PlantClinic

THE VITAL SCIENCE AT WORK

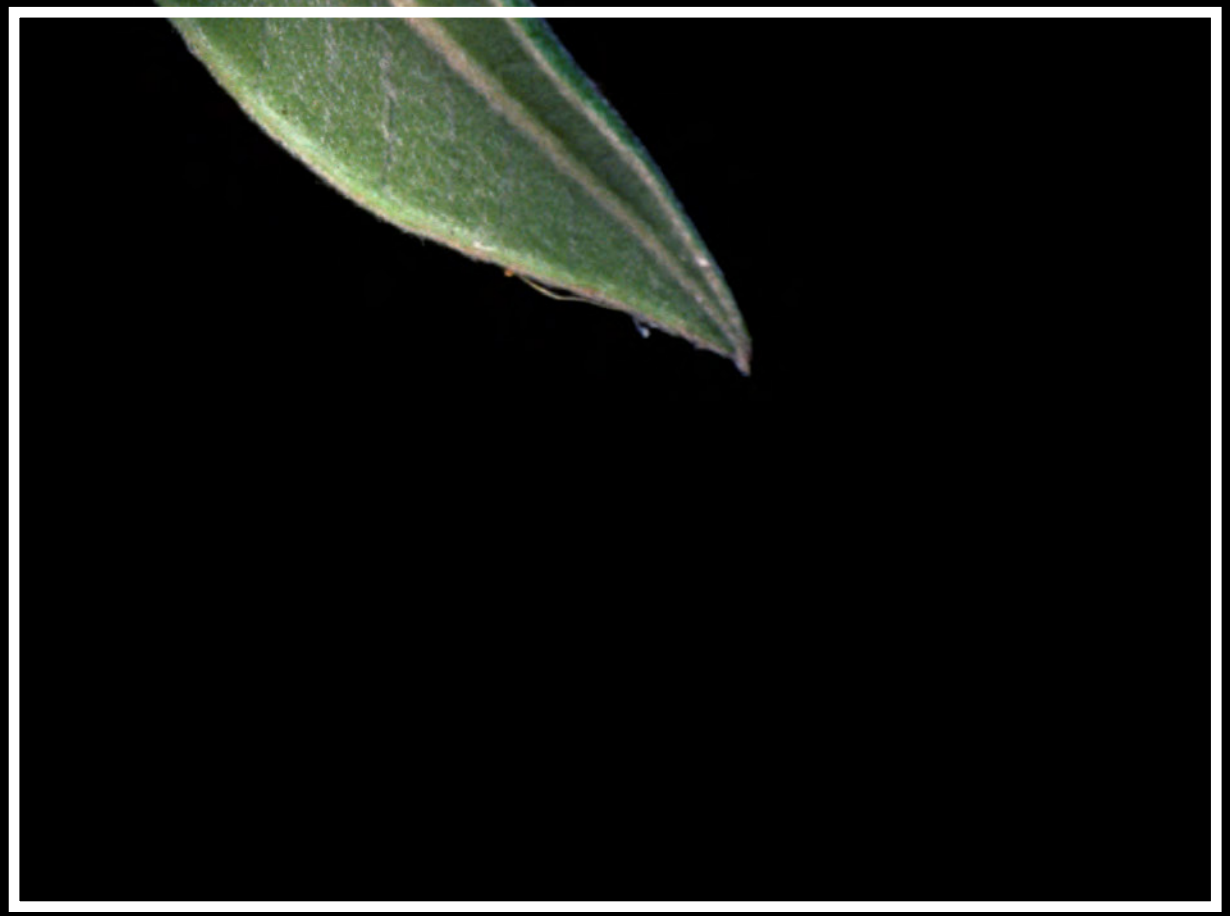
Who we are

The PlantClinic offers a wide range of plant disease diagnostics, pathogen detection and plant DNA identification services to the public, industry and government agencies.

This service is not for profit and all revenue goes towards the PlantClinic service, development of new diagnostic techniques and research into plant diseases that threaten our forests, parks and gardens.

Why?

Plant diseases threaten our biodiversity and have a devastating impact on our native flora and fauna. Diagnostics is the first step in plant disease management and is integral to our future plant health and conservation efforts.



