Western Australian Surveillance Plan

for *Austropuccinia psidii* (Myrtle Rust)

Document control information

Version	Document author	Document reviewed by	Submission date
Draft	Mariana Campos	Simon McKirdy, Murdoch University	08 November 2021
V0	Mariana Campos Shane Tobe	Simon McKirdy, Murdoch University	17 December 2021
V1	Mariana Campos	Laura Fagan, DPIRD Michael Robinson, APBSF Justin Bellanger, South Coast NRM	29 April 2022

This document should be cited as:

Campos M, Tobe S. 2022. Western Australian Surveillance Plan for *Austropuccinia psidii* (myrtle rust) V1. Document prepared for the Department of Primary Industries and Regional Development, Government of Western Australia by Murdoch University. 47 pages.

Acknowledgements:

This work was funded by the Australian Plant Biosecurity Science Foundation (PBSF-035). We thank them for their support to this research, and to their CEO Michael Robinson for reviewing the work. We also thank Simon McKirdy, Laura Fagan and Justin Bellanger for their reviews, and Emer O'Gara, Darryl Hardie, Nicole Hammond, Sonya Broughton, Justin Bellanger, Geoff Pegg, Francisco Tovar, Kylie Ireland, Gavin Hunter, and Tracey Steinrucken for engagement throughout the project and hopefully beyond it.

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1. Situation

At the time of submission of this Plan (April 2022), no incidences of *Austropuccinia psidii* have been recorded in Western Australia.

Austropuccinia psidii (G. Winter) Beenken (syn. *Bullaria psidii, Dicaeoma psidii, Puccinia psidii, Uredo rangelii*), commonly known as myrtle rust (also guava rust, eucalyptus rust and 'ohi'a rust) is a rust fungus with a broad range of hosts in the Myrtaceae family that often affects new growth.

To date, 524 hosts have been identified (Soewarto et al. 2019), with no apparent association between the susceptibility of hosts and the phylogenetic relatedness of taxa (Morin et al. 2012), although species susceptibility has been documented to increase with increased inoculum pressure (Ireland & Pegg 2020). Further, different races of *A. psidii* have different levels of aggression (Almeida et al. 2021).

Myrtle rust was first described as *Puccinia psidii* in southern Brazil (Almeida et al. 2021) but is assumed to be endemic to neighbouring countries (CABI 2021). It was reported in Florida in 1977 and Hawaii in 2005, spreading quickly to other countries thereafter (Figure 1).

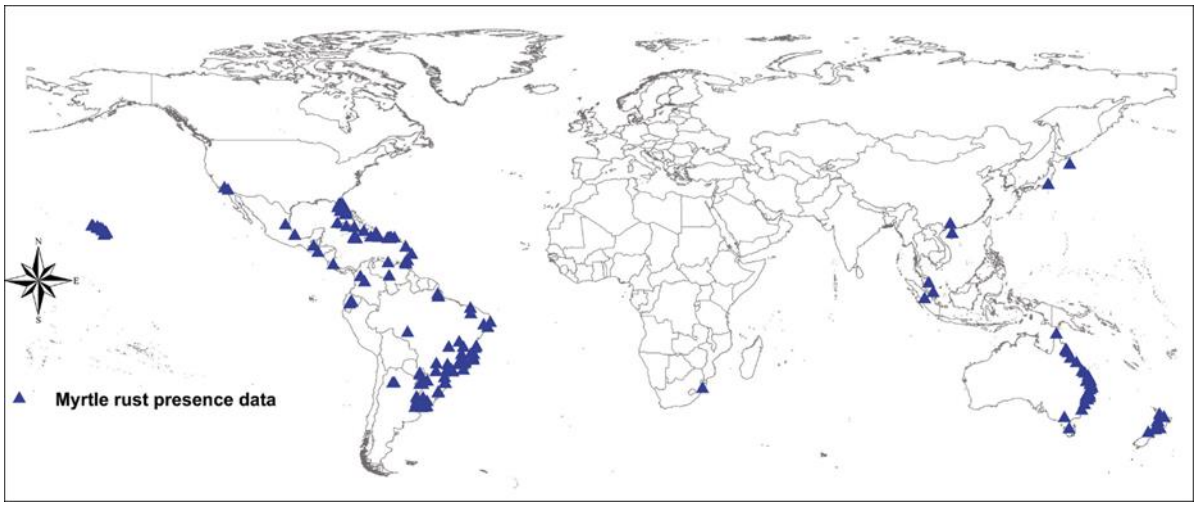

Figure 1: Current global distribution of myrtle rust caused by* Austropuccinia psidii *(reproduced from Narouei-Khandan et al. 2020).

Austropuccinia psidii was introduced to New South Wales, Australia in 2010 (DAWE 2021) and quickly spread to the eastern coast of Australia. It was declared ineradicable from NSW in April 2010 and from QLD in December 2010 (Invasive Species Council 2017). The impact of repeated infection on some species has resulted in severe decline and tree death, and in cases, to their listing as endangered species (Pegg et al. 2018a).

Compared to the east coast of Australia, fewer records have been made in Tasmania and the Northern Territory since discovery in 2015 (Tasmanian Government 2020 and Westaway 2016, 2018 respectively), while South Australia and Western Australia remain free from the rust (Berthon et al 2018).

The current known distribution of myrtle rust in Australia is shown in Figure 2.

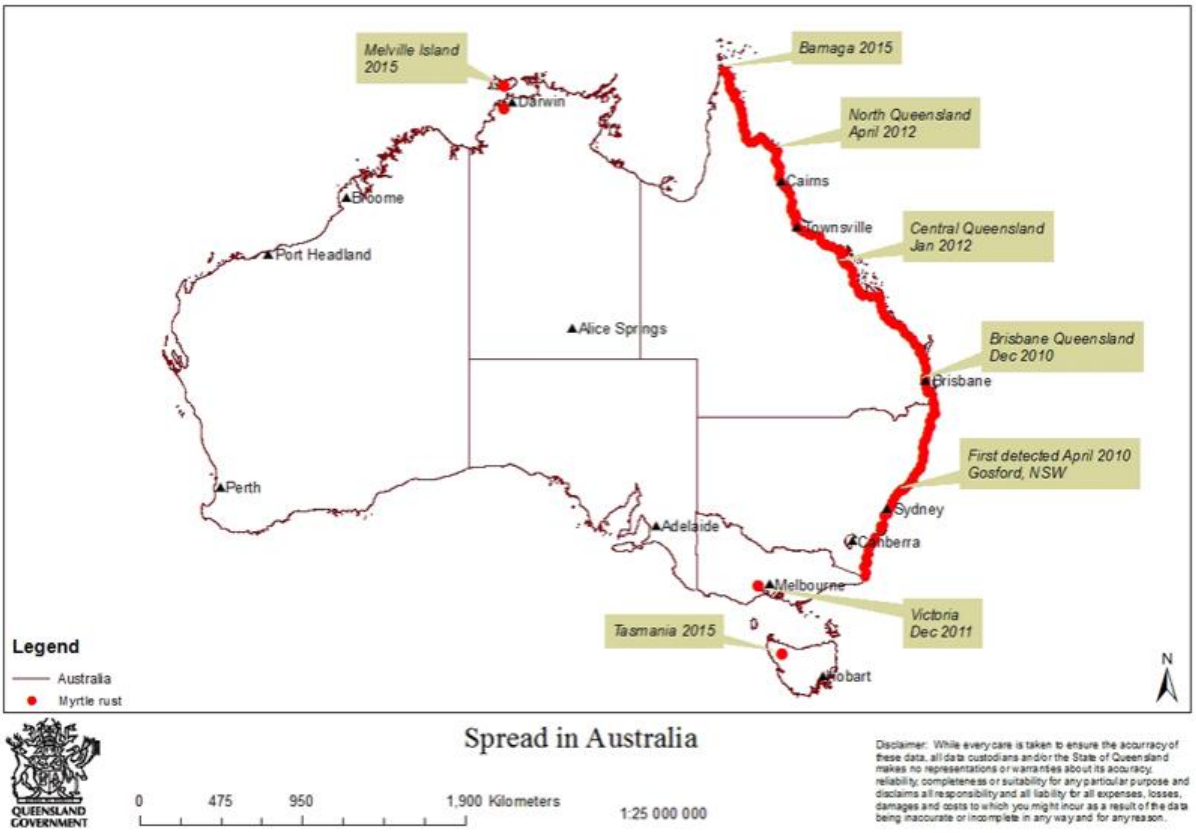

Figure 2: Known distribution of* Austropuccinia psidii *in Australia (reproduced from Pegg et al. 2018b).

