



Martin Peacock Tree Care
Arboricultural & Horticultural Consultancy

Arboricultural Impact Assessment Report (Stage 1)

Royal Prince Alfred Hospital Redevelopment
Camperdown
NSW

Prepared by: Martin Peacock Tree Care

Date: 14th November 2022

Phone: 0405 221 056

Email: martin@martinpeacocktreecare.com.au

Web: www.martinpeacocktreecare.com.au

Contents

	Page no.
1.0 Summary	page 3
2.0 Introduction	page 4
3.0 Scope of The Report	page 5
4.0 Observations	page 6
5.0 Discussion	page 6
6.0 Recommendations	page 9
Caveats & Limitations	
References	
Appendix A – Tree Assessment Results	
Appendix B – Pruning Specification	
Appendix C – Pathology Trest Results – T596	
Appendix D – Methodology	

1.0 Summary

- 1.1 This Arboricultural Impact Assessment Report (Stage 1) provides an assessment of one hundred and five (105) trees growing within the Royal Prince Alfred Hospital and the University of Sydney campuses.
- 1.2 The development proposal includes:
 - Redevelopment of the eastern section of the Hospital campus
 - Ancillary works within the western section of the Hospital campus
 - Tree removal and supplementary tree planting around the western edge of the University of Sydney Oval no. 1.
- 1.3 As part of the proposed redevelopment works a total of seventy-one (71) trees are to be removed including: fifty-five (55) trees within the Hospital East campus, seven (7) trees within the Hospital West campus and nine (9) trees on the western edge of the University of Sydney Oval no. 1.
- 1.4 The trees proposed for removal include a number of specimens listed on the City of Sydney Councils Significant Tree Register (rear garden group). A *Statement of Heritage Impact* which details the heritage significance of these trees has been prepared as part of the development proposal. The *Statement of Heritage Impact* should be read in conjunction with this Report.
- 1.5 The development proposal includes the retention of thirty-four (34) trees including: twenty-four (24) trees within the Hospital East campus, three (3) trees within the Hospital West campus and seven (7) trees on the western edge of the University of Sydney Oval no. 1.
- 1.6 The landscaping component of the development proposal includes the installation of seventy-nine (79) new trees within the Hospital East campus. These trees are to be installed in both deep soil and on-structure landscape areas. An additional nine (9) tree are to be installed around the western edge of the University of Sydney Oval no. 1 as part of the Tree Replantation Strategy.
- 1.7 A comprehensive assessment of the impact of development upon the trees proposed for retention cannot be undertaken at this stage as detailed plans are yet to be prepared. To minimise the impact of development encroachment within the Tree Protection Zone areas of the trees proposed for retention, the detailed plans will include elements of tree sensitive design. An Arboricultural Impact Assessment Report (Stage 2) will be prepared based on these detailed plans. This Report will also include a Tree Protection Specification detailing specific, tree protection measures and tree sensitive construction methods which will be utilised to minimise the impact of the works upon the trees.

2.0 Introduction

2.1 This Arboricultural Impact Assessment Report (Stage 1) has been prepared for Royal Prince Alfred (RPA) Hospital in relation to the proposed redevelopment of the Hospital Campus.

2.2 Site description:

The Royal Prince Alfred (RPA) Hospital campus is located in Sydney's inner west suburb of Camperdown, within the City of Sydney Local Government Area. The campus is situated between the University of Sydney to the east and the residential area of Camperdown to the west. A north-south arterial road (Missenden Road) divides the campus into two distinct portions, known as the East and West Campuses. The northern boundary of the campus is defined by the Queen Elizabeth II Rehabilitation Centre and the southern extent of the campus is defined by Carillon Avenue. The works are proposed to both the East and West Campuses, as well as some off-site works occurring within the University of Sydney.

The site comprises the following land titles:

East campus:

- Lot 1000 DP 1159799 (12 Missenden Road, Camperdown, 2050).

West campus:

- Lot 11 DP 809663 (114 Church Street, Camperdown, 2050); and
- Lot 101 DP 1179349 (68-81 Missenden Road, Camperdown 2050).

Off-site works are proposed on University of Sydney land, known as Lot 1 DP 1171804 (3 Parramatta Road, Camperdown, 2050) and Lot 1001 DP 1159799 (12A Missenden Road, Camperdown, 2050).

2.3 Project background:

In March 2019, the NSW Government announced a significant \$750 million investment for the redevelopment and refurbishment of the RPA Hospital campus. The Project will include the development of clinical and non-clinical services infrastructure to expand, integrate, transform and optimise current capacity within the hospital to provide contemporary patient centred care, including expanded and enhanced facilities.

The last major redevelopment of RPA Hospital was undertaken from 1998 to 2004 projected to 2006 service needs. Since then, significant growth has been experienced in the volume and complexity of patients, requiring significant investment to address projected shortfalls in capacity and to update existing services to align with leading models of care.

The redevelopment of RPA Hospital has been the top priority for the Sydney Local Health District since 2017 through the Asset Strategic Planning process, to achieve NSW Health strategic direction to develop a future focused, adaptive, resilient and sustainable health system.

2.4 Description of development:

Alterations and additions to the RPA Hospital East Campus, comprising:

- Eastern wing: A new fifteen (15) storey building with clinical space for Inpatient Units (IPU's), Medical Imaging, Delivery, Neonatal and Women's Health Services,

connecting to the existing hospital building and a rooftop helicopter landing site (HLS);

- Eastern extension: A three (3) storey extension to the east the existing clinical services building to accommodate new operating theatres and associated plant areas;
- Northern expansion: A two (2) storey vertical expansion over RPA Building 89 accommodating a new Intensive Care Unit and connected with the Eastern Wing;
- Internal refurbishment: Major internal refurbishment to existing services including Emergency Department and Imaging, circulation and support spaces;
- Enhanced Northern Entry/ Arrival including improved pedestrian access and public amenity;
- Demolition of affected buildings, structures and trees;
- Changes to internal road alignments and paving treatments; and
- Landscaping works, including tree removal, tree pruning, and compensatory tree planting including off-site on University of Sydney land.

Ancillary works to the RPA Hospital West Campus, comprising:

- Temporary helicopter landing site above existing multi storey carpark;
- Re-routing of existing services; and
- Associated tree removal along Grose Street.

- 2.5 This report provides an assessment of one hundred and five (105) trees growing at Royal Prince Alfred Hospital and the University of Sydney campuses.

3.0 Scope of The Report

- 3.1 This Arboricultural Impact Assessment Report (AIA) has been prepared in accordance with *Australian Standard: AS 4970—2009 Protection of Trees on Development Sites* (AS4970) to provide an assessment of the impact of the proposed development works upon the subject trees. The assessment criteria include:

- Conduct at ground level, a visual inspection of the subject trees and their growing environment
- Assess the physiological and structural condition of the subject trees
- Determine the useful life expectancy, quality and value(s) of the subject trees
- Award a retention category for the subject trees
- Assess relevant plans and documentation to determine the potential impacts of the proposed development upon the subject trees
- Make recommendations for retention, removal or remedial works to the subject trees and/or implementation of tree protection measures as appropriate

- 3.2 The following plans/documentation were referenced in the preparation of this report:

- Contour & Detail Survey (Rev D), dated 05.10.21 – prepared by RPS
- SSDA Landscape Report (Revision B), dated 02.11.22 – prepared by Turf

- 3.3 The trees covered by this report include a number of trees listed on the City of Sydney Significant Tree Register (identified as Rear Garden Group). It should be noted that this AIA Report considers the arboricultural and amenity value of the subject trees only. An assessment of the heritage/cultural value of the trees is detailed in the *Statement of Heritage Impact* which should be referenced in conjunction with this Report.