Service Summary & Cost Information

SERVICE	DESCRIPTION	SUBSTRATE	PRICE (EX GST)
Phytophthora & Pythium	Detection of *Phytophthora* and *Pythium* species from soil, plant tissue, mulch, water, and growing media. The service also attempts to identify the detected *Phytophthora* and/or *Pythium* to the species taxonomic level using a DNA sequencing and analysis approach.	**Soil and Plant**	**$175 per sample**
Phytophthora (Genus Only)	Detection of the genus *Phytophthora* from soil, plant tissue, mulch, water, and growing media. Please note that this service does not attempt to identify the detected *Phytophthora* to the species taxonomic level.	**Soil and Plant**	**$115 per sample**
Wood Decay Fungi Detection	For the detection and identification of the major genera of wood decay fungi (*Armillaria*, *Phellinus* & *Ganoderma*) from wood tissue or fungal mycelium. This service cannot be used for soil samples.	**Plant/Wood**	**$250 per sample**
Plant Pathogen Detection & Basic Identification	A general service for the detection and basic identification of fungal and bacterial plant pathogens from plant tissue and agar cultures using a DNA sequencing approach. Resulting DNA sequences are matched to species using the Basic Local Alignment Search Tool (BLAST) algorithm against reference sequences in public sequence databases.	**Plant**	**$290 per sample**
Plant, Fungal & Insect Barcode Sequencing Service	DNA extraction and Sanger sequencing of commonly used plant, insect and fungal barcodes for identification and research. Sequence data is provided in both the 5' & 3' directions. This is a data only service with no analysis and is aimed at research scientists. For a list of routinely used barcoding loci please refer to the detailed information provided on page 11.	**Plant, Culture & Insect**	**$95 per sample/locus**
Basic Plant Identification (from root & other plant tissues)	Identification of plants from their roots and other tissues using a DNA sequencing approach. The service is accurate to genus and usually to species but cannot differentiate between individuals of the same species. This service does not assess the taxonomic reliability of the matched reference sequence or provide higher resolution analyses or interpretation. If this is required, please select the High Resolution Fungal and Plant Identification service.	**Plant**	**$295**
High Resolution Fungal & Plant Identification	High resolution fungal and plant identification to the species level using a phylogenetic approach. This service includes a review of the relevant literature and interpretation of the results. This service is appropriate when very accurate identification is required (e.g. biosecurity, legal etc.). This service cannot differentiate between individuals of the same species.	**Plant, Culture & Insect**	**Please enquire for a fee proposal.**

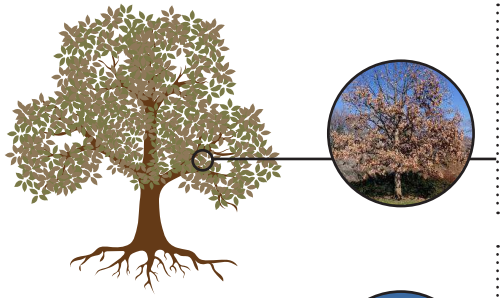
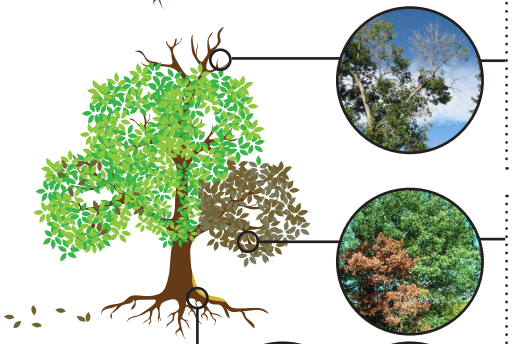
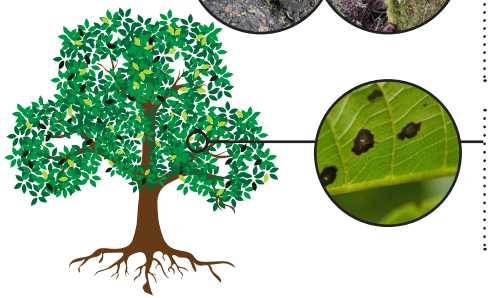
PlantClinic

For all enquiries and variations please contact Dr Matthew Laurence **matthew.laurence@botanicgardens.nsw.gov.au**

Which Service?

Plant Diseases

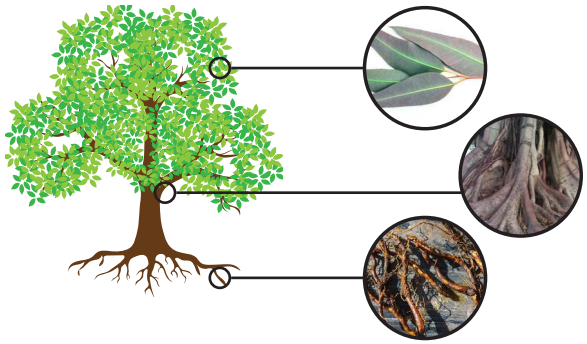
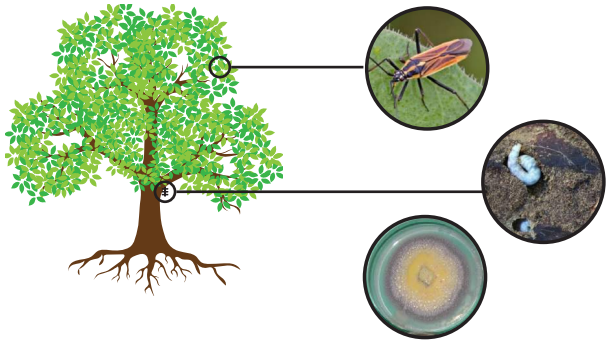
CLICK BUTTONS BELOW FOR FURTHER INFORMATION ON SERVICES.