To our knowledge, no systematic surveillance or monitoring program exists for Australia (Fernandez Winzer et al. 2019).

Despite the lack of records in Western Australia to date, models have shown that the State is susceptible to myrtle rust both in the tropical north (Singh et al 2016, Narouei-Khandan et al. 2020) and the temperate southwest (Kriticos et al 2013, Singh et al 2016, Narouei-Khandan et al. 2020). Based on the known host list (Soewarto et al. 2019, Appendix 1) and vegetation mapping of the state (DPIRD 2021), risk maps were created for the state (Appendix 2).

Western Australia declared a ban on trade of myrtaceous species from other Australian mainland states and territories in February 2011 (McDonald 2012) and from Tasmania in 2015 (DPIRD 2015) to prevent the incursion of myrtle rust.

Pathways for myrtle rust are, however, not exclusive to soil or nursery trade. The spores can be transported by people on their clothing and luggage, as well as wind currents (CABI 2021 and references therein). In addition to these, animal activity is another recognised way in which long distance dispersal of rust pathogens can occur (Nagarajan & Singh 1990).

Key parameters used to guide this surveillance plan are wind, climatic suitability, human travel pathways, and host susceptibility and density.

2. Aim, Scope and Objectives

The aim of this surveillance plan is to establish a framework for undertaking field surveys in Western Australia to provide evidence *Austropuccinia psidii* is absent from the State. In the event of an

incursion, this plan will also guide the early detection and rapid response to stop the fungal pathogen from spreading further.

The scope of this Plan is to enable general and targeted surveillance, both visual and molecular. Specific surveillance activities in this plan will:

- target native and alien species in the Myrtaceae family (host plants) for visual surveillance within the local government areas of interest (detailed in Sampling Locations, Section 6.1);
- target eDNA sampling on key areas for spore introduction via multiple pathways (detailed in Sampling Locations, Section 7.1);
- completed by officers of the DPIRD and/or persons who are appointed inspectors under the *Biosecurity and Agriculture Management Act 2007* and persons defined as assistants to inspectors under that Act;
- completed on or before December 2023.

The surveillance activities in this plan will not:

- provide data outside the local government areas of interest, unless there is a direct link to an infected premise, and
- aim to deliver data to support whole of state area freedom from *Austropuccinia psidii* claims (data that represents testing to achieve a 95% confidence level).

The objectives of this Surveillance Plan are to:

(1) designate reporting channels; roles and responsibilities;
(2) delineate target areas for visual and eDNA surveillance;
(3) delineate timeframes for surveillance; and
(4) establish surveillance and sampling protocols.

This document should be considered in conjunction with the Myrtle Rust Contingency Plan and Communications Plan, both developed by the Department of Primary Industries and Regional Development (DPIRD; Trend et al. 2021).

3. Targets and outputs of surveillance activities

The targets of the visual surveillance activities are to:

- identify and document the location of myrtle rust host species in areas of interest;
- assess the likelihood of each host plant on a premise of interest being an untraced plant; and
- inspect host plants on premises of interest to detect visual signs of myrtle rust.

The targets of eDNA surveillance activities are to:

- collect samples from areas of interest;
- analyse and report the samples for presence of *Austropuccinia psidii* DNA;
- upon positive result, direct visual targeted surveillance to the area.

Note that a positive result from the eDNA surveillance does not mean that the disease is present, but rather, that the pathway for spore or DNA introduction is open.

The outputs of surveillance activities are:

- data (presence or absence) for host plants and/or for myrtle rust in premises of interest within areas of interest from visual surveillance (data collected by direct premise visit, by direct phone call and premise visit or by public reporters);
- data (presence or absence) for *A. psidii* DNA in eDNA samples collected from areas of interest; and
- management of suspect premises in accordance with approved standard operating procedures produced by DPIRD.

4. General Approach

4.1. Assumptions for sampling

Assumption 1: Western Australia has two regions of climatic suitability: Northern Province (in particular Central Kimberley), and South-West Province. Using the precautionary principle, all areas of suitability in both Climex and Climatch models (Kriticos et al 2013, Singh et al 2016, respectively) are considered suitable areas for the pathogen (Figure 3).

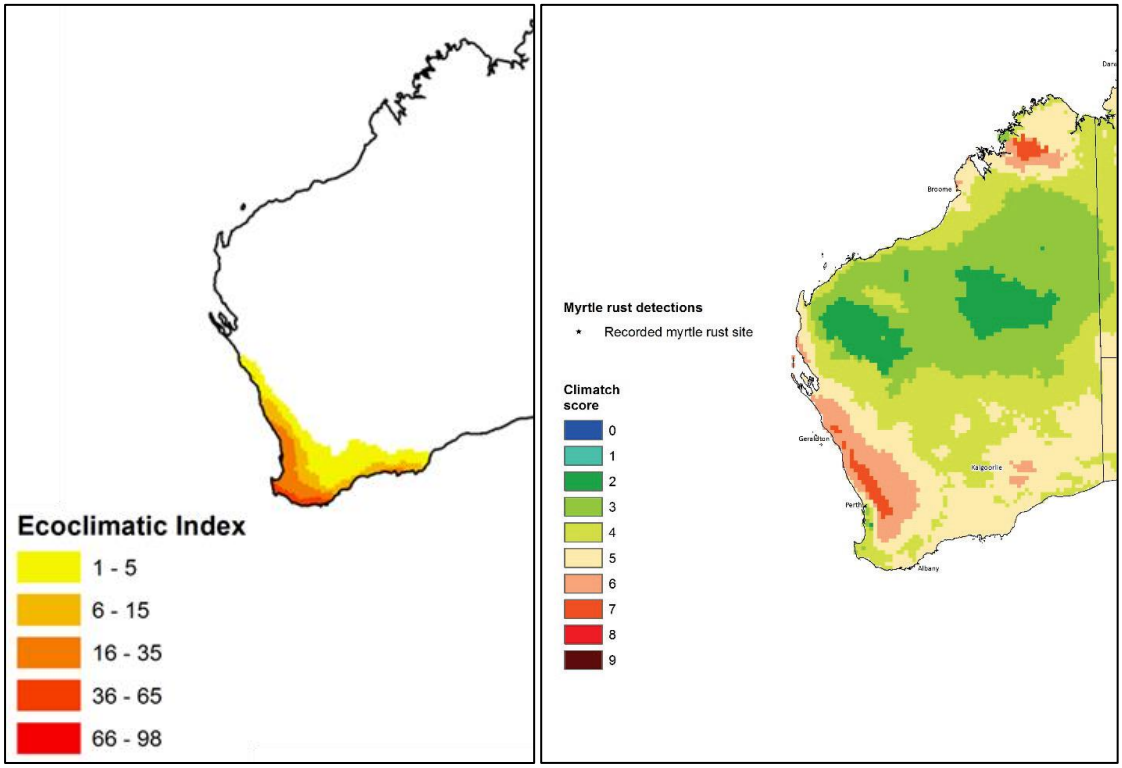

***Figure 3: Climex model (modified from Kriticos et al. 2013), left; Climatch model (reproduced from Singh et al. 2016), right. Higher scores indicate higher climatic suitability for* Austropuccinia psidii.**

Assumption 2: Myrtaceae species (both known hosts and potential hosts, Figure 4) are common in the Northern Province and ubiquitous in the Southwestern Province of Western Australia.

