Subcommittee on Plant Health Diagnostics

National Diagnostic Protocol

Colletotrichum lentis

The cause of lentil anthracnose

NDP 44 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 https://www.plantbiosecuritydiagnostics.net.au/work/subcommittee-on-plant-health-diagnostics/

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 *Colletotrichum lentis* 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: sphd@agriculture.gov.au

Contents

1 INTRODUCTION

Anthracnose of lentil (*Lens culinaris*, Fabaceae) is caused by *Colletotrichum lentis*. It was first described in Canada as the lentil-attacking form of *Colletotrichum truncatum* (Morrall 1988). This fungus is known to infect lentil and other Fabaceae crop but has not been recorded in Australia. *Colletotrichum lentis* is characterised morphologically by its straight to slightly curved, fusoid conidia that are gradually tapered to acute ends and by the globose appressoria with an entire margin (Damm *et al*. 2014). *Colletotrichum lentis* is also distinguished from other species of *Colletotrichum* by DNA sequence analyses of the internal transcribed spacer (ITS) region of the ribosomal DNA, partial region of the beta-tubulin and glyceraldehyde-3-phosphate dehydrogenase nuclear DNA (Damm *et al*. 2014).

1.1 Disease cycle

Colletotrichum lentis can be spread in several forms, such as by infected seed, and as microsclerotia on infested trash. Seed infection levels of 2 – 3% have been reported from severely diseased crops (Buchwaldt *et al*. 1996), but generally levels of 0.5 – 1% are more common.

The pathogen can survive as microsclerotia on crop residues for up to four years. Infested trash, which includes pod walls and small stem fragments, can carry microsclerotia, which can initiate infection in subsequent lentil crops. When the plant debris decomposes the microsclerotia can survive free in the soil. Survival of *C. lentis* microsclerotia increased from 12 months, when left on the soil surface, to 4 years when buried in lentil debris (Buchwaldt *et al*. 1996).

Spores are initially dispersed from old lentil debris near the soil surface to newly establishing lentil plants by rain splash. Symptoms usually appear 7 – 8 days following infection. Newly infected plants produce new generations of spores that disperse to infect neighbouring plants by rain splash. Spores are produced in large numbers on infected host material, even leaflets that have dropped onto the ground. Observations have revealed that the number of rainfall events is more important than the total amount of rainfall. Once the pathogen has entered the plant the fungus will grow regardless of temperature and humidity. Chongo and Bernier (2000) found that the optimal conditions for lentil anthracnose development were temperatures of 16 – 24°C in conjunction with 18 – 24 hours of leaf wetness.

Field pea crops have been considered to play a role in the carryover of lentil anthracnose inoculum at low levels when rotated with lentil under field conditions (Morrall. 1997).

1.2 Host range

Host range of *C. lentis* in field studies (Gossen *et al*. 2009, Damm *et al*. 2014) and under growth-cabinets conditions (Gossen *et al*. 2009).

Field	**Growth room**	
Lens culinaris (lentil)	*Lens culinaris* (lentil)	*Vicia americana* (narrow-leaf vetch)
Pisum sativum (field pea)	*Cicer arietinum* (chickpea)	*Vicia faba* (faba bean)
Vicia faba (faba bean)	*Pisum sativum* (field pea)	

2 TAXONOMIC INFORMATION

2.1 Taxonomic description

Kingdom: Fungi
Phylum: Ascomycota
Order: Pezizomycotina
Class: Sordariomycetes
Family: Glomerellaceae
Genus: *Colletotrichum*
Species: *Colletotrichum lentis* Damm, 2014

Common name: Lentil anthracnose

2.2 Morphological description (Damm *et al*. 2014)

Sexual morph not observed. *Asexual morph* on synthetic nutrient-poor agar medium (SNA) (Nirenberg, 1976). *Vegetative hyphae* 1.5–11 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae or on or close to chains or clusters of pale to dark brown, verruculose, cylindrical to subglobose, cells. *Setae* pale to medium brown, smooth-walled, 40–85 µm long, 1–3-septate, base ± inflated, sometimes constricted at the basal septum, 5–6 µm diam, tip round. *Conidiophores* hyaline, smooth-walled, septate, branched, to 30 µm long. *Conidiogenous cells* hyaline, smoothwalled, cylindrical to ampulliform, 9–28 × 3.5–5 µm, sometimes intercalary (necks not separated from hyphae by septum) and sometimes polyphialides observed, opening 1–2 µm diam, collarette 0.5–1 µm long, periclinal thickening observed. *Conidia* hyaline, smooth-walled, aseptate straight to slightly curved, fusiform with ± acute ends, (13–)16–20(–26) × 3–4(–5) µm, av. ± SD = 18.1 ± 2.0 × 3.5 ± 0.4 µm, L/W ratio = 5.1, conidia of strain CBS 127605 shorter, measuring (13–) 15–17.5(–19.5) × 3–3.5(–4) µm, av. ± SD = 16.3 ± 1.4 × 3.4 ± 0.2 µm, L/W ratio = 4.8. *Appressoria* single or in loose groups, medium brown, smooth-walled, globose, subglobose to elliptical in outline, with an entire margin, (5–) 5.5–7.5(–9) × (3.5–)4.5–6(–6.5) µm, av. ± SD = 6.4 ± 0.8 × 5.2 ± 0.6 µm, L/W ratio = 1.2.