EXAMPLE	SYMPTOMS	POSSIBLE CAUSES	COLLECT	PLANTCLINIC SERVICE
	Entire crown affected evenly, with leaf chlorosis, browning or death affecting entire crown evenly (with or without retained leaves) usually with rapid onset.	• **Environmental stress factors** e.g. herbicide toxicity, soil pH, drought, water logging • **Mechanical** e.g. root severance, soil compaction • **Plant pathogen** active in the roots and/or vascular system.	Soil Plant Tissue	**Phytophthora & Pythium** **Plant Pathogen Detection & Basic Identification**
	General dieback and decline over a longer period of time, which can be evenly distributed over the entire crown. Results in the characteristic 'dead top' often seen in mature trees.	• **Environmental stress factors** e.g. herbicide toxicity, soil pH, drought, water logging • **Mechanical** e.g. root severance, soil compaction • **Plant pathogen** active in the roots and/or vascular system.	Soil Plant Tissue	**Phytophthora & Pythium** **Plant Pathogen Detection & Basic Identification**
	Dieback restricted to single branch and associated with wounds or lesions on the branch, trunk or root system.	• **Environmental stress factors** e.g. herbicide toxicity, soil pH, drought, water logging • **Mechanical** e.g. root severance, soil compaction • **Plant pathogen** causing branch/trunk wounds	Plant Tissue	**Plant Pathogen Detection & Basic Identification**
	Trunk/root wounds with wood decay.	• **Mechanical damage** with secondary decomposing fungi • **Wood decay fungal pathogens**	Plant/ Fungal Tissue	**Wood Decay Fungi Detection**
	Leaf lesions/spots which can be evenly distributed over the entire crown or restricted to sections.	• **Contact toxicity** e.g. herbicide, salinity, air pollution, sun scorch • **Plant pathogen** active in the leaves	Plant Tissue	**Plant Pathogen Detection & Basic Identification**

Samples may need more than one service, for all questions please contact **matthew.laurence@botanicgardens.nsw.gov.au**

Identification Services

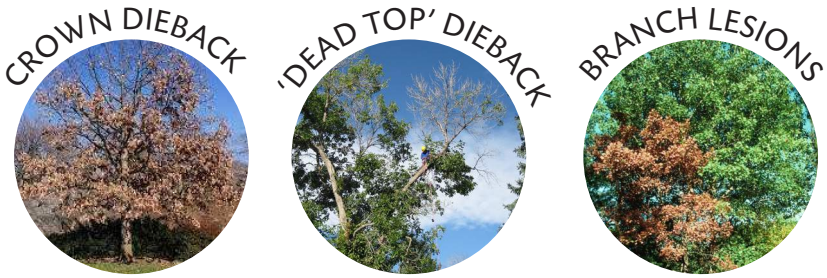
CLICK BUTTONS BELOW FOR FURTHER INFORMATION ON SERVICES.

EXAMPLE	TISSUE/SAMPLE TYPE	PLANTCLINIC SERVICE
	**Leaf, Trunk or Root Sample** • This service can identify plants from their roots and other plant parts using DNA sequences of the standard plant barcodes described in CBOL Plant Working Group. Specifically, the nuclear ribosomal DNA internal transcribed spacer regions 1 and 2 (ITS) and the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene regions. • Resulting DNA sequences are matched to species using the BLAST algorithm against reference sequences in public sequence databases. The service is accurate to genus and usually to species but cannot differentiate between individuals of the same species. Further, this service does not assess the taxonomic reliability of the matched reference sequence or provide higher resolution analyses or interpretation. If high level resolution is required (e.g. for legal cases) then please refer to our High Resolution Plant Identification Service.	**Basic Plant Identification**
	**Insect, Grub, Plant or Fungal Culture** • This service provides DNA extraction and Sanger sequencing of commonly used plant, insect and fungal barcodes for identification and research. Sequence data is provided in both the 5' & 3' directions. This is a data only service with no analysis and is aimed at research scientists. For a list of routinely used barcoding loci please refer to service information sheet page 11.	**Plant, Fungal & Insect Barcode Sequencing**
	Insect, Plant or Fungal Culture • High resolution insect, plant or fungal culture identification to the species taxonomic rank using a phylogenetic approach. This service includes a review of the associated literature and interpretation of the results. This service is appropriate when very accurate identification is required (e.g., biosecurity, legal etc.). This service cannot differentiate between individuals of the same species.	**High Resolution Fungal & Plant Identification**