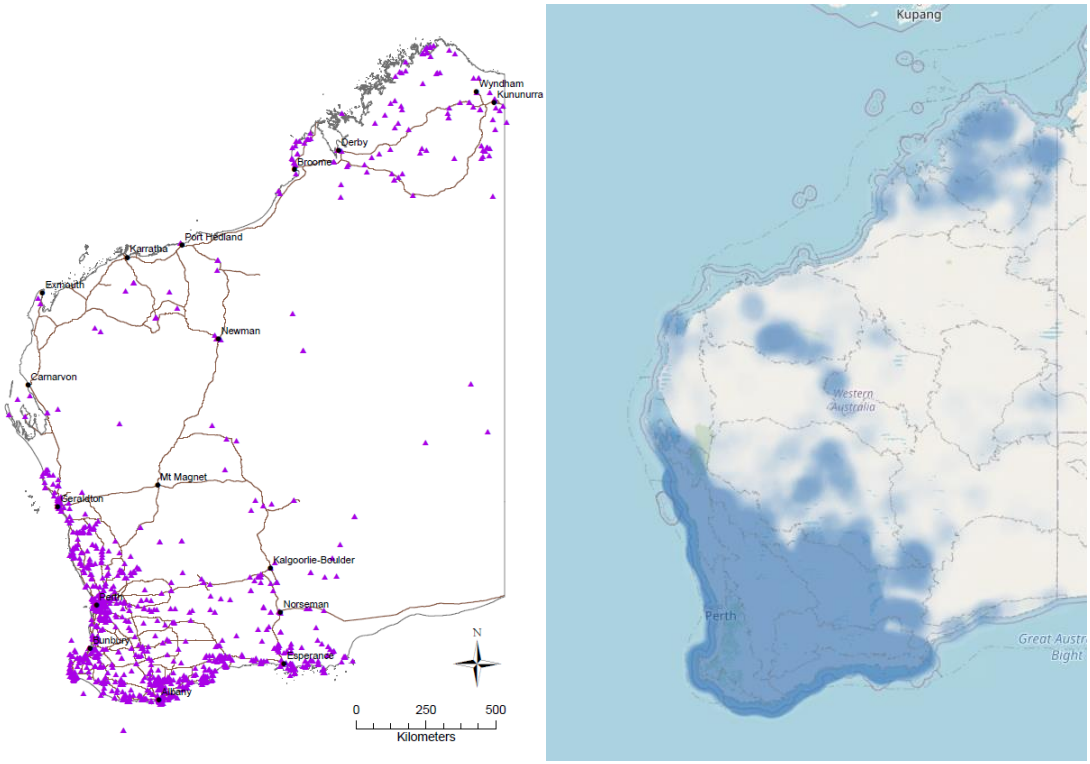

Figure 4: Known host species (left) and all Myrtaceae species (right) in Western Australia. For more information on the maps, please refer to Appendix 2.

Assumption 3: Altered landscapes (with irrigation or climatically buffered) in or out of the modelled areas (Figure 3) may also be suitable to establishment of myrtle rust. Nurseries and private gardens are premises of interest because they are often irrigated, with higher host plant density, promote plants' new growth through pruning and hedging, and have higher exposure to humans.

Assumption 4: Within the known host species lists (Appendix 1, Soewarto et al. 2019), the best to target for surveillance are *Syzygium* and *Melaleuca* in the Northern Province and *Agonis flexuosa* and *Chamelaucium uncinatum* in the South-West Province. These species were selected due to a combination of their known susceptibility, distribution, and ease of identification. Note that susceptibility varies between and within provenances of a given species (Pegg et al. 2018a), and all Myrtaceae species could potentially be hosts.

Assumption 5: Winds can carry spores and therefore, are a possible pathway of entry. Wind currents from infested areas in the Northern Territory could transport spores to the Northern Province of Western Australia, and cyclonic winds could carry spores from the Timor Sea region into Western Australia.

Assumption 6: People and their luggage are a pathway for the spores. Major ports, airports, roads and significant destinations are important sampling points.

Assumption 7: Interstate transport of contaminated Myrtaceae species is not an open pathway for introduction of myrtle rust into Western Australia. Since the trade ban on Myrtaceae into Western Australia in 2010, little or no susceptible material would have come into the State. It is possible illegal trade occurs; but due to its illegal nature, very difficult to trace.

Assumption 8: Citizen scientist reports contribute largely to the surveillance efforts (Toome-Heller et al. 2020). Reports from individuals will trigger further investigation from relevant authorities.

4.2. Surveillance approaches

Visual surveillance (opportunistic and targeted) and eDNA sampling are proposed as complementary approaches to the surveillance program.

Opportunistic visual surveillance can be conducted by trained staff (DPIRD, DBCA and other agencies and industries) on other duties as well as citizen scientists, whereas targeted surveillance should be undertaken by DPIRD personnel or authorised persons under the *Biosecurity and Agriculture Management Act 2007*. The process, target sampling areas and timing of surveys are detailed in Section 6.

The proposed eDNA surveillance involves sampling of environmental DNA from areas of interest to investigate whether there are open pathways of introduction of myrtle rust spores or DNA into the state. Sampling can be undertaken by trained staff or volunteers and submitted for molecular identification as detailed in Section 7.

5. Strategy and Tactics

On-ground visual surveillance is not feasible for the entire area of modelled climatic suitability, and pathways of interest, so a combination of two strategies (visual and eDNA surveillance) should be used.

If any sample from either strategy returns a positive result, further surveillance of a broader area around the positive should be undertaken, following the rigorous containment and decontamination procedures detailed in the Contingency Plan.

Any confirmed positives from visual surveillance samples would indicate that the disease is present and should trigger the alert phase if the emergency incident response, which will either lead to an operational phase or a stand-down phase (if the outbreak is deemed ineradicable).

Any positive result from eDNA sampling would indicate that spores or exogenous DNA are present in the environment, which does not necessarily mean that there are infected plants. What this result indicates is that there is an open pathway for the spores to arrive at the sampling location. This should trigger an investigation and intensification of eDNA sampling in the surrounding areas. In laboratory and field studies (Beresford et al. 2020), the latent period was observed to be 5 to 7 days and spore production started one week after the end of the latent period and peak spore production lasted for two weeks, continuing for another 1-2 months. If no visual signs or additional positive eDNA samples are returned within 2 months, further sampling may cease and baseline eDNA sampling is to be reinstituted.

The tactics for surveillance will include:

- change in communication focus to target groups of interest identified through previous and current incident reporting data;
- more direct engagement with targeted groups based on field intelligence and other demographics to obtain information regarding the absence or presence of host plants;
- investigating and/or inspecting premises of interest which have been identified as targets of surveillance activities; and

- inspecting premises of interest in response to public and industry phone enquiries or reports submitted via MyPestGuide® Reporter, including plants with visual signs of infection (symptomatic) and plants without visual signs of infection (asymptomatic).

6. Visual Surveillance

Visual surveillance relies on the observation of symptoms of the disease (Figure 5). The most common expression of myrtle rust is infection on the leaves (although occasionally on juvenile stems and shoot tips), with rust sori apparent; and in some hosts, there can be a chlorotic area around the infection (Toome-Heller et al. 2020). Myrtle rust can also affect developing fruit, causing it to prematurely drop (Sutherland et al. 2020).

Figure 5: Austropuccinia psidii severity levels from relatively tolerant (a, b) to extremely susceptible (g, h). Image reproduced from Pegg et al. (2014).

No physical samples should be taken during opportunistic surveillance, even by trained staff, as there is little ability to undertake thorough containment and disinfection procedures with no previous planning. Instead, trained staff (DPIRD, DBCA, NRMs, FPC and NGIWA) should record both absence and suspect positive records through photographs submitted to MyPestGuide® Reporter application or MAX surveillance reporting system.

If plants show visual symptoms of myrtle rust disease, only DPIRD staff (unless otherwise directed) undertaking targeted surveillance should include physical samples of the pathogen (see Section 6.3 on how to collect physical samples).

The identification of the host plant should be done in the field or through images when possible. If a sample must be taken, a healthy representative branch of the plant should be collected, then double-bagged into ziplock plastic bags, and disinfected for delivery to the herbarium or taxonomist.

There are currently 524 species of Myrtaceae known to be vulnerable to the rust, a number likely to increase if the pathogen arrives in Western Australia. Of these, species selected for targeted visual surveillance in natural ecosystems are *Syzygium* and *Melaleuca* species in the Northern Province of the state; and *Agonis flexuosa* and *Chamelaucium uncinatum* in the South-West Province. Targeted visual surveillance of nurseries and private gardens containing Myrtaceae species should also be undertaken. A breakdown on known susceptible species by region is presented in Appendix 1.

6.1. Locations

Targeted visual surveillance should be undertaken in the areas with an overlap of climate suitability, presence of host species and stronger likelihood of arrival of spores. Based on this reasoning, premises of interest are:

- Nurseries, community gardens, and private gardens along the Great Northern Highway between Kununurra and Broome;
- Nurseries in the South-West Province (from Geraldton to Esperance, and 50 km inland);
- Caravan, trailer parks, and camping grounds in the Northern and South-West Provinces;
- Community gardens and horticultural areas, targeting (but not limited to) *Psidium* (e.g., guava), *Syzygium* (e.g., lilly pilly, rose apple), and *Backhousia citriodora* (lemon myrtle);
- Myrtaceae vegetation in the Derby-Gibb-Prince Regent River zone, targeting (but not limited to) *Melaleuca* and *Syzygium*;
- Myrtaceae vegetation along hiking and biking trails in the South-West Provinces targeting (but not limited to) *Agonis flexuosa* (peppermint) and *Chamelaucium uncinatum* (Geraldton wax);
- Kings Park Botanic Gardens, Bold Park Botanic Gardens, Araluen Botanic Park, Wanneroo Botanic Gardens, Australian Wildlife Park Albany.