Figure 1. Appresoria (L-Q) and conidia (R-S) of *C. lentis* (Damm *et al*. 2014).

3 DETECTION

Stems, leaves, inflorescences, pods and seeds are affected by *C. lentis*. There are several common foliar diseases of lentil in Australia, these include Ascochyta blight (*Ascochyta lentis*) (Figure 14) and Botrytis grey mould (*Botrytis cinerea* and *B. fabae*). Lentil anthracnose may be easily mistaken for one of these common foliar diseases, but there are several key symptoms that are unique to this disease. These include the formation of microsclerotia and setae within lesions and lesions on the stem that are sunken into the plant tissue.

3.1 Symptoms

3.1.1 Leaf lesions and premature leaf drop

In most lentil crops, the first symptoms of anthracnose appear before flowering, when the plants have 8 to 12 nodes on the main stem. This is also the time when the first tendrils form, and approximately a week before flowers start to open. If there is a large amount of inoculum in the field the first symptoms may appear earlier. The initial symptoms of lentil anthracnose are greenish water-soaked lesions on the lower stems and leaves that become necrotic with time. Tan coloured lesions of variable size develop on the lower leaflets and the most severely affected leaflets die prematurely and drop to the ground. Creamy white lesions are also sometimes evident on the upper foliage (Figures 2-4).

Figure 2. *Colletotrichum lentis* causes tan coloured lesions of variable size to develop on the lower lentil leaflets. The most severely affected leaflets die and drop to the ground. (Photograph courtesy of L. Buchwaldt, Agriculture and Agri-Food Canada).

Figure 3. Early stages of leaf lesion development by *C. lentis*. (Photograph courtesy of R. Morrall, University of Saskatchewan).

Figure 4. Early stages of anthracnose leaf lesion caused by *C. lentis*. (Photograph courtesy of R. Morrall, University of Saskatchewan).

3.1.2 Stem lesions

Lesions on stems develop soon after the appearance of leaf lesions, during flowering, primarily at the base of the plant. Stem lesions may be small, brownish with a black border, or larger, stretching along the stem. In favourable conditions white to pale orange spore masses will form within stem lesions. As the season progresses, more and more golden-brown lesions develop at the stem base, as well as on the upper part of the stems, and many stems are girdled. (Figures 5-7) Finally there is a marked blackening of old infected tissues due to the production of stromatic mycelium (microsclerotia) under acervuli.

Figure 5. Early stem lesion development caused by *C. lentis* prior to the appearance of microsclerotia. (Photograph courtesy of G. Chongo, Agriculture and Agri-Food Canada).

Figure 6. Early lentil anthracnose stem lesions caused by *C. lentis* in the field. Note the development of microsclerotia within the lesions. (Photograph courtesy of G. Chongo, Agriculture and Agri-Food Canada).

Figure 7. A heavily infected lentil plant with *C. lentis* showing numerous stem lesions. Stem lesions may be small, brownish with a black border, or larger, stretching along the stem. (Photograph courtesy of L. Buchwaldt, Agriculture and Agri-Food Canada).

3.1.3 Microsclerotia (survival structures) (Bailey et al. 2003):

Small, pinhead-sized fungal structures (microsclerotia) form on the older infected plant tissue. They may be seen with the unaided eye in the centre of stem lesions or more easily with a hand lens (10-15x magnification). Each microsclerotia consists of a few hundred cells with thick, black cell walls that protect the fungus from colonisation by other micro-organisms. Microsclerotia enable the fungus to survive between lentil crops either on the plant debris or free in the soil. They remain viable longer when buried in the soil by tillage than when left exposed to weather extremes on the soil surface. These fungal structures survive on dead lentil debris or in the soil during periods when a host is not available (Figures 8-11).