4.0 Observations

4.1 The trees have been assessed in accordance with *Australian Standard AS4970 (2009) Protection of trees on development sites* (AS4970). Full details of the assessment and a summary of the trees' Retention Values are listed in Appendix A - Tree Assessment Schedule.

4.2 The tree assessment process includes the allocation of a Retention Category for each tree. **The allocation of a Retention Category is a requirement of AS4970** and provides and overview of the quality and value of trees on site. Retention Categories are a guide only and do not take into account design considerations/constraints relating to the development proposal. **It should be noted that Retention Categories are not a schedule for tree removal or retention.**

4.3 Trees Proposed for Retention

The following table indicates the thirty-four (34) trees to be retained and their location:

<u>Location</u>	<u>Tree number</u>
RPA East campus	1-16, 20, 21, 30, 54-57, 127
RPA West campus	2001-2003
Sydney University Oval no.1	590, 597, 598, 1191, 1237-1239

The majority of the trees listed above will be subject to encroachment from development works, to varying degrees. The use of tree sensitive design and construction methods and the establishment of Tree Protection Zone (TPZ) areas will be required to minimise the impact of the works upon the trees.

4.4 Trees Proposed for Removal

The following table indicates the trees seventy (71) to be removed and their location:

<u>Location</u>	<u>Tree number</u>
RPA East campus	17-19, 22 (group of 7 trees), 23 (group of 3 trees), 24 (group of 10 trees), 25 (group of 2 trees), 31-53, 118, 128, 129, 591 (tagged as a University tree)
RPA West campus	2000 (group of 7 trees)
Sydney University Oval no.1	126, 585-588, 593-596

5.0 Discussion

5.1 A comprehensive assessment of the impact of development upon the trees proposed for retention cannot be undertaken at this stage as detailed plans are yet to be prepared. To minimise the impact of development encroachment within the Tree Protection Zone areas of the trees proposed for retention, the detailed plans will include elements of tree sensitive

design. An Arboricultural Impact Assessment Report (Stage 2) will be prepared based on these detailed plans. The Stage 2 Report will also include a Tree Protection Specification detailing specific, tree protection measures and tree sensitive construction methods which will be utilised to minimise the impact of the works upon the trees.

- 5.2 The tree identification number and Tree Protection Zone (TPZ)/Structural Root Zone (SRZ) areas of all trees proposed for retention should be included in all relevant plans going forward. In the event that encroachment from development can be minimised to an area no greater than 10% of a TPZ and outside of the SRZ (i.e. Minor Encroachment), tree sensitive design and construction methods will not be required. However, any encroachment from development which represents an area greater than 10% of a TPZ, or within the SRZ (i.e. Major Encroachment) requires arboricultural input to inform the design in order to minimise the impact upon the tree.
- 5.3 Tree sensitive design and construction methods which should be considered when designing and building in a TPZ area include:
- Maintaining existing ground levels – even minor changes in level (+/-) need to be considered as part of the TPZ encroachment calculations due to the potential for root impacts
 - Pavements installed at existing grade need to consider the impact of excavation for the installation of slabs and sub base layers
 - Pavements, slabs and sub base layers installed above existing grade should avoid excessive compaction of the sub grade
 - Above grade pavements should be constructed on a 'no fines' sub base layer or permeable slab and utilise permeable surfacing materials
 - Structures should be designed and constructed above existing grade using isolated, piered footings
 - At the time of construction, the first 600mm in depth of each pier location should be excavated by hand. Where significant roots are identified (as determined by the Project Arborist) the design should allow for relocation of the pier
 - Construction access requirements for machinery movements and scaffolding/hoarding installation should consider the crown form of the trees. Major pruning to provide temporary access is not acceptable and the detailed design should consider how the installation of structures could impact adjacent trees
 - Smaller diameter branches may be pushed or tied back to provide construction access. However, the feasibility of doing so needs to be confirmed by the Project Arborist during the detailed design stage
 - Sediment and erosion control measures should be designed to avoid excavation of trenches and pits in TPZ areas
 - Services should be located outside of TPZ areas. Where this cannot be achieved, trenches should be excavated using tree sensitive methods (as specified by the Project Arborist) and conduits/pipework installed to avoid significant roots. Alternatively, under boring can be considered where approved by the Project Arborist

- Soft landscaping works should avoid mechanical cultivation/ripping of garden beds and lawns
 - Where an encroachment cannot be minimised through the use of tree sensitive design, root mapping should be undertaken as part of the detailed design stage. Root mapping results will determine whether root pruning can be undertaken, or redesign is required to allow for the retention of significant roots
- 5.5 The establishment of TPZ areas will be required prior to the commencement of construction works. The TPZ of each tree is listed in the Tree Assessment Schedule (Appendix A). The TPZ is a radial measurement taken from the centre of the trunk at ground level. It will not be possible to install TPZ fencing at the perimeter of each tree's TPZ area due to the requirement for construction access. TPZ fencing can be set back where appropriate ground protection is installed to the unfenced area of the TPZ. Alternatively, trunk and ground protection can be installed in lieu of fencing. Tree specific TPZ requirements should be determined through consultation between the Project Arborist and the Project Manager prior to installation.
- 5.6 TPZ areas should be regularly inspected by the Project Arborist throughout the construction stage of the project. The TPZ should remain out of bounds other than for approved development works, and should not be used for storage of waste or construction materials, vehicle parking etc. Any works within a TPZ area should be approved, supervised and documented by the Project Arborist.
- 5.7 Tree 54 *Cinnamomum camphora* (Camphor Laurel) is likely to require significant pruning as part of the proposed development works. An indicative building footprint is provided in the supplied plans, however a detailed assessment of the impact of pruning on the crown form of the tree is required during the detailed design stage. In the event that the pruning works cannot be undertaken in accordance with *Australian Standard AS4373 Pruning of Amenity Trees* (AS4373), the tree will have to be removed.
- 5.8 Trees 2001 – 2003 *Ficus macrocarpa* var. *hillii* (Hills Fig) will require Crown Lifting to provide additional vertical clearance over Grose Street. The proposed pruning works are minor in nature and can be undertaken in accordance with AS4373 (refer Appendix C – Pruning Specification). Based on the TPZ areas of the trees, the proposed additions (awning) to the carpark on the opposite side of Grose Street represent a Minor Encroachment only (i.e. <10% of the TPZ area) and should not significantly impact the trees.
- 5.9 The development proposal includes the removal of seventy-one (71) trees across the RPA East, RPA West and University campuses. The landscape proposal includes the installation of seventy-nine (79) new trees within the RPA East campus and nine (9) trees around the western edge of the University Oval no 1.
- 5.10 It should be noted that Tree 591 is located on the University side of the existing, chain link boundary fence, however the survey drawing indicates the tree is located within the RPA East Campus.
- 5.11 Within the University, Trees 595 and 596 *Ficus macrocarpa* var. *hillii* (Hills Fig) which are large, late mature specimens will need to be removed due to crown conflict with the proposed East Wing Building. The extent of pruning that would be required to accommodate the building (based on an onsite assessment) would remove a significant proportion of the

trees' crowns and the works could not be undertaken in accordance with *Australian Standard AS4373 (2007) Pruning of amenity trees*. Trees 126 *Polyscias elegans* (Celery Wood), 588 and 594 *Cinnamomum camphora* (Camphor Laurel) and 593 *Ficus macrocarpa* var. *hillii* (Hills Fig) are smaller specimens of lesser visual significance. These trees are subject to suppression from the larger adjacent trees, which has impacted their development and amenity value. Removal of the larger adjacent trees will expose the poor form of these smaller trees and may also increase the potential for branch failures due to altered wind loading.