PlantClinic TURN AROUND TIME

Samples may need more than one service, for all questions please contact **matthew.laurence@botanicgardens.nsw.gov.au**

PHYTOPHTHORA & PYTHIUM SERVICE



Substrate: Soil and Plant Material

Phytophthora and *Pythium* are microscopic plant pathogens that cause *Phytophthora* Root Rot, Damping-Off as well as foliar diseases across a broad host range. Soil-borne species primarily attack the root system of plants, reducing the root volume, and thus impeding water and nutrient uptake. The above ground symptoms are therefore similar to water stress and are exacerbated by drought or low rainfall events. *Phytophthora* Root Rot can lead to rapid death in highly susceptible species and immature plants or a slow decline in mature or less susceptible species.

The PlantClinic offers diagnostic services for the detection of *Phytophthora* and *Pythium* from soil, roots and plant material. The method is based on a combination of traditional baiting followed by total community DNA extraction and detection with a *Phytophthora* genus specific Taqman™ assay followed by Sanger sequencing of the ATP synthase protein 9 (*atp9*) and NADH dehydrogenase subunit 9 (*nad9*) gene regions. *Pythium* is detected using Sanger sequencing of the nuclear ribosomal DNA, internal transcribed spacer 1 and 2 (ITS) with primers specific to Oomycetes. Resulting *atp9*/*nad9* and ITS sequences are used for species identification with the BLAST that matches the sequences to references in publicly available databases. The BLAST algorithm returns a list of species that are most similar to the sample DNA based on sequence identity (similarity) and usually returns multiple species with only the top three matches included.

This service does not assess the taxonomic reliability of the matched reference sequence or provide higher resolution analyses or interpretation.

If this is required, please select the:

High Resolution Fungal & Plant Identification

PlantClinic TURN AROUND TIME

SAMPLING STRATEGY

The sampling strategy is important for both the *Phytophthora* detection and management recommendations. *Phytophthora* can have a patchy distribution at the individual plant and site scale and therefore representative sampling is imperative. At the site scale there are a number of variables that can be taken into account to maximise the likelihood of detection.

- Firstly, *Phytophthora* is associated with plant roots, so samples should be collected within the plant/tree drip line.
- Trees/shrubs with dieback symptoms typical of *Phytophthora* (wilting yellowing and dieback etc.) should be sampled preferentially. However, the pathogen may no longer be present if the host is completely dead.
- *Phytopthora cinnamomi* is an introduced species and therefore distribution is often linked to human activity. Therefore, sites of adjacent to human disturbance (roads, tracks etc.) have higher risk of *Phytophthora* dieback.
- **Vegetation type** There is variation in *Phytophthora* susceptibility, with some species acting as 'indicators' of dieback caused by *P. Cinnamoni* e.g. *Xanthorrhoea*.
- Topography can also influence distribution as this pathogen is spread in association with free water. Areas of poor drainage and/or open textured soil are more conducive to *Phytophthora*.
- The proximity to infected areas, especially sites down slope.
- **Temporal factors** This pathogen is more active in warm moist conditions between 15-30℃.
- Many other variables both known and unknown will also affect distribution, including interactions with the previously described variables.

See next page for how to collect soil samples

PHYTOPHTHORA & PYTHIUM SERVICE

SOIL COLLECTION

Start clean, keep it clean, finish clean!

When collecting multiple samples, it is important to disinfect ALL tools between composite samples (e.g. with 70% methylated spirits).

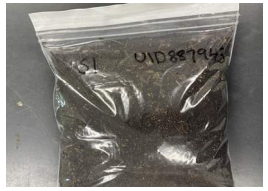
1. Take multiple cores from within the dripline, and combine into a single composite sample.

2a. Start by removing mulch and leaf litter layer.

b. Use a trowel or ideally a soil corer to take a soil core to around 10-15cm depth.

c.Try to sample mostly root material.

3. Combine all individual cores into a single composite sample.



You should end up with 3-5 cups of soil/ root mix per composite sample.

Label bags with your sample number and your unique identifying number (UID) which is referred to on P.14.