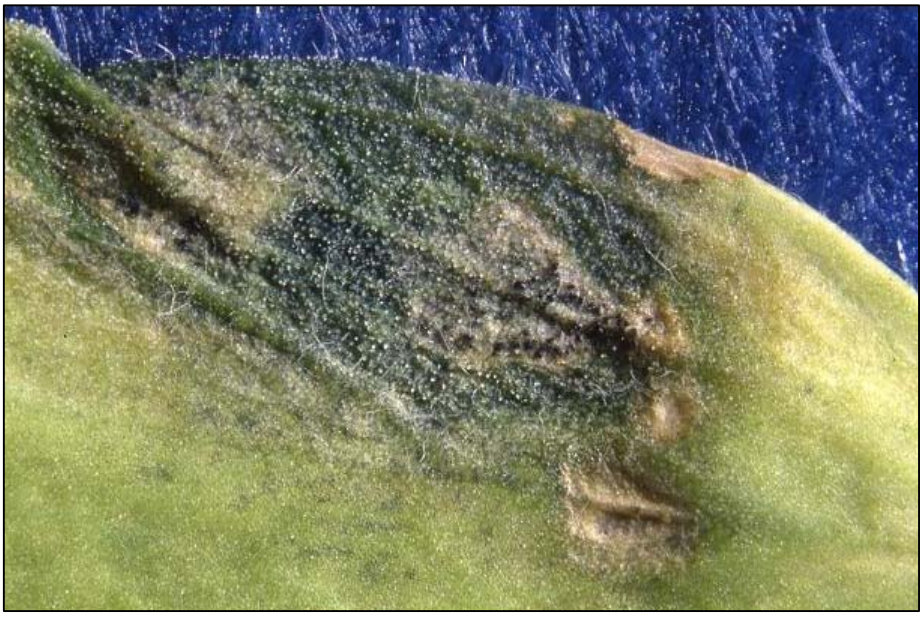


Figure 8. A maturing leaf lesion with spore masses and microsclerotia of *C. lentis* present on a lentil leaf. (Photograph courtesy of R. Morrall, University of Saskatchewan).

Figure 9. A mature leaf lesion with fully formed microsclerotia of *C. lentis* present on a lentil leaf. (Photograph courtesy of R. Morrall, University of Saskatchewan).

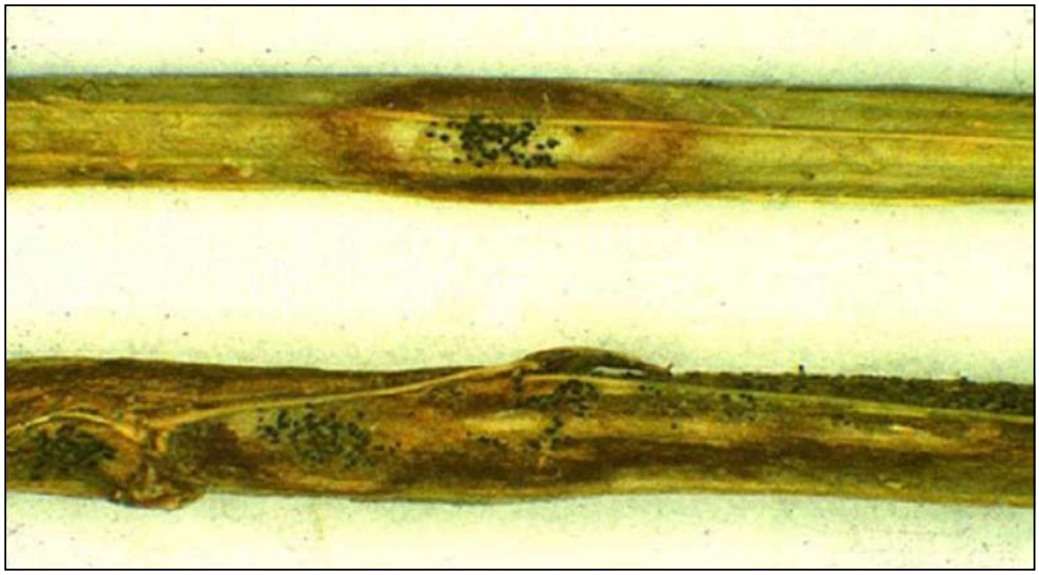


Figure 10. An image of the small, pinhead sized fungal structures (microsclerotia) that form within stem lesions caused by *C. lentis.* (Photograph courtesy of L. Buchwaldt, Agriculture and Agri-Food Canada).