Opportunistic visual surveillance should be undertaken in any of the areas modelled as suitable for the pathogen (see Figure 1 in this report) by both trained officers, industry members, and citizen scientists. Engagement with industry and community will follow the Communications Plan. The areas of interest for opportunistic surveillance are:

- Broome and Derby to Kununurra;
- Kalbarri to Esperance;
- Rottnest Island, Penguin Island and other offshore islands.

6.2. Timing

Visual surveillance should occur when conditions for germination of spores are optimal. While highest infection has been observed at the range of 20-25°C, germination of uredinial spores is optimal with high leaf wetness or humidity and 18°C temperature (Piza & Ribeiro 1988), although this optimal point varied between 12°C and 20°C depending in the origin of the spores (Auer et al. 2012).

The most suitable periods for sampling are shown in Table 1. Altered habitats (gardens, nurseries, and irrigated areas) are expected to be climatically buffered and are suitable for surveillance throughout the year.

Table 1: Recommended surveillance periods for different regions in Western Australia.

Targeted region	Ideal targeted surveillance period
Derby to Ord River	June
Carnarvon to Geraldton	March (before Easter), September (before school holidays)
Geraldton to Jurien Bay	September (before school holidays)
Jurien Bay to Mandurah	March (before public holidays), September (before school holidays)
Mandurah to Augusta	March (before public holidays), September (before school holidays)
Augusta to Albany	November
Albany to Israelite Bay	November
Dwellingup to Mount Barker	March (before public holidays)
Dumbleyung to Ravensthorpe	March (before public holidays), September (before school holidays)

Targeted visual surveillance of touristic locations should occur prior to peak tourist season (i.e., before school holidays and public holidays).

Targeted visual surveillance of nurseries, horticultural areas, community and private gardens, and botanical parks; as well as all opportunistic surveillance, may be conducted year-round.

6.3. Collecting Samples

Only plants observed showing signs of myrtle rust disease will be physically sampled in accordance with the relevant Standard Operating Procedure (SOP) developed by DPIRD. Sampling methodology was based on the work done by MPI in New Zealand (Toome-Heller et al. 2020), where samples of the spores were taken in the field directly into microtubes; with the tubes disinfected and double-bagged into ziplock plastic bags, again disinfected for delivery to the laboratory. This method reduces handling of contaminated material outside of the source of infection and streamlines the sample preparation in the laboratory for PCR analyses.

Samples collected following the SOP will be diagnosed at Murdoch University, who are currently equipped to analyse samples. At the time of submission of this document (April 2022), no National Diagnostic Protocol had been developed for *Austropuccinia psidii* (National Plant Biosecurity Diagnostic Network 2022).

Biosecurity precautions

Any inspection, sampling and handling of samples will be in accordance with the national “Arrive Clean, Leave Clean” guidelines (Commonwealth of Australia 2015), approved Standard Operating Procedures and Work Instructions.

All DPIRD staff and contractors will take precautionary measures to avoid further spread of myrtle rust during surveillance work.

Legislation and Regulatory Authority

Surveillance and any subsequent regulatory activity will be carried out under the provisions of the *Biosecurity and Agriculture Management Act 2007*.

Section 42 of the Act allows officers of the Department to carry out operational work necessary or conducive to the control of a declared pest (i.e., myrtle rust) on or in relation to any place. This allows them to conduct enquiries, enter properties to make and record observations, and collect diagnostic samples.

An inspector appointed under section 162 of the Act may exercise the powers of an inspector. Actions that require the powers of an inspector include:

(1) Entering a premise with assistants (note: contractors are not officers of the department so not able to act under the provision of s42 so require the supervision of an inspector) - s65
(2) Requiring information, including trace forward and trace back information, if the person does not co-operate with an enquiry - s67
(3) Accessing relevant records detailing the transport, possession, supply, or distribution of potential carriers - s66
(4) Seizing an organism or potential carrier until it is determined whether it is infected or infested with a declared pest -s73(1)(iii), and
(5) Directing a person from whom an organism or potential carrier is seized to keep it under specified conditions –s73(2)(a).

The resource implications of the above are that an inspector will need to be a part of each team conducting premise visits in order for any seizures *in situ* to be put into effect at the time of inspection.

Alternatively, an inspector contactable by telephone may verbally give a direction under s67 to detain plants *in situ* until they attend the premise and effect the seizure. This would allow the surveillance and sampling to be undertaken by officers of DPIRD.

7. eDNA Surveillance

The eDNA sampling, unlike visual observations, does not necessarily require plants being infected by myrtle rust spores, but rather, spore movement into an area. The eDNA samples consist of vials with either water or air samples. The scientific grounds are based on two validated studies: (1) rainfall collection of spores has been successfully used previously for soybean rust, *Phakopsora pachyrhizi* with passive collectors fitted with a filter for spore capture (Isard et al. 2011); and (2) dry spore trapping for myrtle rust using a Burkard 7-day recording spore trap from Burkard Manufacturing Co. Ltd, Rickmansworth, UK (Tessman et al. 2001).

The field methodology for eDNA collection for the detection of myrtle rust spores is currently being developed. This section will be updated once the information on how to sample for myrtle rust DNA is available.

7.1. Locations

The most significant location for eDNA sampling targeting wind movement is in the region closest to the Northern Territory, where known positives have occurred. However, strong cyclonic winds may disperse the spores over very long distances, potentially bringing spores from the Timor Sea region to the north-western coast of Western Australia. Select towns for sampling are proposed in Table 2.

The human pathways should focus on roads, shipping ports, major domestic and international airports, and touristic sites and areas of high traffic (Table 2). Specific locations for targeted eDNA

within those areas should be selected with field intelligence information, aiming to capture areas of highest passenger flow (such as natural or man-made bottlenecks) or areas where spores would aggregate (downwind, onto a valley or water channel).

7.2. Timing

The logic applied to eDNA sampling is for it to be undertaken at periods where sporulation is highest in the 'source' areas; that is, locations where the pathogen is present and uncontrolled. Given that different climatic regions in the eastern states and internationally will have sporulation peaks at different times, eDNA sampling can be conducted throughout the year at ports and airports. Arterial roads can also be sampled year-round or at periods of highest activity.

Table 2: Recommended eDNA sampling times for different regions in Western Australia, based on key pathways. Note that for ports and airports, sampling can occur year-round but is preferred at peak activity.

Location	Wind and cyclones	Interstate roads	Ports	Airports	Tourist or high-traffic destinations
Wyndham	December to February		peak		
Kununurra	December to February	year-round		peak	
Lake Argyle	December to February				
Sturt Creek		year-round			
Halls Creek	November to February	year-round			
Mitchell Plateau	post-cyclones				
Yampi Sound			peak		
Derby	post-cyclones		peak		
Broome	post-cyclones		peak	peak	peak tourist season
Port Hedland			peak		
Karratha					year-round
Dampier			peak		
Onslow/Ashburton			peak		
Exmouth					peak tourist season
Learmonth				peak	
Kalbarri					year-round
Geraldton			peak	peak	year-round
Perth				peak	year-round
Fremantle			peak		year-round
Kwinana			peak		
Rottnest Island					year-round
Bunbury			peak		
Busselton					year-round
Margaret River					year-round
Augusta					peak tourist season
Walpole					peak tourist season
Albany			peak		year-round
Esperance			peak		peak tourist season
Israelite Bay					peak tourist season
Eucla		year-round			
Kalgoorlie				peak	

8. Resources

Resourcing will involve government and non-government agencies. A Memorandum of Understanding (MOU) has been drafted by DPIRD detailing the parties engaged at present and their role in surveillance.