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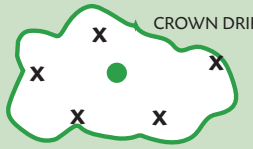
TURN AROUND TIME

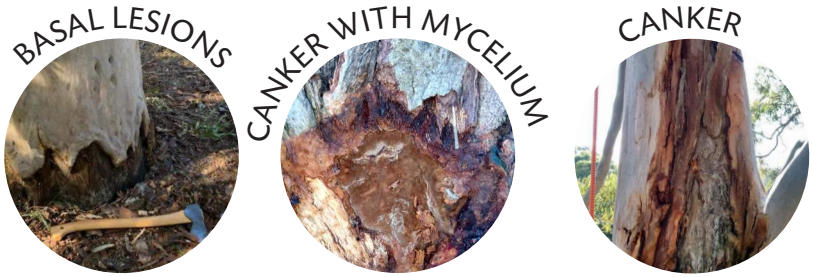
PLANT SCALE



Detection can be maximised by taking a composite soil sample within the drip line of the tree away from the main stem/trunk.

A composite soil sample consists of multiple soil cores combined into one bag.

WOOD DECAY FUNGI SERVICE



What is Wood Decay Fungi?

Wood decay fungi have the ability to degrade lignin and cellulose which are the main chemical components of wood tissue responsible for structural integrity. These fungi can therefore contribute to structural failure (Figure 1a). Although living trees can maintain their structural integrity with large amounts of decay, the rate of decay and therefore prognosis is partly dependent on the fungal species.

Furthermore, some wood decay fungi are able to attack the living sapwood of trees, resulting in general dieback symptoms (Figure 1b).

How does PlantClinic Test?

The PlantClinic offers a diagnostic service for the major genera of wood decay fungi.

This method uses a total DNA extraction approach followed by the multiplex PCR described in Guglielmo et al. (2007). This method can detect *Armillaria* spp., *Ganoderma* spp., *Inonotus*/*Phellinus* group. Other fungal genera can be detected on specific request.

PlantClinic TURN AROUND TIME

Fig 1a. *Armillaria* showing basal lesions and mushrooms

Fig 1b. General dieback 'deadtop' symptoms caused by *Armillaria*.

SAMPLE COLLECTION

Armillaria Sample Collection

1. *Armillaria* is a fungal root rot pathogen that also infects the sapwood and is often associated with a basal lesion.

2. Mushrooms are the spore-bearing structures of *Armillaria* and are produced in autumn/ early winter. However, not all infections produce mushrooms. If mushrooms are observed, then package and send to the PlantClinic.

3. Wrap the mushroom in absorbent towelling. Place in zip-lock bag and label with your sample number.

4. Outside of mushroom season, fungal mycelium can often be found under the bark and cortical tissue of the lower trunk and major roots.

Collect and package bark and mycelium in the same way as mushrooms.

PlantClinic

TURN AROUND TIME

When collecting multiple samples, it is important to disinfect tools between samples (e.g. with 70% methylated spirits).

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It is VERY IMPORTANT not to breach the tree's natural defences and spread the pathogen into healthy functional wood.

Therefore, ONLY take samples from decayed tissue.

NB You might want to consider engaging an appropriately qualified arborist to collect the appropriate tissue samples.
We recommend contacting the Institute of Australian Consulting Arboriculturists for a Level 5 Arborist in your area.

DECAYED WOOD SAMPLE COLLECTION

1. Samples from non-living decayed tissue for wood decay fungi service can be collected with a standard 8-10mm drill at slow speed.
2. Collect drill shavings in a zip-lock bag
3. Take multiple cores to represent the decayed area.
4. Combine the drill shavings from the multiple cores into a single ziplock bag. Label bags with your sample number.

PLANT PATHOGEN DETECTION & BASIC IDENTIFICATION SERVICE

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When collecting multiple samples, it is important to disinfect all tools between samples (e.g. With 70% methylated spirits).

This service is designed to detect plant pathogens associated with diseased plant parts. Plant parts include roots, stems, shoots, foliage, flowers and fruit.

This service uses a combination of total community DNA extraction with traditional methods of selective agar media to detect and recover plant pathogens. Pathogen identification uses Sanger sequencing of commonly used barcodes followed by a BLAST analysis that matches the pathogen's sequences to references in publicly available databases.

The BLAST algorithm returns a list of species that are most similar to the sample DNA based on sequence identity (similarity) and usually returns multiple species with only the top three matches included in the report.

This service does not assess the taxonomic reliability of the matched reference sequence or provide higher resolution analyses or interpretation. If this is required, please select the:

High Resolution Fungal & Plant Identification

SAMPLE SELECTION

Plant pathogens are usually easier to detect in tissue that is in the transition from healthy to diseased. Although the pathogen may still be present in dead tissue, this area is quickly colonised by decomposing fungi. This complicates the detection of the causal organism. Dead tissue is NOT suitable for pathology testing.