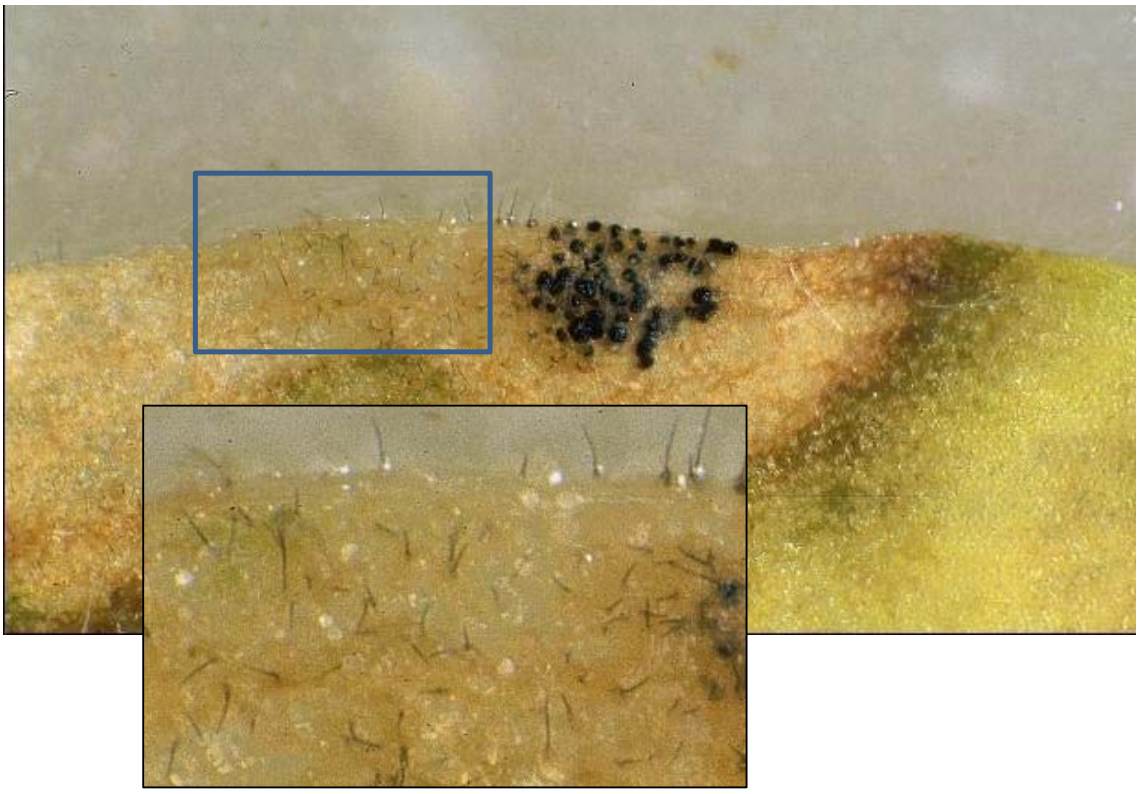


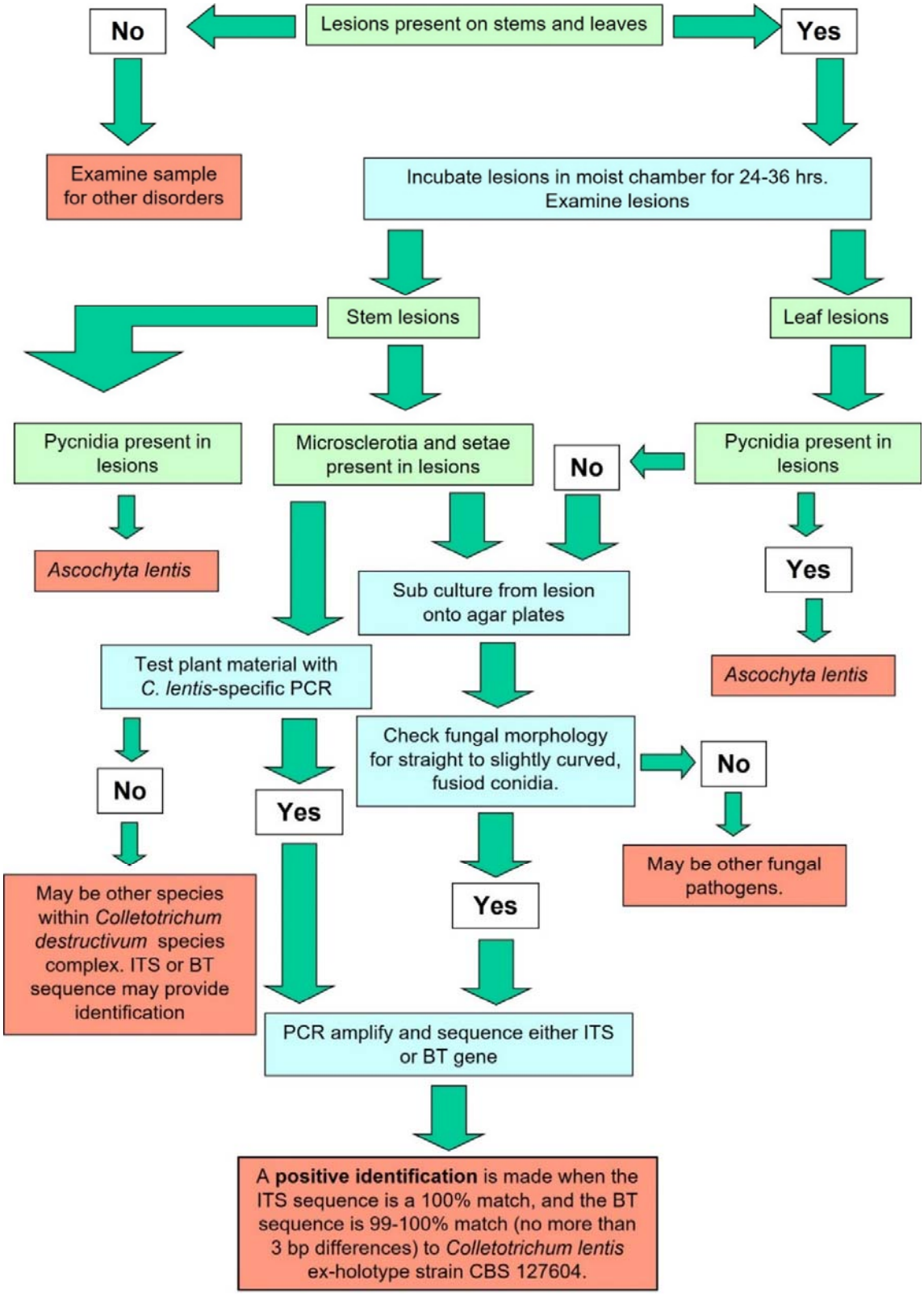
Figure 11. Close up image of a stem lesion caused by *C. lentis* showing setae and microsclerotia development. (Photograph courtesy of L. Buchwaldt, Agriculture and Agri-Food Canada).

3.1.4 Wilt

Anthracnose causes defoliation and stem girdling, which inhibits utilisation of water and nutrients. The fungus penetrates the vascular tissue and girdles the stem resulting in wilting of the entire plant. As a result, large areas of brown and dying plants can be found in the field (Figure 12).

Figure 12. Scattered wilting and dying plants within a lentil crop caused by *C. lentis* (infection near Regina, Canada 2005; Source: Unknown).

Figure 13. Flow diagram of the protocols for the analysis of a suspect plant sample.



4 IDENTIFICATION

Colletotrichum lentis can only be confirmed by DNA sequence analysis, as morphology is unreliable for the identification of *Colletotrichum* species. The preliminary diagnostic test for the detection of *C. lentis* is the species-specific conventional PCR assay (Section 4.3.3.1), followed by confirmation based on DNA sequence analysis of the ITS or BT genes against the *C. lentis* ex-holotype strain CBS 127604 (Section 4.3.3.2).

NB: The species-specific conventional PCR assay was designed to identify C. lentis at the time of publication. Users should be aware of any later changes in taxonomy that may impact on identification and ensure quality and quantity checks on DNA.

4.1 Morphological examination

Morphological characters, while not being diagnostic to the level of host specific strain, can confirm the presence of *Colletotrichum* species.

4.1.1 Preliminary examination

The infected plant material is examined for the presence of microsclerotia or other fruiting bodies under the dissecting microscope. If fruiting bodies are present their identity should be determined under the compound microscope (i.e., microsclerotia or pycnidia). If fruiting bodies are found to be pycnidia, the causal pathogen is most likely *A. lentis*. To be certain of the identity of the pathogen infected plant tissue should be plated onto agar media (Section 4.1.4).