Community participation as citizen scientists is strongly encouraged and promoted through the Communications Plan. Community members should be directed to engage in visual surveillance in the following locations:

- Private gardens (all citizen scientists)
- Community gardens (special interest groups)
- Touristic locations (all citizen scientists)
- Parks and reserves (special interest groups such as "Friends of …")
- Trails (special interest groups such as Bibbulmun Track and Munda Biddy Trail Foundations)

8.1. Field Activity Resources – Labour

Targeted visual surveillance can be undertaken by trained staff from DPIRD, DBCA, Forest Products Commission (FPC), Nursery and Garden Industry Western Australia (NGIWA) and Natural Resource Management (NRM) groups in Western Australia. Their roles in surveillance have been outlined in the Memorandum of Understanding (MOU) prepared by DPIRD (Draft 1 dated 21 October 2021). Opportunistic visual surveillance can also be undertaken by the trained staff listed above and citizen scientists, which may lead to resources being expended in communications and follow-ups.

Sample collection for eDNA analyses should be undertaken by trained staff mentioned above, and samples sent to Murdoch University for analyses (Appendix 3).

Until a positive sample is obtained, there is no dedicated allocation of funds or person-hours to myrtle rust surveillance, and both opportunistic surveillance and sampling for eDNA are expected as in-kind contribution from the groups listed above.

Prior to an incursion, targeted visual surveillance aims at early detection and rapid response. Estimated annual resources for surveillance prior to an incursion are:

- Northern Province: 10 person-days including four days of travel time. This estimate is based on: the time required for surveillance based on the approach agreed, the capability of staff to visit 10 premises (private residences, camping grounds, caravan parks) per day, being at least 5 in each town/locality on the sealed road between Broome and Kununurra.
- South-West Province: 20 person-days including travel time. This estimate is based on: the time required for surveillance based on the approach agreed; the capability of staff to visit 30 premises (private residences, camping grounds, caravan parks) per day, visiting at least 20 towns/localities on sealed roads.
- Natural environment areas: targeted visual surveillance should be undertaken by DBCA and, as much as feasible, incorporated into existing activities. Four person-days (including travel) would be required for visual surveillance of the natural environment of the Derby-Gibb-Prince Regent River area, and 15 person-days (including travel) would be required for visual surveillance of National Parks in the South-West province. This estimate is based on: the capability of staff to traverse 8-10 km on foot per day; the time required for surveillance based on the approach agreed; and the ability to visit 5 or more localities per day, covering at least 40 localities in the South-West.

Labour resourcing for calling and tracing questions should be considered a replacement to site visits, not an addition to them. Specific Standard Operating Procedures (SOPs) and Work Instructions (WI)

will need to be developed prior to deployment to field surveillance by the agency(ies) responsible for surveillance. SOPs required may include (but not only):

- SOP: Decontamination protocols for personnel and equipment
- SOP: Decontamination protocols for vehicles
- SOP: Premise Classification
- SOP: Surveillance for myrtle rust outside the RA
- SOP: Trace premises outside a Control Area
- WI: Sample collection for myrtle rust
- WI: Inspection of host plants for myrtle rust

Sampling for eDNA should be incorporated into existing activities and rely, as much as feasible, on *in-kind* personnel costs and collaboration with industries, other agencies, and volunteers. The estimated labour needed to engage stakeholders, inform how sampling should be undertaken, and receive samples is four person-days per year.

8.2. Field Non-labour Resources

Each team will require a vehicle, mobile devices, first-aid kits and emergency beacons or satellite phones. Inspectors will require a seizure receipt book and blank copies of any Direction to do certain activities (such as confiscate plants as per SOPs).

Travel and accommodation will likely be required for officers and inspectors deployed to regional locations. DPIRD and DBCA staff located in regional offices should be requested to assist with surveillance in their local areas, where possible.

Observations should be recorded using the MyPestGuide® Reporter application which includes the GPS location, photographs, and logs any eDNA samples collected.

Sampling of eDNA requires deployment of eDNA traps that collect air or water from rainfall or streams. There are three sampling options (*yet to be tested and rolled out; this section will be updated once results are available*):

1. Rainfall sampling with filter. Set up following methodology in Holliday et al. (2013): “passive rain collectors were 29 cm in diameter and designed with a filter assemblage for immediate filtration and spore capture on a cellulose nitrate filter (8.0-µm pore size) in the field”. Filters to be placed in a sterile tube or bag.
2. Water stream sampling. Sample water from a stream on the day following a rainfall event in a sterile plastic tube with 50-200ml capacity.
3. Dry spore trapping. Burkard 7-day recording spore trap (Burkard Manufacturing Co. Ltd, Rickmansworth, UK) or rotary cylinder trap.

In order to increase feasibility of deployment and sample recovery, the testing of traps will assess both the trap’s success in detecting myrtle rust eDNA, the trap’s cost, and the ease of use/deployment.

8.3. Testing and Diagnostic Services

The images submitted via MyPestGuide® Reporter application will be triaged by DPIRD staff who manage the app submissions and forwarded to plat pathologists if deemed necessary. If a positive is

suspected, a team will be deployed to the location following the protocols established in the Contingency Plan.

The eDNA samples are to be submitted to the Forensic Laboratory at Murdoch University for genetic analyses. Murdoch University is equipped to analyse 500 further samples with funds provided by a grant (Australian Plant Biosecurity Science Foundation PBSF035). Beyond that, it is anticipated that DPIRD will oversee any testing, and operational funds for eDNA sample analyses are to be sought.

Appendix 3 details the development of the genetic test and its sensitivity, as well as the protocol for sample submission.

8.4. Other Resources

DBCA offer a non-accredited course on dieback (*Phytophthora cinnamomi)* called Green Card, this is delivered to staff and contractors, FPC and NRM and industry workers. The Green Card online course, has seven modules on dieback and one on myrtle rust, made available in mid-December 2020. This has been used as a tool to keep up awareness on myrtle rust in Western Australia. The myrtle rust awareness module covers what myrtle rust is, where it occurs and why it is important; and shows images of the symptoms (O'Gara 2021).

DBCA also have information on their website that can assist with diagnostic for the general public, as well as links to other sources of information:

- (https://www.dpaw.wa.gov.au/mobile/management/pests-diseases/206-myrtle-rust).
- https://www.dbca.wa.gov.au/parks-and-wildlife-service/threat-management/plant-diseases/myrtle-rust

DPIRD have My Pest Guide where suspect occurrences can be reported online or via the app, and also have information on their website and links to information from other states and departments:

- https://www.agric.wa.gov.au/pests-weeds-diseases/mypestguide
- https://www.agric.wa.gov.au/plant-biosecurity/myrtle-rust-threat-western-australia

9. Budget

Funding for annual targeted visual surveillance is expected to be allocated by one or multiple parties involved in the Memorandum of Understanding.

If sampling for eDNA can be undertaken as *in-kind* labour from government agencies, industries and volunteers, the cost involved will be setting up and retrieving samples (*to be determined upon completion of field trials but estimated to be less than 15 dollars per sample including shipping*). Analyses of up to 500 eDNA samples by Murdoch University is free of charge due to funding provided by the Australian Plant Biosecurity Science Foundation for the development of the molecular assay. Beyond the first 500 samples, the estimated cost per sample (as of April 2022) is between AUD$9-19 for consumables, plus between AUD$10-36 for technician labour to process the samples (costs vary depending on economy of scale, with cheaper options above 500 samples per year).

In accordance with the Contingency Plan, if myrtle rust is detected in Western Australia, DPIRD will lead the initial inter-agency response for up to three weeks and/or up to $100 000 to scope out the extent of the outbreak.

10. Survey design

The design of the surveillance is targeted and is biased towards detection of host plants likely to be infected with myrtle rust. Hence, traditional probabilistic statements about the surveillance design cannot be made.

The population units in this surveillance plan are individual premises and individual myrtle rust host plants on premises.

10.1 Performance indicators

This surveillance plan, if effective will have:

- Visited and visually inspected or contacted by phone at least 5 localities and at least 50 properties in the Northern Province
- Visited and visually inspected or contacted by phone at least 20 localities and at least 300 properties in the South-West Province
- Confirmed the presence/absence of Myrtaceae plants on 90% of premises of interest
- Visually inspected 100% of Myrtaceae plants on those premises of interest identified through direct telephone contact or self-reporting
- Assessed 100% of Myrtaceae plants inspected for likelihood of being a trace plant
- Managed 100% of suspect premises in accordance with approved SOPs
- Generated MyPestGuide® reports in both the South-West and Northern Provinces of Western Australia

11. Triggers

The surveillance plan will be reviewed if any of the following triggers are met:

(1) Detection of myrtle rust outside of the designated Quarantine Area on premises:
   - that are not directly linked to current, known IP or SP; or
   - that are directly linked to current, known IP or SP, requiring further trace forwards

(2) Myrtle rust is confirmed in a commercial nursery or other production premise.