Please send specimens with the full range of symptoms from healthy to completely dead. We can then select the best tissue for pathology testing.

EXAMPLES

Some examples of the pathogens and diseases and we routinely detect & diagnose:

- Fusarium Wilt of the Canary Island Date Palm (Fig 1c).
- Myrtle Rust (*Austropuccinia psidii*)
- Cypress Canker
- Rhizoctonia diseases
- Eucalyptus Leaf Blight

Fig 1a. Showing canker cross section with disease margin.

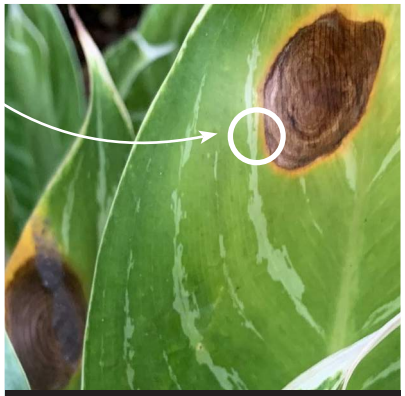

Fig 1b. Showing leaf spot with disease margin

Fig 1c. Typical Fusarium wilt symptoms

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TURN AROUND TIME

BASIC PLANT IDENTIFICATION

Basic Plant Identification

The PlantClinic offers a plant identification service from their roots and other tissues to genus and species level. Our method of identification is based on DNA extraction followed by polymerase chain reaction (PCR), Sanger sequencing and comparison of the resulting sequences with those in the National Center for Biotechnology Information (NCBI) global database. This technique is therefore dependent on successful DNA extraction and the availability of DNA sequences from the target species in the NCBI database.

The service is accurate to genus and usually to species but cannot differentiate between individuals of the same species.

This service does not assess the taxonomic reliability of the matched reference sequence or provide higher resolution analyses or interpretation.

If this is required, please select the: **High Resolution Fungal & Plant Identification**

SAMPLE COLLECTION

Successful DNA extraction is largely dependent on the quality of the specimen we receive. We have found that **living roots greater than 10mm** diameter give more reliable results. Please refer to the figures for examples of good specimens. Unfortunately, we must still charge for this service even if the DNA extraction is unsuccessful to cover our costs.

Please also be aware that more than one tree species/individual's roots may be involved and can complicate the results.

PlantClinic TURN AROUND TIME

Fig 1a. **Leaf sample**

Fig 1b. **Living root sample greater than 10mm diameter**

PLANT, FUNGAL & INSECT BARCODE SEQUENCING

What is Barcode Sequencing?

DNA barcoding is a method of species identification using a short section of DNA from a specific gene or genes. This service offers DNA extraction and Sanger sequencing of commonly used plant, insect and fungal barcodes for identification and research.

Sequence data is provided in both the 5′ & 3′ directions. This is a data only service with no analysis and is aimed at research scientists. For a list of routinely used barcoding loci please refer to list below. Other loci can be sequenced on request.

BARCODES

PLANT BARCODES

- The nuclear ribosomal DNA, internal transcribed spacer 1 and 2 (*ITS*)
- The maturase K (*matK*) gene region
- The ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene region

FUNGAL BARCODES

- The nuclear ribosomal DNA, internal transcribed spacer 1 and 2 (*ITS*)
- The RNA polymerase II largest (*RPB1*) and second largest (*RPB2*) subunits
- The translation elongation factor 1-alpha (*EF-1α*) locus

INSECT BARCODE

- The mitochondrial cytochrome c oxidase subunit I (*COI*) gene

PlantClinic TURN AROUND TIME

Fig 1a. **Insect samples in hand-sanitiser gel for submission to PlantClinic**

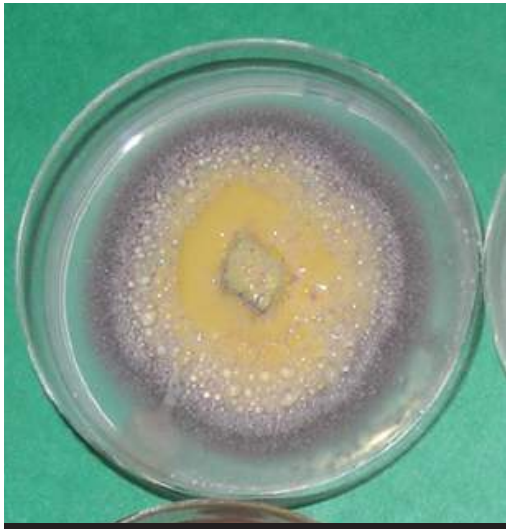
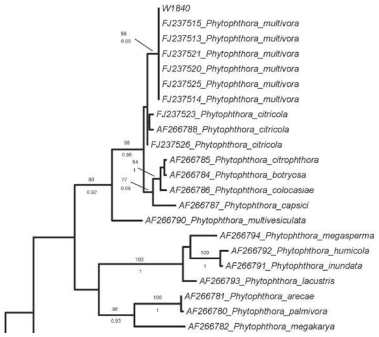