4.1.2 How to distinguish between anthracnose and Ascochyta blight symptoms.

Colletotrichum lentis and *A. lentis* can cause similar symptoms on leaves and pods but are slightly different on the stems. Stem symptoms are different since anthracnose penetrates deeper and creates dents in the stem surface, while ascochyta lesions are more superficial. To determine which disease is present, it is helpful to look for lesions where the pathogen has produced pinhead-sized, black structures. In an ascochyta lesion, these structures (pycnidia) are almost round, rarely touching one another, and partially embedded in the plant tissue (Figure 14). If anthracnose is present, the structures (microsclerotia) are irregularly shaped, and some are clumped together (Figures 9-11). Most characteristic of anthracnose, however, are the tiny black hairs (setae) sticking up from the lesion, which can be seen with the naked eye or a hand lens at 10 – 15 magnifications. Setae will grow out of the lesion even if microsclerotia are not present (Figure 11). Microsclerotia of anthracnose are easiest to observe in stem lesions, and pycnidia of ascochyta are often found in leaf lesions (Bailey *et al*. 2003).

Figure 14. Leaf lesion caused by *Ascochyta lentis* on lentil. Pycnidial fruiting bodies are clearly seen within the lesion (Photograph T. Bretag, Department of Primary Industries, Victoria).

4.1.3 Encouraging setae development.

The appearance of setae within a suspect lesion is a characteristic feature of lentil infected by *C. lentis*. Often setae can be encouraged to develop within 24 hours if infected plant pieces are placed within moist chambers. Microsclerotia, while also characteristic of the disease, will take several more days to develop.

Portions of plants with lesions can be incubated within a moist chamber to induce the formation of setae. Infected plant parts can include both leaf and stem lesions. Microsclerotia will also form under these conditions but will take several more days. Setae can develop overnight under ideal conditions, but usually take 24 – 36 hours. The easiest moist chambers to prepare are made by placing moist filter paper, dampened with distilled water, in the bottom of a petri dish and placing the specimen on the moist paper. These chambers allow the specimen to be sealed within the plate and can be viewed under a dissecting microscope without disturbing the specimen.

Normally moist chambers are incubated at room temperature with diurnal lighting. Specimens should not require surface sterilising as this may inhibit fungal growth. However, any plant tissue covered with soil should be washed in gently running tap water prior to setting up in a moist chamber.

4.1.4 Isolation of pathogen from infected plant specimen

General items required

- Infected plant sample.
- Compound microscope with 10, 20, 40 and 100x objectives.
- Dissecting microscope up to 50x magnification.
- Scalpel blades.
- Dissecting needle.
- >95% w/v ethanol.
- Sterile distilled water.
- 2% bleach solution.
- Sterile petri dishes – 90 x 15 mm
- Oatmeal agar.

Agar plate recipes

½ strength oatmeal agar (commercial mix)

Oatmeal agar	36 g
Distilled water	1 L

The above recipe should produce approximately 50 plates.

If a commercial mix of oatmeal agar is not available, then the following recipe can be used.

½ strength oatmeal agar (using rolled oats)

Oatmeal (rolled oats)	30 g
Agar	20 g
Distilled water	1 L

In a commercial blender, blend 30g of rolled oats in 600ml of distilled water, and heat to 45 – 55°C. Then add 20g agar dissolved in 400ml of distilled water. Autoclave at 121°C for 30 mins.

Method - Preliminary examination

The infected plant material is examined for the presence of microsclerotia or other fruiting bodies under the dissecting microscope. If fruiting bodies are present their identity should be determined under the compound microscope (i.e., microsclerotia or pycnidia). If fruiting bodies are found to be pycnidia, the causal pathogen is most likely *A. lentis* (see Figure 14~~Figure 14~~).

Isolation from infected plant material

Infected plant pieces should be cut using a scalpel from the edge of the lesion. Old, infected tissues from the centre of the lesions often contain secondary organisms, which invade and colonise the dying tissue. These often include bacteria. Any fruiting bodies that are present should also be plated onto agar media.

Tissue pieces to be plated out should be briefly sterilised in 2% bleach solution to remove any surface contaminants. Tissue should be fully submerged in the solution for at least 60 secs, and up to 2 mins. The length of time will vary according to the tissue type (i.e. stem or leaf material) and the size of the piece. Following submergence in bleach, the tissue pieces should be rinsed in sterile distilled water and allowed to dry on clean filter paper before placing on agar plates.

The plates should be sealed with parafilm incubated at 18 – 22°C for 7 – 10 days. This allows adequate time for fungi within the tissue pieces to grow onto the agar.

It may become necessary to sub-culture from the initial plates within several days of plating out. Often secondary fungi are fast growing and may contaminate other cultures growing on the plate making sub-culturing necessary to maintain a pure culture. If this needs to be done, ensure that the plates are clearly labelled and noted on the specimen worksheet.

Once conidia develop, the morphological features of the specimen can be compared to the description of *C. lentis* (Section 2.2). Also refer to for conidia and appressoria illustrations.

