh* (223bp & 211bp) that are different from the bands amplified from *Psph* (225bp and 205bp) and from *Pst* (217bp and 199bp). No band was amplified from *Psp*
- Host tissue does not amplify similar band sizes.
- No observed overlap of band size with other pathogens tested.
- RJ24 is the preferred marker for a diagnostic test.

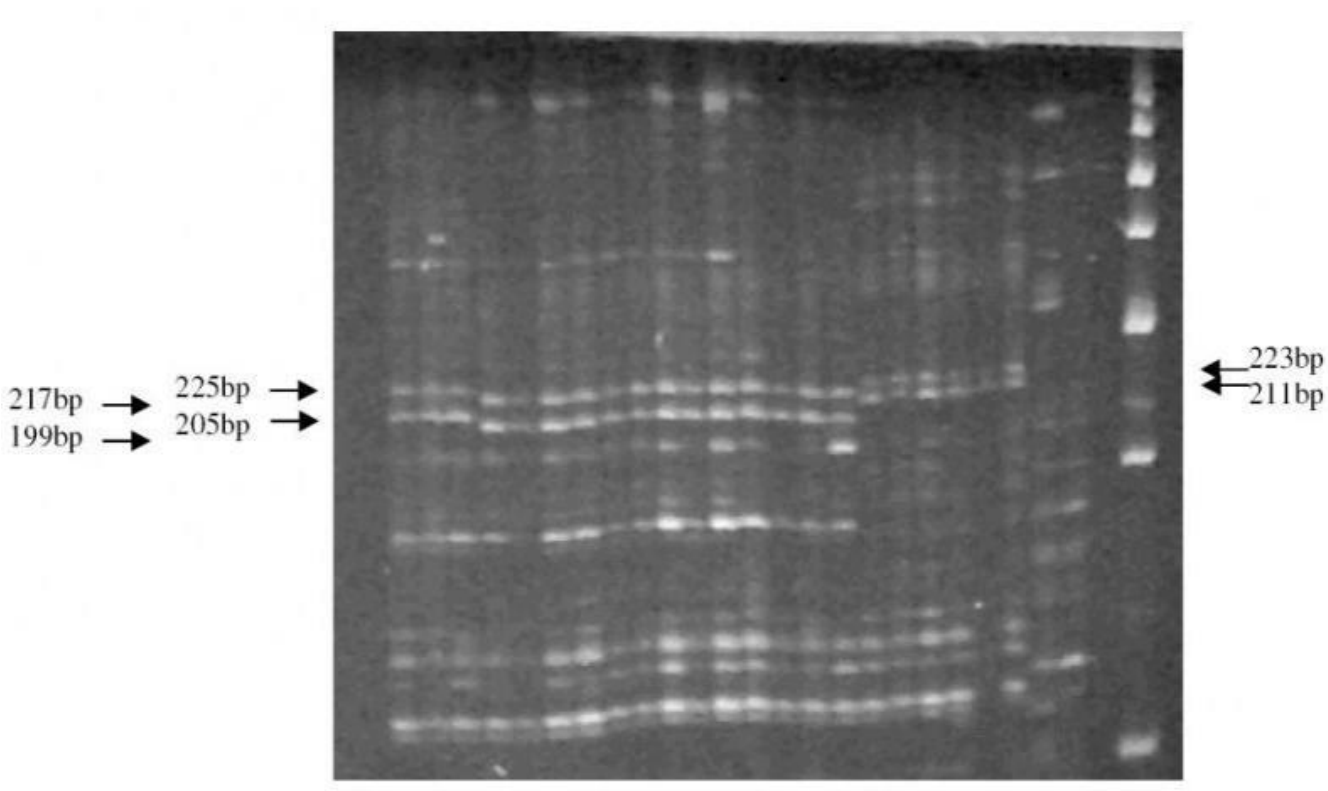

Figure 9. Amplification products using SSR primers RJ24. Lanes 1-3 - *P.s. pseudo-hordei* (*Psph*). Lanes 4-13 - *P.s. tritici* (*Pst*). Lanes 14-19 - *P.s. hordei* (*Psh*). Lanes 20-21 - *P.s. poae* (*Psp*).

SUNIPst10-06

- SUNIPst10-06 amplifies 2 bands for *Pst* (either 350 and 423 or 358 and 406) that are different to at least one of the two bands amplified for *Psph* (350 and 369) and the single band amplified for *Psh* (410).
- Host tissue did not amplify similar band sizes.

SUNIPst09-48

- SUNIPst09-48 amplifies a single band for *Psh* (200 bp) and two bands for *Pst* (either 206 and 224 or 212 and 215) and for *Psph* (206 and 215). It is not able to distinguish between Psph and *Pst*.
- Host tissue from a wild barley grass (*Hordeum* sp.) did amplify in a similar region, but barley host tissue did not.