- 5.12 Trees 590, 597 and 598 are a row of large *Cinnamomum camphora* (Camphor Laurel) which are located immediately to the east of the University trees that are proposed for removal. These trees will provide a significant screen between the proposed East Wing Building and the Oval, reducing the visual impact of tree removals to some extent. However, these trees are late mature specimens with an estimated Useful Life Expectancy (ULE) tending towards the lower end of the 15–40-year range. Therefore, a Tree Succession Strategy has been devised which forms part of the landscape proposal.
- 5.13 Assessment of Tree 596 identified decay and bark splitting in the lower trunk and buttress roots which can be symptomatic of a number of species of fungal pathogen. To better determine the ULE of the tree, tissue samples were collected for laboratory analysis at the Royal Botanic Gardens Sydney Plant Clinic. The test results have returned a positive result for *Armillaria* sp. (Honey Fungus).

Armillaria is a serious fungal pathogen causing a white rot (selective delignification) of woody tissues in the root system and lower trunk of infected trees. *Armillaria* predominately spreads through the soil and is likely to infect adjacent trees, with some species being more susceptible than others. Older, less vigorous or, physiologically stressed trees are also more susceptible to infection. The presence of this pathogen inevitably reduces the ULE of Tree 596. It is not possible to accurately predict the rate of tree decline however crown symptoms (i.e. thinning) are likely to become apparent before the trees structural integrity is significantly compromised.

Based on the trees late mature growth stage and presence of *Armillaria*, the ULE of Tree 596 is likely to be towards the lower end of the 5–15-year range. Therefore, when considering the impact of development related tree removals at the University, it should be noted that it is highly likely that Tree 596 will need to be removed in the short to medium term regardless of any development.

The presence of *Armillaria* will need to be considered as part of the tree succession strategy within the University. Ideally the stump and root crown of any trees approved for removal should be excavated rather than ground out, to remove as much woody material as possible. Dead root material in ground provides a food source for the pathogen. Any excavation of roots and stumps will need to consider minimising potential root impacts on the trees that are being retained.

- 5.14 The Tree Succession Strategy which forms part of the landscape proposal includes the installation of nine (9) advanced size new trees of various species around the Oval edge. The proposed removal of trees within the University will provide the additional space and solar access required for the establishment and development of the new trees, which will provide screening of the proposed East Wing Building. The installation of healthy new trees

will ensure the canopy cover and amenity around the Oval edge is maintained over the long term as Trees 590, 597 and 598 are late mature specimens with a relatively short ULE.

6.0 Recommendations

6.1 The following trees are proposed for removal:

<u>Location</u>	<u>Tree number</u>
RPA East campus	17-19, 22 (group of 7 trees), 23 (group of 3 trees), 24 (group of 10 trees), 25 (group of 2 trees), 31-53, 118, 128, 129, 591
RPA West campus	2000 (group of 7 trees)
Sydney University Oval no.1	126, 585-588, 593-596

In addition, Trees 2001 – 2003 are proposed for pruning. Trees approved for removal shall be identified and marked on site by the Project Arborist prior to removal. Tree removal and pruning works shall be undertaken by a qualified Arborist (minimum AQF level 3) covered by adequate third party, public liability insurance.

Pruning works shall be undertaken in accordance with *Australian Standard AS4373 Pruning of Amenity Trees*. Arborists and ground staff shall comply with the *Work Cover Code of Practice for the Amenity Tree Industry* (refer Appendix B – Pruning Specification).

Methodologies for tree stump removal within TPZ areas of retained trees shall be approved by the Project Arborist. As much of the stump, root crown and primary woody roots of Tree 596 shall be excavated to remove *Armillaria* infected material.

- 6.2 Further assessment of the proposal shall be undertaken by the Project Arborist as part of the detailed design stage to determine the potential impact of development upon the trees proposed for retention. To minimise development impacts, tree sensitive design and construction methods shall be utilised within TPZ areas (refer point 5.3).
- 6.3 Prior to the commencement of construction works TPZ areas shall be established for trees: 1-16, 20, 21, 30, 54-57, 127, 590, 597, 598, 1191, 1237-1239 and 2001 – 2003. Tree specific TPZ requirements shall be based on the Arboricultural Impact Assessment (Stage 2) and determined through consultation between the Project Arborist and the Project Manager prior to installation.
- 6.4 TPZ areas shall be maintained and regularly inspected by the Project Arborist throughout the constructing stage of the project. The TPZ shall not be used for storage of waste or construction materials, vehicle parking or any other construction related activities. The Project Arborist shall be notified prior to the undertaking of any approved development works within a TPZ area. All works within a TPZ area shall supervised and document by the Project Arborist.
- 6.5 New trees shall be grown and supplied in accordance with *AS:2303 2018 Tree stock for landscape use*. Advanced size trees shall be inspected in the nursery by the Project Arborist

to ensure the quality of plant material and compliance with *AS:2303*. Poor quality or damaged stock shall be rejected and replaced. The planting and aftercare of the trees shall be undertaken by a qualified horticulturalist (minimum AQF level 3).



Martin Peacock

BSc (hons.) Arboriculture (UK)
Higher National Diploma Arboriculture (UK)
National Diploma Horticulture (Arb.) (UK)
Diploma Horticulture (Landscape Design) (AUS)



Caveats & Limitations

The subject trees were inspected from the ground only, using the methodology detailed in this report.

The findings of this report are based on the observations made at the time of inspection and from the information contained within plans/documentation provided by the Project Manager.

The report reflects the subject trees as found at the time of inspection. There is no warranty or guarantee, expressed or implied, that problems or deficiencies with the site or the subject trees may not arise in the future. Any changes to development proposals or tree management works beyond those recommended in this report may alter the findings of the report.

References

Australian Standard: *AS 4970 - 2009 Protection of trees on development sites*. Standards Australia GPO Box 476, Sydney, NSW, 2001.

British Standard Institution (2005). *Guide for Trees in relation to construction*. BSI, 2 Park Street, London W1A 2BS.

Draper B.D. and Richards P.A. (2009), *Dictionary for Managing Trees in Urban Environments*, Institute of Australian Consulting Arboriculturists (IACA), CSIRO Publishing, Collingwood, Victoria, Australia.