Fig 1b. **Fungal culture**

HIGH RESOLUTION FUNGAL & PLANT IDENTIFICATION

This service provides high resolution fungal and plant identification to the species level using a phylogenetic approach. This service includes a review of the associated literature and interpretation of the results. This service is appropriate when very accurate identification is required (e.g. biosecurity, legal etc.). This service cannot differentiate between individuals of the same species. To differentiate between individuals of the same species please email PlantClinic to request a fee proposal.



Phylogenetic Tree

PlantClinic TURN AROUND TIME

HOW TO COLLECT & PACKAGE YOUR SAMPLES

See **barcoding section** for appropriate specimens for this service.

Leaf (1a) Ideally dry leaf specimens in silica prior to sending or wrap in absorbent paper towelling.

Mycelium, mushrooms and bracket fungi (1b) Wrap fungus in absorbent towelling and place in a zip-lock bag.

Plate culture (1c) Seal plate with parafilm or cling wrap and place in a zip-lock bag.

Label bags with your sample number.

Fig 1a. Leaf specimen

Fig 1b. Mushroom specimen

Fig 1c. Fungal plate cultures

HOW LONG WILL MY SAMPLES TAKE TO PROCESS?

Service Processing Timeline

The idealised timeline for each service is outlined below. The lab operates on a weekly cycle with samples processed each Monday. Samples received after Monday will enter in the next week's cycle. Please note that the timeline is indicative only and times can vary according to unforeseen processing and/or diagnostic issues.

SERVICE	WEEK 1					WEEK 2					WEEK 3		
	MON	TUE	WED	THUR	FRI	MON	TUE	WED	THUR	FRI	MON	TUE	WED
Phytophthora & Pythium (PHY)	Setup	Incubation				Harvest	DNA Test(s)	Third Party Sequencing Service			Results/Reporting		
Wood Decay Fungi Detection (WDF)	Setup	DNA Test(s)	Third Party Sequencing Service			Results/Reporting							
Plant Pathogen Detection & Basic Identification (PDI)	Setup	Incubation					DNA Test(s)	Third Party Sequencing Service			Results/Reporting		
Plant, Fungal & Insect Barcode Sequencing Service (BC)	Setup	DNA Test(s)	Third Party Sequencing Service			Results/Reporting							
Basic Plant Identification (from Root & other plant tissues) (PI)	Setup	DNA Test(s)	Third Party Sequencing Service			Results/Reporting							
High Resolution Fungal & Plant Identification	Setup	DNA Test(s)	Third Party Sequencing Service			Results/Reporting					Analysis		