4.3 Virulence on differential wheat and barley cultivars for detection of Psh.

The barley cultivar differentials are used as a confirmatory test because of the length of time it takes to complete. The barley differential test will differentiate *Pst* and *Psph* infections from *Psh* infections and differentiate between four pathotypes of *Psh*.

This procedure was not verified for this protocol. Differential cultivar virulence is a specialist test currently undertaken in Australia by the Plant Breeding Institute of the University of Sydney. This laboratory has the barley stripe rust differential lines and the capacity to use different f. spp. of rusts to serve as control checks.

It is advisable to send any material needing pathotype analysis on a differential set to this laboratory:

Australian Cereal Rust Survey
Plant Breeding Institute, the University of Sydney,
107 Cobbitty Road, Cobbitty, NSW, 2570.

It is important that rusted plant material be stored and transported in paper packaging and never in plastic.

4.3.1 Equipment required

1. constant temperature room capable of maintaining a temperature of 10±2 ºC
2. potting mix & pots

Specific items

1. 3 sets of differentials (see Table)
2. Rust of one pathotype each of *Pst* and *Psph* to use for comparison.
3. Inoculation apparatus (Pressurised spray gun for distributing spores suspended in mineral oil over leaf surfaces)
4. Incubation facilities (Dew chambers with water trays and a plastic hood in cool room).
5. Plant growth facilities with sufficient light for growing seedlings and temperature control set at 17 ºC
6. Inoculating mineral oil (Shellsol T®, Isopar L® or similar)
7. Personal protective clothing and equipment including lab coat, gloves, goggles and respirator while using mineral oil for inoculations.

4.3.2 Method

- Germinate 5–8 seeds of the barley differential genotypes shown in Table in pots with four clumps per pot.
- Suspend urediniospores in light mineral oil (Shellsol T®) and spray a fine mist over plant leaves in a spray chamber. Spray above plants and allow mist to fall onto the leaves. Note: Too much oil can burn leaves, inhibit infection . or induce a susceptible reaction on the leaf above the burn site.
- Leave to dry for 5 min.
- Place inoculated plants in a tray of water in a dew chamber at 10°C under plastic covers with the edges of the plastic cover under water in the tray to allow for dew development for 18 to 24 hr to initiate infection and then move to a growth chamber set at 17°C for symptom development.
- Record infection type data from 14 d after inoculation according to the PBI 0-4 scale for infection type (Table 2) (McIntosh *et al*. 1995).

- Correlate infection type with host and/or resistance gene to determine *forma specialis* and pathotype (Table).

Table 2 Scale of infection for rust symptoms

Value	Necrosis or Chlorosis	Sporulation	Host rating
0	None	None	Resistant (R)
;	Flecks	None	R
;N	Necrotic areas without sporulation	None	R
1	Necrotic and/or chlorotic areas with restricted sporulation	Trace	R
2	Moderate sporulation with necrosis and/or chlorosis	Moderate	R
3	Abundant sporulation with chlorosis but no necrosis	Abundant	Susceptible (S)
4	Abundant sporulation without chlorosis	Abundant	S

Table 3 Barley varieties used as differential testers for diagnosis of barley stripe rust. The expected reaction type of four different pathotypes of barley stripe rust and of wheat stripe rust and barley grass stripe rust on each variety is shown.

Differential Tester	Barley stripe rust pathotype				Wheat stripe rust	Barley grass stripe rust
	Race 23	Race 24	Race 24	Race 57		
Cambrinus	R	S	S	R	R	R
Astrix	R	S	S	R	R	R
Agio	R	S	S	R	R	R
Bigo	R	R	S	R	R	R
Varunda	R	R	S	R	R	R
Mazurka	R	R	S	R	R	R
Atem	S	S	S	S	R	R
Keg	S	S	S	S	R	R
Sultan	S	S	S	S	R	R
Berac	S	S	S	S	R	R
Heils Franken	R	S	R	R	R	Unknown
Topper	S	S	S	S	R	S
Fong Tien	S	S	S	S	S	Unknown

5 CONTACTS FOR FURTHER INFORMATION

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8 APPENDICES

8.1 Stages of Development

Puccinia striiformis is an obligate pathogen which grows only on living host plants (Adams, 1997). It survives between seasons on volunteer wheat, barley and some wild grasses. The amount of over-summering rust available for the following year depends on the number and susceptibility of volunteer plants, which in turn is a function of moisture in the off-season. Only one infected leaf per 30 ha of regrowth needs to survive the summer to produce severe rust infections (Hollaway, 2005). Rust spores are spread by wind to initiate and spread infections.

So far, the alternate host for *Puccinia striiformis* has not been shown to play a role in the epidemiology of stripe rust diseases in Australia. As a result, teliospores to do not appear to play a role in disease survival.