Appendix A - Tree Assessment Results

Tree No.	Species	DBH (mm)	Height (m)	Radial Crown Spread (m)	Health Rating	Structural Rating	Comments	Age Class	ULE (years)	Quality & Value	Retention Category	Radial TPZ (m)	Radial SRZ (m)
<u>RPA Hospital East Campus</u>													
1	<i>Ficus microcarpa</i> var. 'Hillii' (Hills Weeping Fig)	1100	22	14	Good	Good	Small (<25mmØ) & Wound(s), various stages of decay. Retain – No works in TPZ	Mature	15-40	High	A: Priority for Retention	13.2	3.5
2	<i>Ficus microcarpa</i> var. 'Hillii' (Hills Weeping Fig)	1050	20	10	Good	Good	Small (<25mmØ) & deadwood in low volumes. Partially suppressed. Wound(s), various stages of decay. Retain – No works in TPZ	Mature	15-40	High	A: Priority for Retention	12.6	3.4
3	<i>Angophora costata</i> (Sydney Red Gum)	650	18	6	Good	Good	Partially suppressed. Retain – No works in TPZ	Mature	15-40	Moderate	A: Priority for Retention	7.8	2.8
4	<i>Flindersia australis</i> (Crow's Ash)	550	16	5	Good	Good	Partially suppressed. Wound(s), early signs of decay. Retain – No works in TPZ	Mature	15-40	Moderate	A: Priority for Retention	6.6	2.6

5	<i>Flindersia australis</i> (Crow's Ash)	550	16	5	Good	Good	Partially suppressed. Wound(s), early signs of decay. Retain – No works in TPZ	Mature	15-40	Moderate	A: Priority for Retention	6.6	2.6
6	<i>Flindersia australis</i> (Crow's Ash)	450	19	9	Fair	Good	Crown density 50- 75%. Small (<25mmØ) deadwood in low volumes. Partially suppressed. Retain – No works in TPZ	Mature	15-40	Moderate	A: Priority for Retention	5.4	2.4
7	<i>Ficus microcarpa</i> var. 'Hillii' (Hills Weeping Fig)	800	20	12	Good	Good	Small (<25mmØ) & medium (25-75mmØ) deadwood in low volumes. Partially suppressed. Wound(s), various stages of decay. Retain – No works in TPZ	Mature	15-40	High	A: Priority for Retention	9.6	3.1
8	<i>Stenocarpus</i> <i>sinuatus</i> (Queensland Firewheel Tree)	250	7	3	Good	Good	Partially suppressed. Wound(s), early signs of decay. Retain – Major Encroachment pavement	Mature	15-40	Low	B: Consider for Retention	3.0	1.9

9	<i>Ficus microcarpa</i> var. 'Hillii' (Hills Weeping Fig)	1100	22	14	Good	Good	Small (<25mmØ) deadwood in low volumes. Partially suppressed. Wound(s), various stages of decay. Retain – Major Encroachment pavement	Mature	15-40	High	A: Priority for Retention	13.2	3.5
10	<i>Flindersia australis</i> (Crow's Ash)	475	19	6	Good	Good	Crown density 75- 95%. Partially suppressed. Retain – Major Encroachment pavement	Mature	15-40	Moderate	A: Priority for Retention	5.7	2.5
11	<i>Flindersia australis</i> (Crow's Ash)	375	16	5	Good	Good	Small (<25mmØ) deadwood in low volumes. Partially suppressed. Wound(s), early signs of decay. Retain – Major Encroachment pavement	Mature	15-40	Moderate	A: Priority for Retention	4.5	2.2
12	<i>Flindersia australis</i> (Crow's Ash)	500	19	6	Fair	Good	Crown density 50- 75%. Small (<25mmØ) deadwood in low volumes. Small (<25mmØ) epicormic growth in low volumes. Partially suppressed. Retain – Major Encroachment pavement	Mature	15-40	Moderate	A: Priority for Retention	6.0	2.5

13	<i>Flindersia australis</i> (Crow's Ash)	500	19	5	Good	Good	Small (<25mmØ) deadwood in low volumes. Partially suppressed. Wound(s), early signs of decay. Retain – Major Encroachment pavement	Mature	15-40	Moderate	A: Priority for Retention	6.0	2.5
14	<i>Ficus microcarpa</i> var. 'Hillii' (Hills Weeping Fig)	1000	21	14	Good	Good	Small (<25mmØ) deadwood in low volumes. Partially suppressed. Wound(s), various stages of decay. Retain – Major Encroachment pavement	Mature	15-40	High	A: Priority for Retention	12.0	3.3
15	<i>Ficus microcarpa</i> var. 'Hillii' (Hills Weeping Fig)	1000	22	12	Good	Good	Small (<25mmØ) deadwood in low volumes. Partially suppressed. Failed inclusion. Wound(s), various stages of decay. Retain – Major Encroachment pavement	Mature	15-40	High	A: Priority for Retention	12.0	3.3
16	<i>Flindersia australis</i> (Crow's Ash)	500	16	5	Good	Good	Heavily suppressed. Retain – Major Encroachment pavement	Mature	15-40	Moderate	A: Priority for Retention	6.0	2.5

17	<i>Syagrus romanzoffianum</i> (Cocos Palm)	150	4	2	Good	Good	Self-sown weed species Remove – road alignment	Semi mature	<5	Low	C: Consider for Removal	3.0	n/a
18	<i>Lophostemon confertus</i> (Brush Box)	500	8	5	Fair	Good	Crown density 75-95%. Small (<25mmØ) deadwood in low volumes. Partially suppressed. Remove – road alignment	Mature	15-40	Moderate	B: Consider for Retention	6.0	2.5
19	<i>Magnolia grandiflora</i> (Bull Bay Magnolia)	450	8	5	Fair	Fair	Crown density 50-75%. Small (<25mmØ) deadwood in low volumes. Partially suppressed. Wound(s), early signs of decay. Remove – road alignment	Mature	15-40	Moderate	B: Consider for Retention	5.4	2.4
20	<i>Brachychiton acerifolius</i> (Illawarra Flame Tree)	350	7	3	Good	Good	Small (<25mmØ) deadwood in low volumes. Retain – no works in TPZ	Mature	15-40	Moderate	B: Consider for Retention	4.2	2.1

21	<i>Jacaranda mimosifolia</i> (Jacaranda)	300	6	5	Good	Good	Retain – no works in TPZ	Mature	15-40	Moderate	B: Consider for Retention	3.6	2.0
22	<i>Corymbia maculata/citriodora</i> (Spotted Gum)	400 max.	16 max.	4 max.	Good	Good	Group of 8 trees. Partially suppressed. Remove – pavement	Early mature	15-40	Low	C: Consider for Removal	4.8	2.3
23	<i>Corymbia citriodora</i> (Lemon Scented Gum)	300 max.	12 max.	4 max.	Good	Good	Group of 3 trees. Partially suppressed. Remove – pavement	Early mature	15-40	Low	C: Consider for Removal	3.6	2.0
24	<i>Corymbia citriodora</i> (Lemon Scented Gum)	300 max.	12 max.	4 max.	Good	Good	Group of 10 trees. Partially suppressed. Remove – pavement	Early mature	15-40	Low	C: Consider for Removal	3.6	2.0
25	<i>Pyrus sp.</i> (Ornamental Pear)	125	6 max.	3 max.	Good	Good	Group of 2 trees. Remove – pavement	Semi Mature	15-40	Low	C: Consider for Removal	2.0	1.5