4.2 Molecular method

4.2.1 Laboratory supplies

- Protective gloves
- 2.0, 200, and 1000 µL sterile barrier pipette tips
- 2.0, 20, 200, and 1000 µL pipette tips
- Microcentrifuge
- Microcentrifuge tubes 1.5 mL
- 0.2 mL PCR tubes
- Thermocycler
- Gel tray with suitable comb/s, electrophoresis tank and powerpack
- UV transilluminator
- Camera/gel documentation system

4.2.2 DNA extraction

Genomic DNA (gDNA) may be extracted from pure fungal cultures and infected plant tissues using commercially available plant DNA extraction kits; e.g. QIAGEN Plant DNeasy kit, Bioline ISOLATE II Plant DNA extraction kit, etc. The preference is for gDNA to be eluted in nuclease-free H_2O. Store gDNA at -20°C.

For the development of this protocol, QIAGEN Plant DNeasy Mini kit was used, and the gDNA were eluted in a total of 100µL PCR-grade H_2O.

4.2.3 Conventional PCR

Primers and expected product sizes

For amplification and sequencing of the ITS region:

V9G (forward) 5'-TTACGTCCCTGCCCTTTGTA-3'
ITS4 (reverse) 5'-TCCTCCGCTTATTGATATGC-3'
Expected product size: 560bp

For amplification and sequencing of the BT region:

T1 (forward) 5'- AACATGCGTGAGATTGTAAGT-3'
Bt2b (reverse) 5'- ACCCTCAGTGTAGTGACCCTTGGC-3'
Expected product size: 513bp

Species-specific diagnostic primers designed based on the BT region:

Clentis-tub2F (forward) 5'- AGGCAAAACATCTCTGGCGA-3'
Clentis-tube2R (reverse) 5'- ACTGGGGTACGAGAATAACT-3'
Expected product size: 184bp

PCR reagents

For DNA sequencing analysis, the preference is to use a high-fidelity PCR master mix to avoid the introduction of PCR errors into the product for DNA sequencing purposes, and to minimise pipetting errors in the preparation of PCR master mix. High-fidelity PCR master mixes can be purchased on a

commercial basis, e.g. Phusion® High-Fidelity PCR Master Mix, Platinum® Taq DNA polymerase High Fidelity, etc.

For the development of this protocol, Phusion® High-Fidelity PCR Master Mix in HF Buffer was used for PCR of the ITS and BT region, and the MyTaq™ Master Mix was used for the species-specific PCR.

DNA gel running buffer – TBE Buffer

Tris-Borate-EDTA (TBE) gel running buffer can be purchased commercially in concentrated liquid format. Follow the manufacturer's instructions to dilute it to a 1X concentration. Alternatively, it can be made up from the following components:

	Per 1L	Final conc.
Tris base	54.0 g	0.4 M
Boric acid	27.5 g	0.05 M
0.5M EDTA pH 8.0	20.0 mL	0.001 M

Dissolve components in 1L distilled water. Store at room temperature.

Agarose gel

	Per 100 mL	Conc.
DNA grade agarose	1.0 g	1%
	1.5 g	1.5%
TBE	100 mL	1x

Dissolve the molecular biology-grade agarose in TBE buffer in a heat-proof glass container (e.g. beaker or Schott bottle) by heating in a microwave. Once the bottle is slightly cool to the touch, pour into the gel tray with comb. It will take approximately 30 mins to set at room temperature (20-22°C).

WARNING: The container and the content are extremely hot. Handle the container with care using heat-proof gloves.

DNA Loading dye and DNA stain

Certain dye powders are hazardous in concentrated forms. Therefore, due to the workplace health and safety considerations of dye powders, DNA loading dye should be purchase from commercial companies.

Either Ethidium Bromide or GelRed® may be used to stain double-stranded DNA to visualise it on an agarose gel.

This protocol has been developed using GelRed® by adding 10 µL of a 10,000X concentration (in water) in 1 mL of DNA loading dye. Vortex well to mix, and store in a dark container when not in use.

4.2.4 Colletotrichum lentis-specific PCR on infected plant tissues

Prepare the following PCR mix as described below for the number of test samples, the Positive Control, a blank, and one extra.

Reagents	Volume (µL) per reaction
Phusion ® Master Mix (2X)	12.5
Primer CL-BTF (10µM)	0.5
Primer CL-BTR (10µM)	0.5
Nuclease-free H_2O	10.5
Total	24

Add 1 µL from a 1/10 dilution of gDNA as template, place in a thermocycler using the following program:

Initial denaturation	95°C	3 mins	1 cycle
Denaturation	95°C	30 secs	30 cycles
Annealing	60°C	30 secs	
Extension	72°C	30 secs	
Final Extension	72°C	5 mins	1 cycle
Cool	12°C	10 mins	1 cycle

When the PCR is complete, mix 5ml of each PCR sample with 1 ml of DNA loading dye.