(3) There is evidence of non-compliance with quarantine and movement controls resulting in host movement outside of the Quarantine Area.

12. Detection or Suspect Detection

If visual signs are detected from visual surveillance, these are to be reported to the Department of Primary Industries and Regional Development. The preferred reporting method is via the MyPestGuide® Reporter application. If that is not possible, they should be reported to the Pest and Disease Information Service, PaDIS (1800 084 881). DPIRD will manage the data from MyPestGuide® app and phone reports, and follow the Contingency Plan.

If eDNA samples return a positive result from the laboratory, these will be reported to DPIRD matching the MyPestGuide® code. The Contingency Plan is to be followed thereafter.

All positive detections should be biotyped to ensure that the occurrences are of the pandemic biotype, rather than an introduction of the other biotypes – the South African biotype, not yet

recorded in Australia, is of particular importance to Western Australia given international trade and travel. At least ten biotypes have been described worldwide to date (MPI 2019).

Hosts plants should be identified and if new hosts are detected, these should be added to the known host list though publication of a scientific manuscript. Extensive efforts should be placed on back and forward tracing of the pathways from the property or location from where the positive sample originated.

13. Data management and reporting

Data from all surveillance activities will be recorded in MAX. Data will consist of:

(1) Records of conversation with direct contact targets (homeowners, accommodation facilities etc.) to determine if plants of interest are present on the premise, and any information provided during interview that will inform forward or backward tracing.
(2) Records of inspection for any premises visited to determine whether plants of interest are present, and the outcome of that inspection.
(3) Records of any samples collected, and laboratory testing results
(4) Data recorded in MyPestGuide® (by officers or citizen scientists) uploaded to MAX

As a result of the above activities, each premise will be recorded as a case in MAX and have a case number with a status assigned.

Regular situation reports will be generated from MAX against the targets outlined below to assist in showing effectiveness of surveillance:

1) % of target premises per local government area that are successfully contacted either directly or by phone per region
2) % of target premises contacted per local government area upon which presence/absence of myrtle rust is confirmed
3) proportion of accounted/unaccounted for trace host plants per local government area by trace priority
4) % reduction in number of unaccounted for trace host plants per local government area by trace priority (tabulated or graphical)

14. Communication

The communication between agencies hinges on three documents:

- Communications Plan, drafted by DPIRD (Draft 1 dated 19 August 2021),
- Memorandum of Understanding, drafted by DPIRD (Draft 1 dated 21 October 2021),
- Contingency Plan, drafted by DPIRD (Draft 1 dated 21 January 2022).

In addition, the established Myrtle Rust Working Group maintains regular meetings (quarterly or more often) and up to date email and phone contacts for its members, which at the time of submission of this Plan (April 2022), include research institutions (Murdoch University, CSIRO, QDAF) and the parties represented by the above documents (DPIRD, DBCA, FPC, NGIWA and NRMs).

14.1 Community Engagement

Community engagement will follow the National biosecurity engagement communications framework (Australian Commonwealth Government 2013).

The general approach to community engagement is to: provide information to groups of interest on the importance of reporting Myrtaceae plants with symptoms of myrtle rust; encourage individual reporting of suspect myrtle rust occurrences; and make direct contact with persons in charge of certain properties to determine the likelihood of presence/absence of highly susceptible Myrtaceae species and assign risk ratings to these premises for follow up surveillance.

The key messages for surveillance activities are that: the Department of Primary Industries and Regional Development (DPIRD) responds quickly and effectively to contain detections of myrtle rust in Western Australia; the movement of host plants from other States and territories remains restricted; submission of MyPestGuide® Reporter records with photographic records and GPS location to assist DPIRD in finding possibly infected plants; that reporting and not moving suspect diseased plants will give WA the best chance of maintaining disease-free status.

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Appendix 1: Known hosts of *Austropuccinia psidii*

Western Australia flora is rich in Myrtaceae species, with 1,933 species (native and alien) listed as present in the state, of which 1,512 are mapped in the South-West Province and 135 in the Northern Province (Florabase 2021).

In Western Australia there are at least 88 species (native, non-native and cultivated in agriculture) known to be hosts of myrtle rust (Soewarto et al. 2019): 22 are present in the Northern Province; 62 in the South-West Province; three exclusively in the Eremaean where suitable climatic conditions are unlikely (Florabase 2021). We added four species to the list that are known to be cultivated species (Table A1).

Table A1.1: **Known hosts of *Austropuccinia psidii* and their occurrence in Australia and Western Australia (table modified from Soewarto et al. 2019). Data in columns marked with * were sourced from Soewarto et al. 2019; marked with ^ from Florabase (Florabase 2021).**

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Backhousia gundarara M.D.Barrett, Craven & R.L.Barrett	x		x	Yes	N	P2
Corymbia grandifolia (R.Br. ex Benth.) K.D.Hill & L.A.S.Johnson				Yes	N	
Eucalyptus alba Reinw. ex Blume		x		Yes	N	
Eucalyptus tetrodonta F.Muell.				Yes	N	
Eugenia reinwardtiana (Blume) DC.	x	x	x	Yes	N	P1
Leptospermum madidum A.R.Bean	x		x	Yes	N	
Lithomyrtus retusa (Endl.) N.Snow & Guymer	x		x	Yes	N	
Melaleuca nervosa (Lindl.) Cheel	x		x	Yes	N	
Melaleuca viridiflora Sol. ex Gaertn.	x		x	Yes	N	
Melaleuca cajuputi Maton & Sm. ex R.Powell		x		Yes	N	
Syzygium angophoroides (F.Muell.) B.Hyland	x		x	Yes	N	
Syzygium eucalyptoides (F.Muell.) B.Hyland subsp. *eucalyptoides*	x		x	Yes	N	
Syzygium forte (F.Muell.) B.Hyland	x		x	Yes	N	
Syzygium forte subsp. *potamophilum* B.Hyland	x		x	Yes	N	
Syzygium nervosum DC.	x		x	Yes	N	
Syzygium suborbiculare (Benth.) T.G.Hartley & L.M.Perry	x		x	Yes	N	
Melaleuca argentea W.Fitzg.	x		x	Yes	N/ERE	
Melaleuca leucadendra (L.) L.	x	x	x	Yes	N/ERE	

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Osbornia octodonta F.Muell.	x	x	x	Yes	N/ERE	
Eucalyptus websteriana x E. orbifolia		x	x	Yes	ERE	
Eucalyptus woodwardii Maiden		x	x	Yes	ERE	
Eucalyptus xerothermica L.A.S.Johnson & K.D.Hill		x	x	Yes	ERE	
Eucalyptus websteriana x E. crucis		x	x	Yes	ERE/SW	
Eucalyptus youngiana x E. macrocarpa		x	x	Yes	ERE/SW	
Melaleuca cardiophylla F.Muell.		x	x	Yes	ERE/SW	
Verticordia chrysantha Endl.		x	x	Yes	ERE/SW	
Eucalyptus camaldulensis Dehnh.	x	x	x	Yes	N/ERE/SW	
Eucalyptus camaldulensis subsp. *obtusa* (Blakely) Brooker & M.W.McDonald		x		Yes	N/ERE/SW	
Melaleuca viminalis (Sol. ex Gaertn.) Byrnes	x	x	x	Yes	SW/N	P2
Agonis flexuosa (Willd.) Sweet	x	x	x	Yes	SW	
Astartea fascicularis (Labill.) A.Cunn. ex DC.				Yes	SW	
Cyathostemon heterantherus (C.A.Gardner) Rye & Trudgen		x		Yes	SW	
Beaufortia schaueri Preissler ex Schauer		x	x	Yes	SW	
Beaufortia sparsa R.Br.		x	x	Yes	SW	
Calothamnus quadrifidus R.Br.	x		x	Yes	SW	
Calytrix tetragona Labill.		x	x	Yes	SW	
Chamelaucium uncinatum Schauer	x	x	x	Yes	SW	
Corymbia citriodora (Hook.) K.D.Hill & L.A.S.Johnson		x	x	Yes	SW	Alien
Corymbia ficifolia (F.Muell.) K.D.Hill & L.A.S.Johnson		x	x	Yes	SW	
Corymbia maculata (Hook.) K.D.Hill & L.A.S.Johnson		x	x	Yes	SW	Alien
Darwinia citriodora (Endl.) Benth.	x	x	x	Yes	SW	
Eremaea asterocarpa Hnatiuk				Yes	SW	
Eremaea pauciflora (Endl.) Druce				Yes	SW	
Eucalyptus botryoides Sm.				Yes	SW	Alien
Eucalyptus cornuta Labill.		x	x	Yes	SW	
Eucalyptus diversicolor F.Muell.		x	x	Yes	SW	
Eucalyptus forrestiana Diels		x	x	Yes	SW	
Eucalyptus globulus Labill.		x	x	Yes	SW	Alien