30	<i>Cinnamomum camphora</i> (Camphor Laurel)	1200	19	9	Good	Good	Small (<25mmø) & medium (25-75mmø) deadwood in low volumes. Retain – Major Encroachment pavement & landscape features	Late Mature	15-40	High	A: Priority for Retention	14.4	3.7
31	<i>Cinnamomum camphora</i> (Camphor Laurel)	1500	26	9	Good	Good	Small (<25mmø), medium (25-75mmø) deadwood in low volumes. Remove – Major Encroachment building footprint	Late Mature	15-40	High	A: Priority for Retention	15.0	4.1
32	<i>Livistonia australis</i> (Cabbage Tree Palm)	300	7	3	Good	Good	Partially suppressed. Remove – Tree Succession Strategy	Mature	15-40	Low	C: Consider for Removal	4.0	n/a
33	<i>Plumeria acutifolia</i> (Frangipani)	375	6	3	Good	Good	Partially suppressed. Wound(s), early signs of decay. Remove – building footprint	Mature	15-40	Low	C: Consider for Removal	4.5	2.3
34	<i>Cinnamomum camphora</i> (Camphor Laurel)	400	10	5	Good	Good	Partially suppressed. Remove – Major Encroachment building footprint	Mature	15-40	Moderate	B: Consider for Retention	4.8	2.3

35	<i>Celtis sinensis</i> (Chinese Nettle Tree)	500	12	5	Good	Fair	Partially suppressed. Co-dominant inclusions, major. Remove – Major Encroachment building footprint	Mature	15-40	Low	C: Consider for Removal	6.0	2.6
36	<i>Acmena smithii</i> 'Minor' (Dwarf Lilly Pilly)	200	6	3	Good	Good	Crown density 50-75%. Partially suppressed. Remove – building footprint	Mature	15-40	Low	C: Consider for Removal	2.4	1.8
37	<i>Cinnamomum camphora</i> (Camphor Laurel)	1800	26	10	Good	Good	Small (<25mmø), medium (25-75mmø) & large (>75mmø) deadwood in low volumes. Remove – building footprint	Late Mature	15-40	High	A: Priority for Retention	15.0	4.4
38	<i>Persea americana</i> (Avocado)	150	5	2	Good	Good	Partially suppressed. Remove – building footprint	Semi-mature	15-40	Low	C: Consider for Removal	2.0	1.5
39	<i>Cinnamomum camphora</i> (Camphor Laurel)	1100	23	11	Good	Good	Small (<25mmø), medium (25-75mmø) & large (>75mmø) deadwood in low volumes. Partially suppressed. Remove – building footprint	Late Mature	15-40	High	A: Priority for Retention	13.4	3.6

40	<i>Cinnamomum camphora</i> (Camphor Laurel)	1600	25	14	Good	Good	Small (<25mmø), medium (25-75mmø) & large (>75mmø) deadwood in low volumes. Remove – building footprint	Late Mature	15-40	High	A: Priority for Retention	15.0	4.2
41	<i>Ficus microcarpa</i> var. 'Hillii' (Hills Weeping Fig)	1250	24	10	Good	Good	Small (<25mmø), medium (25-75mmø) Wound(s), various stages of decay. Remove – Major Encroachment building footprint	Mature	15-40	High	A: Priority for Retention	15.0	3.8
42	<i>Jacaranda mimosifolia</i> (Jacaranda)	950	16	11	Good	Good	Small (<25mmø), medium (25-75mmø) & large (>75mmø) epicormic growth in low volumes. Partially suppressed. Wound(s), various stages of decay. Remove – building footprint	Mature	15-40	High	B: Consider for Retention	11.4	3.4
43	<i>Melia azedarach</i> (White Cedar)	125 175 200	8	4	Good	Good	Co-dominant inclusions, major. Remove – Major Encroachment building footprint	Early mature	5-15	Low	C: Consider for Removal	3.6	2.0
44	<i>Magnolia grandiflora</i> (Bull Bay Magnolia)	125	5	2	Good	Good	Remove – road	Mature	40+	Low	C: Consider for Removal	2.0	1.5

45	<i>Camellia sasanqua</i> (Camellia)	500	5	3	Good	Good	Wound(s), various stages of decay. Trunk cavity(s), minor. Remove – building footprint	Late Mature	15-40	Low	C: Consider for Removal	6.0	2.6
46	<i>Camellia sasanqua</i> (Camellia)	300	5	3	Good	Fair	Co-dominant inclusions, minor. Wound(s), various stages of decay. Remove – building footprint	Mature	15-40	Low	C: Consider for Removal	3.6	2.1
47	<i>Jacaranda mimosifolia</i> (Jacaranda)	800	15	10	Good	Good	Partially suppressed. Wound(s), various stages of decay. First order branch cavity, minor. Remove – building footprint	Mature	15-40	Moderate	B: Consider for Retention	9.6	3.1
48	<i>Jacaranda mimosifolia</i> (Jacaranda)	750	18	12	Good	Good	Partially suppressed. Wound(s), various stages of decay. Phototropic lean, moderate. Remove – building footprint	Mature	15-40	Moderate	B: Consider for Retention	9.0	3.1
49	<i>Flindersia australis</i> (Crow's Ash)	1000	25	7	Good	Good	Remove – building footprint	Mature	15-40	High	A: Priority for Retention	12.0	3.4
50	<i>Platanus x acerifolia</i> (London Plane Tree)	1300	27	10	Good	Good	Small (<25mmØ) & large (>75mmØ) deadwood in low volumes. Remove – road	Mature	15-40	High	A: Priority for Retention	15.0	3.8

51	<i>Corymbia citriodora</i> (Lemon Scented Gum)	800	22	9	Good	Good	Wound(s), various stages of decay. Remove – road	Mature	15-40	Moderate	B: Consider for Retention	9.6	3.1
52	<i>Cinnamomum camphora</i> (Camphor Laurel)	2000	20	8	Good	Fair	Wound(s), various stages of decay. Trunk cavity(s), major. Remove – road	Late Mature	15-40	High	A: Priority for Retention	15.0	4.6
53	<i>Cedrus sp.</i> (Cedar species)	800	22	6	Good	Good	Partially suppressed. Co-dominant inclusions, minor. Wound(s), no visible sign of decay. Remove – road	Mature	15-40	High	A: Priority for Retention	9.6	3.1
54	<i>Cinnamomum camphora</i> (Camphor Laurel)	2000	22	11	Good	Good	Wound(s), various stages of decay. Retain – Major Encroachment building footprint (Further analysis of development impacts required based on detailed plans)	Late Mature	15-40	High	A: Priority for Retention	15.0	4.6

55	<i>Jacaranda mimosifolia</i> (Jacaranda)	750	16	9	Good	Good	Small (<25mmØ) & medium (25-75mmØ) deadwood in low volumes. Retain – Major Encroachment pavement & landscape	Mature	15-40	High	A: Priority for Retention	9.0	3.1
56	<i>Waterhousia floribunda</i> (Weeping Lillypilly)	225	9	3	Good	Good	Partially suppressed. Retain – no works in TPZ	Mature	15-40	Low	C: Consider for Removal	2.7	1.8
57	<i>Harpullia pendula</i> (Tulipwood)	250	9	4	Good	Fair	Partially suppressed. Co-dominant inclusions, minor. Retain – no works in TPZ	Mature	15-40	Moderate	B: Consider for Retention	3.0	1.9
118	<i>Livistonia australis</i> (Cabbage Tree Palm)	300	6	2	Good	Good	Partially suppressed. Remove – building footprint	Mature	15-40	Low	C: Consider for Removal	3.0	n/a
127	<i>Camellia japonica</i> (Japanese Camelia)	150	2	2	Good	Good	Retain – no works in TPZ	Mature	15-40	Low	C: Consider for Removal	2.2	1.5
128	<i>Camellia japonica</i> (Japanese Camelia)	150	2	2	Good	Good	Remove – road	Mature	15-40	Low	C: Consider for Removal	2.2	1.5
129	<i>Polyscias elegans</i> (Celery Wood)	125	7	2	Good	Good	Partially suppressed Remove – building footprint	Semi Mature	15-40	Low	C: Consider for Removal	2.0	1.5