The optimum temperature for the germination of urediniospores is 10–12°C. Spores germinate over 3–8 hours in conditions of high humidity and temperatures between 5 and 20°C (Park 1990). Infections result by urediniospores producing adhesion pads to maintain contact with the host cuticle, and a germ-tube that grows across the leaf surface and enters the leaf via stomata. An infection peg forms through the stomatal pore and gives rise to a vesicle from which infection hyphae develop that branch out and can infect the whole of the leaf tissue (Park, 2000). The optimum temperature for development of stripe rust in plants is 13-18°C. Under optimum conditions, the time from inoculation to sporulation is 12–13 days (Line, 2002; Davis & Jackson, 2002). Late in the summer, telia develop as black pustules on the leaf.

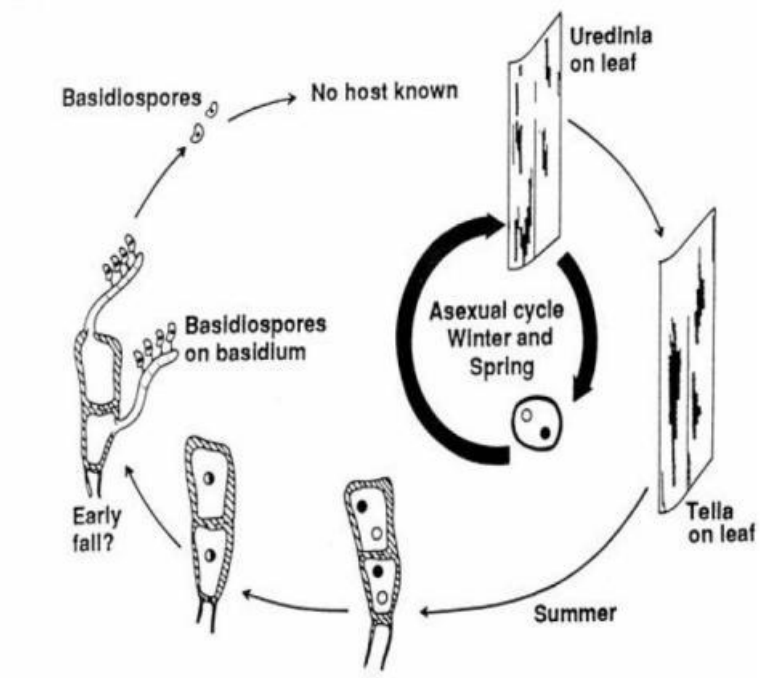

Figure 10 Lifecycle of *Puccinia striiformis*

8.2 Recipes

CTAB Extraction buffer

	500 mL	**final**
Cetyl Trimethyl Ammonium Bromide (CTAB)	10 g	2%
0.5 M EDTA (pH 8.0)	20 mL	20 mM
5 M NaCl	140 mL	1.4 M
Polyvinylpyrrolidone (PVP; 40000 MW)	5 g	1%
1 M Tris-HCl (pH 8.0)	50 mL	100 mM
ddH_2O	290 mL	

- Dissolve solids and mix solutions in 200 mL ddH_2O and then add in the final 90 mL ddH_2O to bring the final volume to 500 mL.
- Autoclave before use.
- Store at room temperature.

5 x TBE Buffer

	1 L	**final**
Tris base	54.0 g	0.4 M
Boric acid	27.5g	0.05 M
0.5 M EDTA pH 8.0	20.0 ml	0.001 M

- Dissolve components in 1 L sterile RO water.
- Store at room temperature.
- Dilute to 1X concentration for use.

6x Loading dye

	50 ml
1 x TE	5 ml
Glycerol	25 ml
Bromophenol blue	0.125 g
Xylene cyanoll FF*	0.125 g

- Make up in 50 ml RO water.
- Store at room temperature.

TE Buffer

	1 L	**final**
1M Tris HCl (pH 8.0)	10 ml	10 mM
0.5M EDTA (pH 8.0)	2 ml	1 mM

- Mix reagents in 1 L sterile RO water.

- Autoclave before use.
- Store at room temperature.
- Stock solutions prepared as described below.

1.0 M Tris HCl (pH 8.0)

- 12.1 g Tris base dissolved in 100 ml sterile RO water.
- pH to 8.0 with concentrated HCl.

0.5 M EDTA (pH8.0) ethylenediamine trisodium acetate

- 18.6 g EDTA dissolved in 100 ml sterile RO water
- pH to 8.0 with NaOH while stirring. Solid will not dissolve until close to pH8.0
- Autoclave before use
- Store at room temperature

8.2.1 Suppliers and catalogue numbers

Chemical	Supplier	Catalogue #
Agarose	Progen	2000011
Boric acid	Sigma	11611
Bromophenol blue	Sigma	B-5525
Chloroform	Merck	10077 6B
CTAB	Merck	102342
EDTA	Sigma	43178-8
Ethanol	Merck	10476.9020
Ethidium bromide	Merck	443922U
HCl	Merck	10307 6P
Isopropanol	Sigma	I-9516
NaCl	Merck	1024.3
NaOH	Merck	106482
Tris base	Merck	108382
Xylene cyanol FF	Aldrich	33594-0