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Eucalyptus gomphocephala A.Cunn. ex DC.		x	x	Yes	SW	
Eucalyptus grandis W.Hill	x	x	x	Yes	SW	Alien
Eucalyptus guilfoylei Maiden		x	x	Yes	SW	
Eucalyptus jacksonii Maiden		x	x	Yes	SW	
Eucalyptus lehmannii (Schauer) Benth.		x	x	Yes	SW	
Eucalyptus marginata subsp. marginata		x	x	Yes	SW	
Eucalyptus megacarpa F.Muell.		x	x	Yes	SW	
Eucalyptus microcorys F.Muell.		x	x	Yes	SW	Alien
Eucalyptus occidentalis Endl.		x	x	Yes	SW	
Eucalyptus pyriformis x *E. macrocarpa*		x	x	Yes	SW	P3
Eucalyptus resinifera Sm.		x	x	Yes	SW	Alien
Eucalyptus rudis Endl.				Yes	SW	
Eucalyptus saligna Sm.	x	x	x	Yes	SW	Alien
Eucalyptus wandoo subsp. *wandoo*		x	x	Yes	SW	
Hypocalymma angustifolium (Endl.) Schauer	x		x	Yes	SW	
Hypocalymma robustum (Endl.) Lindl.		x		Yes	SW	
Kunzea ambigua (Sm.) Druce		x	x	Yes	SW	Alien
Kunzea baxteri (Klotzsch) Schauer		x	x	Yes	SW	
Kunzea ericoides (A.Rich.) Joy Thomps.		x	x	Yes	SW	Alien
Kunzea recurva Schauer				Yes	SW	
Leptospermum laevigatum (Gaertn.) F.Muell.		x	x	Yes	SW	Alien
Leptospermum rotundifolium (Maiden & Betche) Domin	x		x	Yes	SW	Alien
Melaleuca armillaris (Sol. ex Gaertn.) Sm	x		x	Yes	SW	Alien
Melaleuca eurystoma Craven				Yes	SW	
Melaleuca linariifolia Sm.	x	x	x	Yes	SW	Alien
Melaleuca nesophila F.Muell.	x	x	x	Yes	SW	
Melaleuca quinquenervia (Cav.) S.T.Blake	x	x	x	Yes	SW	Alien
Melaleuca sapientes Craven	x		x	Yes	SW	
Melaleuca citrina (Curtis) Dum.Cours.		x	x	Yes	SW	Alien
Pericalymma ellipticum (Endl.) Schauer		x		Yes	SW	