130	<i>Melia azedarach</i> (White Cedar)	250 175 125	8	4	Good	Fair	Co-dominant inclusions, major. Remove – building footprint	Early mature	5-15	Low	C: Consider for Removal	3.6	2.0
131	<i>Melia azedarach</i> (White Cedar)	200	7	3	Good	Good	Bark inclusion(s), minor. Remove – building footprint	Early mature	15-40	Low	C: Consider for Removal	2.4	1.7
591	<i>Cinnamomum camphora</i> (Camphor Laurel)	1100	24	8	Good	Good	Remove - crown conflict with proposed building (Survey confirms tree is within RPA)	Late mature	15-40	High	A: Priority for Retention	12.0	3.4
RPA Hospital West Campus													
2000	<i>Populus simonii</i> (Simons Poplar)	275 max.	11 max.	3 max.	Good/Fair	Good/Fair	Group of 7 trees. Small diameter ($<25\text{mm}\varnothing$) epicormic growth in low volumes. Remove – awning	Early mature	15-40 / 5-15	Low	C: Consider for Removal	3.3	1.9
2001	<i>Ficus macrocarpa</i> <i>var. hillii</i> (Hills Fig)	750 600 200	17	4	Good	Good	Retain – Minor Encroachment awning Crown Lift for road clearance	Late mature	15-40	High	A: Priority for Retention	12.0	3.3
2002	<i>Ficus macrocarpa</i> <i>var. hillii</i> (Hills Fig)	800	16	11	Good	Good	Retain – Minor Encroachment awning Crown Lift for road clearance	Late mature	15-40	High	A: Priority for Retention	9.6	3.0

2003	<i>Ficus macrocarpa</i> <i>var. hillii</i> (Hills Fig)	900	16	10	Good	Good	Retain – Minor Encroachment awning Crown Lift for road clearance	Late mature	15-40	High	A: Priority for Retention	10.8	3.2
<u>University of Sydney Campus</u>													
126	<i>Polyscias elegans</i> (Celery Wood)	75	5	1	Good	Good	University tree. Partially suppressed. Remove – Tree Succession Strategy	Semi Mature	15-40	Low	C: Consider for Removal	2.0	1.5
585	<i>Cupressus sp.</i> (Cypress species)	400	12	3	Fair	Good	University tree. Partially suppressed. Crown density 75- 95%) Remove – infrastructure works	Late mature	15-40	Moderate	B: Consider for Retention	4.8	2.3
586	<i>Cupressus sp.</i> (Cypress species)	250	12	3	Fair	Good	University tree. Partially suppressed. Crown density 75- 95%) Remove – infrastructure works	Late mature	15-40	Moderate	B: Consider for Retention	3.0	1.9
587	<i>Cupressus sp.</i> (Cypress species)	400	12	3	Fair	Good	University tree. Partially suppressed. Crown density 75- 95%) Remove – infrastructure works	Late mature	15-40	Moderate	B: Consider for Retention	4.8	2.3

588	<i>Cinnamomum camphora</i> (Camphor Laurel)	500	20	7	Fair	Good	University tree. Partially suppressed with poor form. Remove – Tree Succession Strategy	Mature	15-40	Moderate	B: Consider for Retention	6.0	2.5
590	<i>Cinnamomum camphora</i> (Camphor Laurel)	1600	25	9	Good	Good	University tree. Retain – Minor Encroachment building	Late mature	15-40	High	A: Priority for Retention	15.0	4.1
593	<i>Ficus microcarpa</i> var. <i>Hillii</i> (Hills Fig)	700	18	6	Fair	Good	University tree. Heavily suppressed with poor form. Crown density 50- 75% Remove – Tree Succession Strategy	Mature	15-40	Moderate	B: Consider for Retention	8.4	2.9
594	<i>Cinnamomum camphora</i> (Camphor Laurel)	300 275	15	5	Fair	Fair	University tree. Heavily suppressed. etiolated form. Remove – Tree Succession Strategy	Mature	5-15	Low	C: Consider for Removal	4.8	2.3
595	<i>Ficus microcarpa</i> var. <i>Hillii</i> (Hills Fig)	1500	24	10	Good	Good	University tree. Partially suppressed. Remove - crown conflict with proposed building	Late mature	15-40	High	A: Priority for Retention	15.0	4.0

596	<i>Ficus microcarpa</i> var. <i>Hillii</i> (Hills Fig)	1800	24	10	Good	Poor	University tree. Basal decay. Partially suppressed. Pathology testing confirms tree infected by Armillaria Remove - crown conflict with proposed building	Late mature	5-15	High	C: Consider for Removal	15.0	4.3
597	<i>Cinnamomum camphora</i> (Camphor Laurel)	1100	25	9	Good	Good	University tree. Partially suppressed. Retain – Minor Encroachment building	Late mature	15-40	High	A: Priority for Retention	13.2	3.5
598	<i>Cinnamomum camphora</i> (Camphor Laurel)	1100	25	9	Good	Good	University tree. Partially suppressed. Retain – Minor Encroachment building	Late mature	15-40	High	A: Priority for Retention	13.2	3.5
1191	<i>Cupaniopsis anacardioides</i> (Tuckeroo)	125	7	4	Good	Good	University tree. Retain – no works in TPZ	Early mature	15-40	Moderate	B: Consider for Retention	2.1	1.5
1237	<i>Cupaniopsis anacardioides</i> (Tuckeroo)	200	7	4	Good	Good	University tree. Retain – no works in TPZ	Early mature	15-40	Moderate	B: Consider for Retention	2.4	1.7
1238	<i>Cupaniopsis anacardioides</i> (Tuckeroo)	275	7	4	Good	Good	University tree. Retain – no works in TPZ	Early mature	15-40	Moderate	B: Consider for Retention	3.3	2.0

1239	<i>Cupaniopsis anacardioides</i> (Tuckeroo)	275	7	4	Good	Good	University tree. Retain – no works in TPZ	Early mature	15-40	Moderate	B: Consider for Retention	3.3	2.0
------	--	-----	---	---	------	------	---	--------------	-------	----------	------------------------------	-----	-----

For Tree Locations Refer: Turf Design Studio – Tree Strategy/Tree Management Plan (L.DA.35), dated 02.11.22

Summary of Trees & retention Values

		Retention Value			Total
		Low	Medium	High	
Existing	East Campus	40	11	28	79
	West Campus	7	-	3	10
	USYD	2	9	5	16
	Sub-total	49	19	28	105
Retain	East Campus	2	4	18	24
	West Campus	-	-	3	3
	USYD	-	4	3	7
	Sub-total	2	7	16	34
Remove	East Campus	39	6	10	55
	West Campus	7	-	-	7
	USYD	2	5	2	9
	Sub-total	48	11	12	71

Appendix B – Pruning Specification




Appendix C – Pruning Specification



Appendix C – Pathology Trest Results – T596

PlantClinic

THE VITAL SCIENCE AT WORK



PlantClinic Report

Client	martin peacock tree care
Attention	martin peacock
Reference	23.069, UID1016085205
Client Reference	USYD T596
Test(s)	Wood Decay Fungi Detection (WDF) service
Sample Received	

Table of Contents

Introduction	1
Summary of Results	3
Discussion	4
Appendix 1 – Client Submitted Data	5
Appendix 2 – Samples Received	6
Appendix 3 – Methodologies	7
Appendix 4 – References	9
Our Team	10



Introduction

The PlantClinic received specimens on the for our Wood Decay Fungi Detection (WDF) service. This service is used for the detection and identification of the major genera of wood decay fungi from wood tissue or fungal mycelium. This test cannot be used for soil samples. 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.