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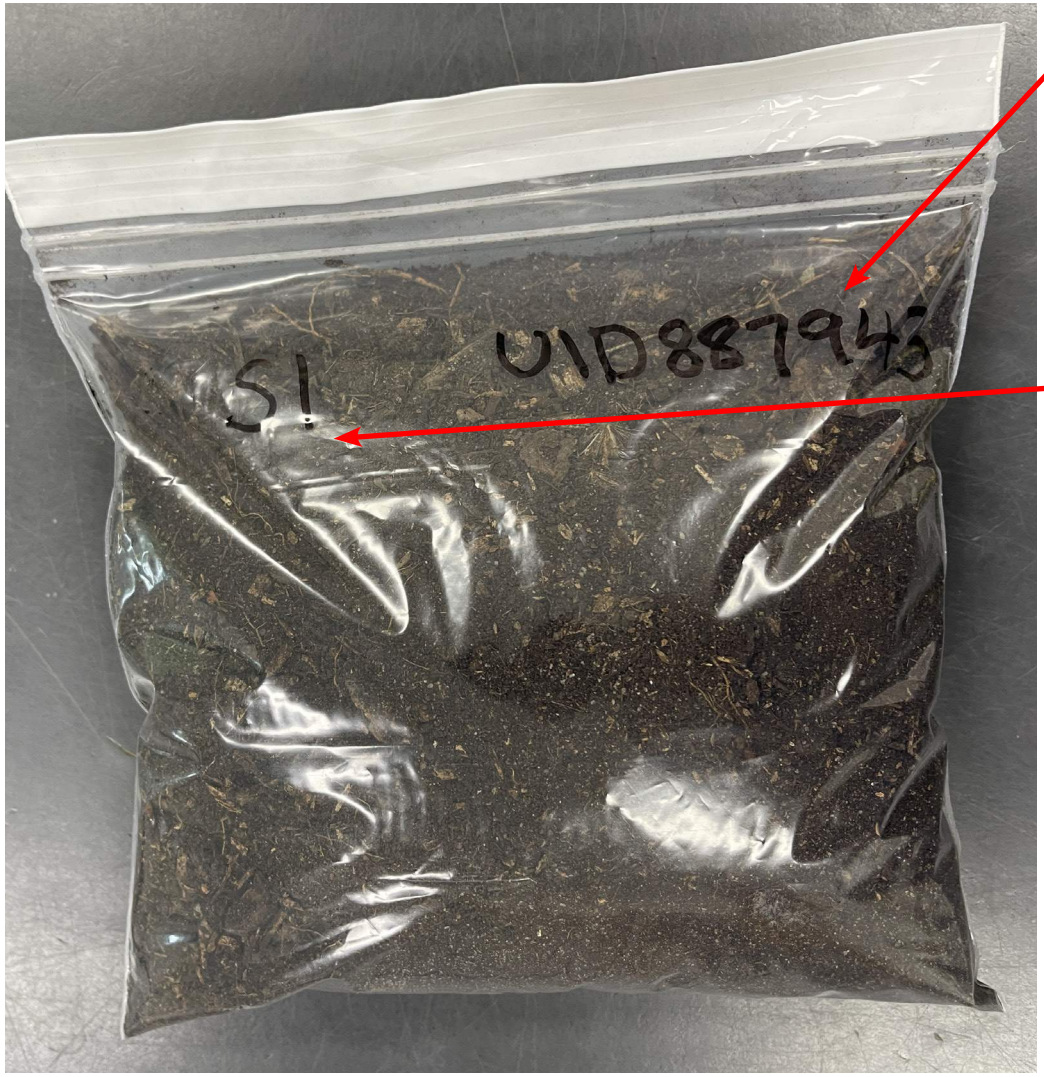
LABELLING YOUR SAMPLES



Sample submission ID (UID) is critical for linking this form with your samples in the laboratory. Please label ALL sample bags with your unique UID number. The UID will be provided once you submit the online form.

Sample Number S1, S2, S3 etc for each individual sample bag. This number correlates to the sample number and services you have requested.

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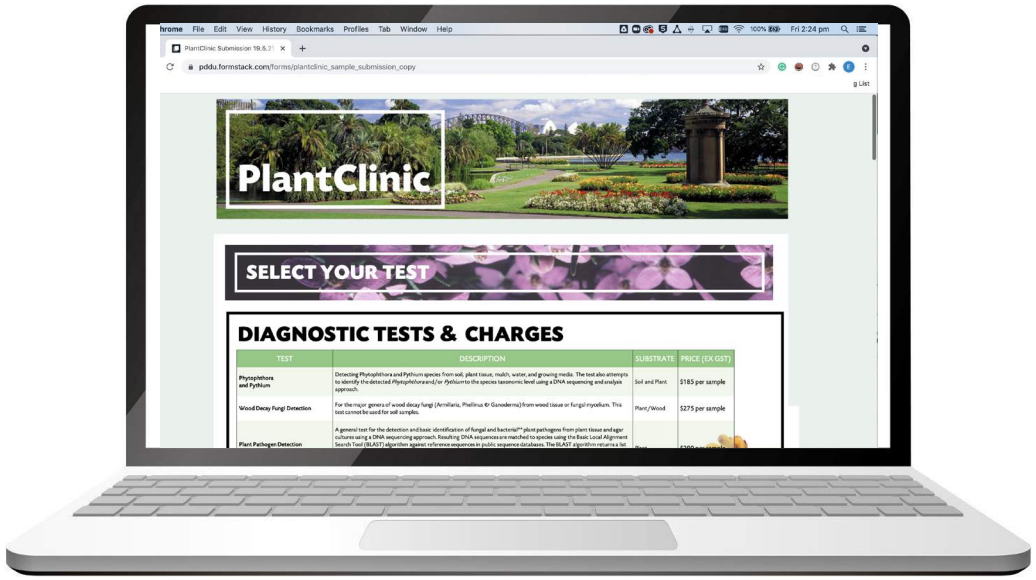
AUSTRALIAN INSTITUTE OF BOTANICAL SCIENCE

SAMPLE SUBMISSION & PAYMENT



1. Complete and submit the on-line form at

https://PDDU.formstack.com/forms/plantclinic_sample_submission_v2

2. Note your Unique UID Number
3. Label your samples
4. Pack your sample(s) and return to **PlantClinic**