Load the samples into a 1.5 % TBE agarose gel.

Run the gel in TBE buffer at 100V for 40 minutes.

Visualise and photograph the gel under UV-light.

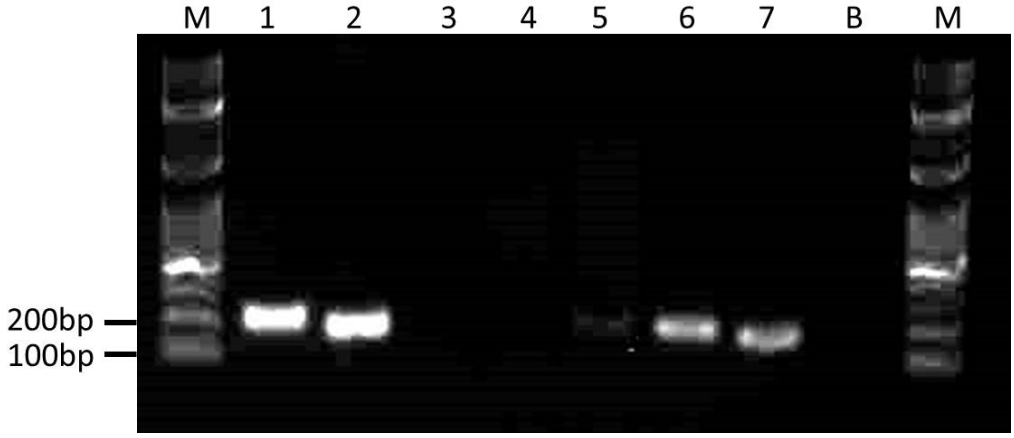


Figure 15. Example DNA electrophoresis gel of species-specific PCR from infected plant tissue. Lane 1: Positive Control 1 *C. lentis* strain CT-21; Lane 2: Positive Control 2 *C. lentis* strain CT-30; Lane 3: Negative Control 1 *Colletotrichum lini*; Lane 4: Negative Control 2: *Colletotrichum higginsianum*; Lane 5: Unknown fungal culture sample CT-64; Lane 6: *Lens culinaris* cv. Eston infected plant tissues 144 hours post-inoculation; Lane 7: *Lens culinaris* cv. Eston infected plant tissues 7 days post-inoculation; Lane B: Blank control; Lane M: DNA Ladder 100bp.

4.2.5 PCR for DNA sequence analysis

Prepare the following PCR mix as described below for the number of test samples, a blank, and one extra.

Reagents	Volume (µL) per reaction
Phusion ® Master Mix (2X)	12.5
Forward Primer (10µM)	0.5
Reverse Primer (10µM)	0.5
Nuclease-free H_2O	10.5
Total	24

Add 1 µL of gDNA from pure fungal culture as template, place in a thermocycler using the following program:

Initial denaturation	98°C	30 secs	1 cycle
Denaturation	98°C	10 secs	30 cycles
Annealing	55 or 60°C*	30 secs	
Extension	72°C	30 secs	
Final Extension	72°C	5 mins	1 cycle
Cool	12°C	10 mins	1 cycle

* Anneal temperatures for the following PCR: ITS at 55°C and BT at 60°C.

When the PCR is complete, mix 2ml of each PCR sample with 1 ml of DNA loading dye.

Load the samples into a 1% TBE agarose gel.

Run the gel in TBE buffer at 100V for 40 minutes.

Visualise and photograph the gel under UV-light.

Sequence PCR product

Once it is confirmed that there is a single PCR product, prepare the PCR product for sequencing. Refer to the sequencing facility's guidelines for sample preparation and shipment.

DNA sequence analysis

Sequences from ITS and BT should only be compared to *C. lentis* ex-holotype strain CBS 127604, GenBank accession JQ005766 (ITS) and JQ005850 (BT). For a positive identification, the ITS sequence of the sample must be 100% match, and the BT sequence must be 99-100% match (no more than 3-bp differences) to the ex-holotype strain.

Positive control DNA

The following genomic DNA are available from the Queensland Plant Pathology Herbarium (BRIP), Brisbane:

- *Colletotrichum lentis* CT-21 = BRIP 63654
- *Colletotrichum lentis* CT-30 = BRIP 63655

5 CONTACTS FOR FURTHER INFORMATION

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