For further details on the samples submitted and the testing methodologies please refer to Appendices 1 -3. A summary of the results is presented in the Summary of Results section of this report.



Summary of Results

PlantClinic Ref.	Sample Type	Client Ref.	Service	Results
23.069		USYD T596	Wood Decay Fungi Detection (WDF)	Armillaria



Appendix 3 – Methodologies

Phytophthora & Pythium Service

The PlantClinic offers a diagnostic service 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.

The soil and/or plant sample is mixed with deionised water to form a soil or plant/water mixture and baited for *Phytophthora* using New Zealand blue lupin seedlings (*Lupinus angustifolius*)¹. The baited soil/water mixture is incubated at room temperature for 7 days. Total DNA is extracted from the blue lupin radicles using a modified version of the Macheray-Nagel (GmbH & Co. KG) NucleoMag Plant kit. *Phytophthora* detection is based on a *Phytophthora* genus specific Taqman™ assay^{2,3} 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 Oomycetes⁴. PCR amplicons are sent to the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales where DNA sequences are determined using an ABI PRISM® 3700 DNA Analyser (Applied Biosystems Inc.).

The Basic Local Alignment Search Tool (BLAST) algorithm is used to match DNA sequences to species⁵. This algorithm compares the similarity of the sample DNA sequence with all samples deposited in publicly available and in-house data bases. The BLAST then returns a list of species that are most similar to the sample DNA based on sequence identity (similarity) and assigns a probability value (E) that gives an indication of the reliability of the match. Because of this, BLAST usually returns multiple species, and we include only the top three matches in our report.

Wood Decay Fungal Detection Service

The PlantClinic offers a diagnostic test for the major genera of wood decay fungi. Total DNA is extracted from the decayed/diseased wood tissue using a modified version of the Macheray-Nagel (GmbH & Co. KG) NucleoMag Plant kit. Detection of wood decay fungi is based on a multiplex PCR that can detect the genera *Armillaria*, *Phellinus* and *Ganoderma*^{6,7} from community DNA. This method cannot be used to identify species in these genera. Species identification and detection of other fungal genera is attempted using fungal specific ITS primers^{8,9} followed by Sanger sequencing using the analysis methods detailed in the Phytophthora & Pythium Service above.

Basic Plant Identification Service

The PlantClinic offers a plant identification service from their roots and other tissues to genus and species level. Total DNA is extracted from the plant specimen(s) using a modified version of the Macheray-Nagel (GmbH & Co. KG) NucleoMag Plant kit. The ribosomal DNA, internal transcribed spacer 1 and 2 (ITS), maturase K (matK) gene and the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene regions are amplified using PCR primers and amplification conditions described in CBOL Plant Working Group¹⁰, Ford et al. 2009¹¹, Hollingsworth et al. 2009¹² and Singh et al. 2012¹³. PCR amplicons are sent to the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales where DNA sequences are determined using an ABI PRISM® 3700 DNA Analyser (Applied Biosystems Inc.).

The Basic Local Alignment Search Tool (BLAST) algorithm is used to match DNA sequences to species⁵. This algorithm compares the similarity of the sample DNA sequence with all samples deposited in publicly available and in-house data bases. The BLAST then returns a list of species that are most similar to the sample DNA based on sequence identity (similarity) and assigns a probability value (E) that gives an indication of the reliability of the match. Because of this, BLAST usually returns multiple species, and we include only the top three matches in our report. This 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 this is required, please select the 'High Resolution Fungal & Plant Identification' service.

Plant Pathogen Detection & Basic Identification Service

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 and published barcodes followed by a BLAST analysis that matches the pathogen's sequences to references in publicly available databases.

Community and fungal isolate DNA is extracted using a modified version of the Macheray-Nagel (GmbH & Co. KG) NucleoMag Plant kit. PCR amplicons are sent to the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales where DNA sequences are determined using an ABI PRISM® 3700 DNA Analyser (Applied Biosystems Inc.).

The Basic Local Alignment Search Tool (BLAST) algorithm is used to match DNA sequences to species⁵. This algorithm compares the similarity of the sample DNA sequence with all samples deposited in publicly available and in-house data bases. The BLAST then returns a list of species that are most similar to the sample DNA based on sequence identity (similarity) and assigns a probability value (E) that gives an indication of the reliability of the match. Because of this, BLAST usually returns multiple species, and we include only the top three matches in our 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' service.

Plant, Fungal & Insect 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.

DNA is extracted from plant & fungal tissues using a modified version of the Macheray-Nagel (GmbH & Co. KG) NucleoMag Plant kit. Insect DNA is extracted using prepGEM® Insect Kit. PCR amplicons are sent to the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales where DNA sequences are determined using an ABI PRISM® 3700 DNA Analyser (Applied Biosystems Inc.). 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 PlantClinic Service Information document. Other loci can be sequenced on request.

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.

DNA is extracted from plant & fungal tissues using a modified version of the Macheray-Nagel (GmbH & Co. KG) NucleoMag Plant kit. Commonly used plant and fungal barcodes for used for the initial identification using the Basic Local Alignment Search Tool (BLAST) algorithm to match DNA sequences to species⁵. The initial identification results are used to guide a literature search for the most appropriate loci for phylogenetic analyses and additional loci are sequenced, if required. PCR amplicons are sent to the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales where DNA sequences are determined using an ABI PRISM® 3700 DNA Analyser (Applied Biosystems Inc.) in both the 5' & 3' directions.

Sequences are aligned for each taxon using the multiple alignment program MAFFT plug-in in the software Geneious¹⁴ with reference taxa selected from the literature and BLAST analysis. Alignments are edited manually using the sequence alignment editing program Geneious and all polymorphisms confirmed by re-examining the electropherograms. Phylogenetic trees are generated under the maximum likelihood criterion using the software RAxML (version 8)¹⁵ plug-in in the software Geneious¹⁴. Resulting phylogenetic trees were visualised and edited using FigTree v1.4¹⁶. Clade stability is assessed by Bayesian inference in MrBayes¹⁷ plug-in in the software Geneious¹⁴ to generate posterior probabilities (PP) for consensus nodes. The MrBayes¹⁷ Monte Carlo Markov Chain is run with 5,000,000 generations, with a subsampling frequency of 1,000 generations and a burn-in length of 1,250 trees.

PlantClinic



Appendix 4 – References

1. Pratt, B. H. & Heather, W. A. Method for rapid differentiation of *Phytophthora cinnamomi* from other *Phytophthora* species isolated from soil by lupin baiting. *Trans. Br. Mycol. Soc.* 59, 87-112 (1972).
2. Miles, T. D., Martin, F. N., Robideau, G. P., Bilodeau, G. J. & Coffey, M. D. Systematic Development of *Phytophthora* Species-Specific Mitochondrial Diagnostic Markers for Economically Important Members of the Genus. *Plant Dis.* 101, 1162–1170 (2017).
3. Bilodeau, G. J., Martin, F. N., Coffey, M. D. & Blomquist, C. L. Development of a multiplex assay for genus- and species-specific detection of *Phytophthora* based on differences in mitochondrial gene order. *Phytopathology* 104, 733–748 (2014).
4. Nikolcheva, L. G. & Bärlocher, F. Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream.
5. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410 (1990).
6. Guglielmo, F., Gonthier, P., Garbelotto, M. & Nicolotti, G. Optimization of sampling procedures for DNA-based diagnosis of wood decay fungi in standing trees. *Lett. Appl. Microbiol.* 51, 90–97 (2010).
7. Guglielmo, F., Bergemann, S. E., Gonthier, P., Nicolotti, G. & Garbelotto, M. A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. *J. Appl. Microbiol.* 103, 1490–1507 (2007).
8. Gardes, M. & Bruns, T. D. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2, 113–118 (1993).
9. Manter, D. K. & Vivanco, J. M. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. *J. Microbiol. Methods* 71, 7–14 (2007).
10. CBOL Plant Working Group *et al.* A DNA barcode for land plants. *Proc. Natl. Acad. Sci. U. S. A.* 106, 12794–12797 (2009).
11. Ford, C. S. *et al.* Selection of candidate coding DNA barcoding regions for use on land plants. *Bot. J. Linn. Soc.* 159, 1–11 (2009).
12. Hollingsworth, M. L. *et al.* Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. *Mol. Ecol. Resour.* 9, 439–457 (2009).
13. Singh, H., Parveen, I., Raghuvanshi, S. & Babbar, S. B. The loci recommended as universal barcodes for plants on the basis of floristic studies may not work with congeneric species as exemplified by DNA barcoding of *Dendrobium* species. *BMC Research Notes* vol. 5 42 (2012).
14. Kearse, M. *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649 (2012).
15. Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313 (2014).
16. Rambaut, A. FigTree v1. 4. (2012).
17. Huelsenbeck, J. P. & Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755 (2001).

Appendix D - Methodology

Data Collection:

The methodology used in this report follows the procedures detailed in *Australian Standard: AS 4970—2009. Protection of Trees on Development Sites*.

The methodology used in this report provides the following information:

1. Tree species - botanical and common name.
2. Age class - Juvenile, semi-mature, mature, senescent.
3. DBH – Diameter at breast height (mm)*
4. Height – estimated total height (m)
5. Crown spread – estimated, average radial crown spread in meters (m)
6. Physiological condition - good, fair, poor
7. Structural condition - good, fair, poor
8. Useful Life Expectancy - <5, 5–15, 15–40, >40 (years)**
9. Quality & Value – A, B, C, D ***
10. Retention Category - Priority for Retention, Consider for Retention, Consider for Removal, Priority for Removal****
11. SRZ – Structural Root Zone radius (m)
12. TPZ – Tree protection Zone radius (m)
13. Comments / Preliminary Management Recommendations

*DBH (Diameter at Breast Height) - Stem /trunk diameter measured at 1.4m above ground level. On sloping ground, measurements will be taken at the mid slope point at the base of the tree. Where a tree stem / trunk begins to branch at a point that is less than 1.4m above ground, a combined stem diameter is calculated using the formula:

$$\text{Total DBH} = \sqrt{\text{DBH}_1^2 + \text{DBH}_2^2 + \text{DBH}_3^2}$$

**Useful Life Expectancy – The estimated lifespan of the tree over which it will positively contribute to the amenity of the area and to the local environment, in a safe, healthy condition.

***Quality & Value – The quality of the tree when compared to an idealised example of the species and the values which the tree provides to the site and local area (see Cascade Chart for Assessment of Tree Quality & Value).

****Retention Category – The subject tree is allocated one of four categories based on a combination of its Quality and Value and Useful Life Expectancy. A certain amount of flexibility may be allowed when allocating a Retention Category, to take into account tree species, significance and site/environmental conditions.

An assessment of the trees condition is made using the Visual Tree Assessment (VTA) method (Mattheck & Breoler, 1994).

Tree assessment results are recorded in the Tree Assessment Schedule (see section 6.0 Results). Note: for trees outside of the site only the species and DBH is recorded for the purposes of calculating the SRZ/TPZ.

This report also references element of the *British Standard BS: 5837 (2005) Trees in Relation to Construction – Recommendations*.

Cascade Chart for Assessment of Tree Quality & Value

(Adapted from British Standard Institution (2005). Guide for Trees in Relation to Construction)

RETENTION CATEGORY & DEFINITION	CRITERIA - SUBCATEGORIES		
	1. Mainly Arboricultural values	2. Mainly landscape values	3. Mainly cultural values, including conservation
Category A High Quality & Value: Those in such a condition as to be able to make a substantial contribution for a minimum of 40 years. Highly significant trees or trees listed on a significant tree register regardless of life expectancy (excluding hazardous trees). Priority for retention.	Trees that are particularly good examples of their species, especially if rare or unusual or essential components of groups or of formal or semi-formal Arboricultural features (e.g. The dominant and / or principal trees within an avenue). Trees that provide a definite contribution to the amenity of the locality.	Trees, groups or woodlands which provide a definite screening or softening effect to the locality in relation to views into or out of the site, or those of particular visual importance (e.g. Avenues or other Arboricultural features assessed as groups).	Trees, groups, remnant bushland or forest of significant conservation, historical, Aboriginal, commemorative or other value. Note: independent ecological/aboriginal/heritage assessment may be required.
Category B Moderate Quality & Value: Those in such a condition as to make a significant contribution for a minimum of 15 years. Consider for retention.	Trees that might be included in the high category, but are downgraded because of impaired condition (e.g. presence of remediable defects including unsympathetic past management and minor storm damage).	Trees situated mainly internally to the site, therefore individually having little visual impact on the wider locality or, trees present in numbers, usually as groups or woodlands, such that they from distinct landscape features, thereby attracting higher collective rating than they might as individuals but which are not, individually essential components of formal or semi formal Arboricultural features (e.g. trees or moderate quality within an avenue that includes better A category specimens).	Trees with clearly identifiable conservation or other cultural benefits.
Category C Low Quality & Value: Those in such a condition as to make a contribution for a minimum of 5 years. Consider for removal.	Trees not qualifying in higher categories. Juvenile, semi mature or small tree species which are considered easily replaceable.	Trees present in groups or woodlands, but without this conferring on them significantly greater landscape value, and / or trees offering low or only temporary screening benefit.	Trees with very limited conservation or other cultural benefits.
Category D Not worthy of retention: Those in such a condition that any existing value would be lost within 5 years and which should in current context, be removed for reasons of sound Arboricultural management. Priority for removal.	Trees that have a serious, irremediable structural defect, such that their early loss is expected due to failure, including those that will become unviable after removal of other trees (i.e. where, for whatever reason the loss of companion shelter cannot be mitigated by pruning). Trees that are dead or are showing signs of significant, immediate and irreversible overall decline Trees infected with a pathogen of significance to the health and/or safety of other trees nearby, or very low-quality trees suppressing adjacent trees of better quality. Trees causing significant damage to structures, where no viable alternatives exist for remedial tree management / modification of structures to enable tree retention. Trees considered a weed species or those listed as noxious weeds. NOTE: Dead or dying trees with hollows or cavities may be of ecological importance. These trees are to be identified and assessed independently of the criteria in this cascade chart. Where category D trees are removed habitat reinstatement may be appropriate.		