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Regelia ciliata Schauer		x		Yes	SW	
Regelia velutina (Turcz.) C.A.Gardner		x	x	Yes	SW	
Syncarpia glomulifera (Sm.) Nied.	x	x	x	Yes	SW	Alien
Thryptomene australis Endl.				Yes	SW	
Thryptomene saxicola (A.Cunn. ex Hook.) Schauer	x		x	Yes	SW	
Verticordia plumosa (Desf.) Druce		x	x	Yes	SW	
Psidium guineense Sw.	x	x		Cultivated		Alien
Psidium cattleyanum Afzel. ex Sabine		x	x	Cultivated		Alien
Psidium guajava L.	x	x	x	Cultivated		Alien
Syzygium jambos (L.) Alston	x	x	x	Cultivated		Alien
Acca sellowiana (O.Berg) Burret	x			No		
Allosyncarpia ternata S.T.Blake		x	x	No		
Angophora costata (Gaertn.) Hochr. ex Britten		*x*	x	No		
Angophora floribunda (Sm.) Sweet	x	x	x	No		
Angophora subvelutina F.Muell.	x		x	No		
Archirhodomyrtus beckleri (F.Muell.) A.J.Scott	x	x	x	No		
Arillastrum gummiferum (Brongn. & Gris) Pancher ex Baill.	x			No		
Asteromyrtus brassii (Byrnes) Craven	x		x	No		
Asteromyrtus magnifica (Specht) Craven		x	x	No		
Austromyrtus dulcis (C.T.White) L.S.Sm.	x	x	x	No		
Austromyrtus dulcis x tenuifolia 'copper tops'	*x*		x	No		
Austromyrtus tenuifolia (Sm.) Burret	x		x	No		
Backhousia angustifolia F.Muell.	x		x	No		
Backhousia bancroftii F.M.Bailey	x		x	No		
Backhousia citriodora F.Muell.	x	x	x	No		
Backhousia enata A.J.Ford, Craven & J.Holmes	x		x	No		
Backhousia hughesii C.T.White	x		x	No		
Backhousia leptopetala (F.Muell.) M.G.Harr.	x		x	No		
Backhousia myrtifolia Hook. & Harv.	x	x	x	No		
Backhousia oligantha A.R.Bean	x		x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Backhousia sciadophora F.Muell.	x		x	No		
Backhousia subargentea (C.T.White) M.G.Harr.	x		x	No		
Backhousia tetraptera Jackes	x		x	No		
Baeckea gunniana Schauer ex Walp.		x	x	No		
Baeckea leptocaulis Hook.f.		x	x	No		
Baeckea linifolia Rudge	x		x	No		
Barongia lophandra Peter G.Wilson & B.Hyland	x		x	No		
Calycorectes pohlianus (O.Berg) Kiaersk.				No		
Campomanesia guaviroba (DC.) Kiaersk				No		
Cloezia artensis (Montrouz.) P.S. Green var. *artensis*	x			No		
Cloezia artensis (Montrouz.) P.S.Green var. *riparia* J.W.Dawson	*x*			No		
Cloezia floribunda Brongn. & Gris	x			No		
Corymbia citriodora subsp. *variegata* (F.Muell.) A.R.Bean & M.W.McDonald	x	x	x	No		
Corymbia ficifolia x *C. ptychocarpa*	*x*		x	No		
Corymbia gummifera (Gaertn.) K.D.Hill & L.A.S.Johnson	x	x	x	No		
Corymbia henryi (S.T.Blake) K.D.Hill & L.A.S.Johnson	x	x	x	No		
Corymbia intermedia (F.Muell. ex R.T.Baker) K.D.Hill & L.A.S.Johnson		x	x	No		
Corymbia tessellaris (F.Muell.) K.D.Hill & L.A.S.Johnson	x	x	x	No		
Corymbia torelliana (F.Muell.) K.D.Hill & L.A.S.Johnson	x	x	x	No		
Corymbia variegata [= citrodora] x C. torelliana		x	x	No		
Darwinia glaucophylla B.G.Briggs		x	x	No		
Darwinia procera B.G.Briggs	x	x	x	No		
Decaspermum humile (G.Don) A.J.Scott	x		x	No		
Decaspermum humile (G.Don) A.J.Scott [Northern metapopulation]	x		x	No		
Decaspermum humile (G.Don) A.J.Scott [Southern metapopulation]	x	x	x	No		
Eucalyptus acmenoides Schauer		x		No		
Eucalyptus agglomerata Maiden	x	x	x	No		
Eucalyptus amplifolia subsp. *amplifolia*		x		No		
Eucalyptus amygdalina Labill.		x	x	No		
Eucalyptus archeri Maiden & Blakely		x	x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Eucalyptus argophloia Blakely		x	x	No		
Eucalyptus baileyana F.Muell.		x	x	No		
Eucalyptus barberi L.A.S.Johnson & Blaxell		x	x	No		
Eucalyptus baueriana Schauer		x	x	No		
Eucalyptus benthamii Maiden & Cambage				No		
Eucalyptus brassiana S.T.Blake		x		No		
Eucalyptus brookeriana A.M.Gray		x	x	No		
Eucalyptus burgessiana L.A.S.Johnson & Blaxell		x	x	No		
Eucalyptus camfieldii Maiden		x	x	No		
Eucalyptus campanulata R.T.Baker & H.G.Sm.		x	x	No		
Eucalyptus camphora F.Muell. ex R.T.Baker		x	x	No		
Eucalyptus carnei C.A.Gardner	x		x	No		
Eucalyptus cephalocarpa Blakely		x	x	No		
Eucalyptus cinerea F.Muell. ex Benth.		x	x	No		
Eucalyptus cloeziana F.Muell.	x	x	x	No		
Eucalyptus coccifera Hook.f.		x	x	No		
Eucalyptus cordata subsp. cordata		x	x	No		
Eucalyptus crebra F.Muell.		x	x	No		
Eucalyptus curtisii Blakely & C.T.White	x		x	No		
Eucalyptus dalrympleana Maiden			?	No		
Eucalyptus deanei Maiden	x	x	x	No		
Eucalyptus deglupta Blume		x		No		
Eucalyptus delegatensis F.Muell. ex R.T.Baker		x	x	No		
Eucalyptus dunnii Maiden	x	x	x	No		
Eucalyptus elata Dehnh.	x	x	x	No		
Eucalyptus fastigata H.Deane & Maiden		x	x	No		
Eucalyptus gillii Maiden		x	x	No		
Eucalyptus globoidea Blakely	x	x	x	No		
Eucalyptus globulus Labill. subsp. *globulus*	x	x	x	No		
Eucalyptus globulus subsp. *bicostata* (Maiden, Blakely & Simmonds) J.B.Kirkp.		x	x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Eucalyptus gunnii subsp. *divaricata* (McAulay & Brett) B.M.Potts		x	x	No		
Eucalyptus gunnii subsp. *gunnii.*		x	x	No		
Eucalyptus haemastoma Sm.		x	x	No		
Eucalyptus johnstonii Maiden		x	x	No		
Eucalyptus laevopinea F.Muell. ex R.T.Baker		x	x	No		
Eucalyptus longirostrata (Blakely) L.A.S.Johnson & K.D.Hill		x	x	No		
Eucalyptus melanophloia F.Muell.		x		No		
Eucalyptus morrisbyi Brett		x	x	No		
Eucalyptus nebulosa A.M.Gray		x	x	No		
Eucalyptus nigra F.Muell. ex R.T.Baker		x		No		
Eucalyptus nitens (H.Deane & Maiden) Maiden		x	x	No		
Eucalyptus nitida Hook.f.		x	x	No		
Eucalyptus obliqua L'Hér.		x	x	No		
Eucalyptus olida L.A.S.Johnson & K.D.Hill	x	x	x	No		
Eucalyptus paniculata Sm.	x			No		
Eucalyptus pellita F.Muell.		x	x	No		
Eucalyptus perriniana F.Muell. ex Rodway		x	x	No		
Eucalyptus pilularis Sm.	x	x	x	No		
Eucalyptus planchoniana F.Muell.	x		x	No		
Eucalyptus populnea F.Muell.		x	x	No		
Eucalyptus propinqua H.Deane & Maiden				No		
Eucalyptus pulchella Desf.		x	x	No		
Eucalyptus punctata A.Cunn. ex DC.	x	x	x	No		
Eucalyptus pyrocarpa L.A.S.Johnson & Blaxell	x			No		
Eucalyptus quadrangulata H.Deane & Maiden		x	x	No		
Eucalyptus radiata A.Cunn. ex DC.		x	x	No		
Eucalyptus radiata subsp. *robertsonii* (Blakely) L.A.S.Johnson & Blaxell		x	x	No		
Eucalyptus regnans F.Muell.		x	x	No		
Eucalyptus resinifera subsp. *hemilampra* (F.Muell.) L.A.S.Johnson & K.D.Hill		x	x	No		
Eucalyptus risdonii Hook.f.		x	x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Eucalyptus robusta Sm.	x	x	x	No		
Eucalyptus rodwayi R.T.Baker & H.G.Sm.		x	x	No		
Eucalyptus rubiginosa Brooker				No		
Eucalyptus scias L.A.S.Johnson & K.D.Hill		x		No		
Eucalyptus siderophloia Benth.	x	x	x	No		
Eucalyptus sieberi L.A.S.Johnson		x	x	No		
Eucalyptus smithii F.Muell. ex R.T.Baker		x	x	No		
Eucalyptus tenuiramis Miq.		x	x	No		
Eucalyptus tereticornis Sm.	x	x	x	No		
Eucalyptus tindaliae Blakely	x	x	x	No		
Eucalyptus torquata Luehm.		x	x	No		
Eucalyptus urnigera Hook.f.		x	x	No		
Eucalyptus urophylla S.T.Blake	x	x	x	No		
Eucalyptus vernicosa Hook.f.		x	x	No		
Eucalyptus × *ambigua* DC.		x	x	No		
Eucalyptus andrewsii Maiden			x	No		
Eucalyptus biturbinata L.A.S.Johnson & K.D.Hill		x	x	No		
Eucalyptus camaldulensis subsp. *simulata* Brooker & Kleinig		x	x	No		
Eucalyptus camphora F.Muell. ex R.T.Baker		x	x	No		
Eucalyptus goniocalyx F.Muell. ex Miq.		x	x	No		
Eucalyptus moluccana Wall. ex Roxb.		x	x	No		
Eucalyptus ovata Labill.		x	x	No		
Eucalyptus pauciflora subsp. *pauciflora*		x	x	No		
Eucalyptus rubida subsp. *rubida*		x	x	No		
Eucalyptus subcrenulata Maiden & Blakely		x	x	No		
Eucalyptus viminalis Labill.		x	x	No		
Eucalyptus viminalis subsp. *viminalis*	x	x	x	No		
Eugenia balansae Guillaumin	x			No		
Eugenia brachythrix Urb.				No		
Eugenia brasiliensis Lam.				No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Eugenia brongniartiana Guillaumin	x			No		
Eugenia bullata Pancher ex Guillaumin	x			No		
Eugenia candolleana DC.				No		
Eugenia capensis subsp. *natalitia* (Sond.) F.White	x		x	No		
Eugenia capensis subsp. *zeyheri* (Harv.) F.White	x		x	No		
Eugenia daenikeri Guillaumin	x			No		
Eugenia erythrophylla Strey	x			No		
Eugenia excorticata J.W.Dawson. ined.	x			No		
Eugenia foetida Pers.				No		
Eugenia gacognei Montrouz.	x			No		
Eugenia hurlimannii Guillaumin	*x*			No		
Eugenia involucrata DC.				No		
Eugenia kanakana N.Snow	x			No		
Eugenia koolauensis O.Deg.	x			No		
Eugenia mouensis Baker f.	*x*			No		
Eugenia munzingeri J.W.Dawson. ined.	x			No		
Eugenia noumeensis Guillaumin	x			No		
Eugenia ovigera Brongn. & Gris	x			No		
Eugenia paludosa Pancher ex Brongn. & Gris	x			No		
Eugenia pitanga (O.Berg) Nied.				No		
Eugenia pyriformis Cambess.				No		
Eugenia stipitata McVaugh				No		
Eugenia stricta Pancher ex Brongn. & Gris	x			No		
Eugenia umtamvunensis A.E.van Wyk	x			No		
Eugenia uniflora L.	x	x	x	No		
Eugenia verdoorniae A.E.van Wyk	x			No		
Gossia acmenoides (F.Muell.) N.Snow & Guymer	x		x	No		
Gossia alaternoides (Brongn. & Gris) N.Snow	x			No		
Gossia bamagensis N.Snow & Guymer	x		x	No		
Gossia bidwillii (Benth.) N.Snow & Guymer	x		x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Gossia floribunda (A.J.Scott) N.Snow & Guymer	x		x	No		
Gossia fragrantissima (F.Muell. ex Benth.) N.Snow & Guymer	x		x	No		
Gossia gonoclada (F.Muell. ex Benth.) N.Snow & Guymer	x		x	No		
Gossia hillii (Benth.) N.Snow & Guymer	x		x	No		
Gossia inophloia (J.F.Bailey & C.T.White) N.Snow & Guymer	x		x	No		
Gossia lewisensis N.Snow & Guymer	x		x	No		
Gossia macilwraithensis N.Snow & Guymer	x		x	No		
Gossia myrsinocarpa (F.Muell.) N.Snow & Guymer	x		x	No		
Gossia pubiflora (C.T.White) N.Snow & Guymer	x		x	No		
Gossia punctata N.Snow & Guymer	x		x	No		
Heteropyxis canescens Oliv.	x			No		
Heteropyxis natalensis Harv.	x	x		No		
Homoranthus croftianus J.T.Hunter	x		x	No		
Homoranthus flavescens Schauer	x		x	No		
Homoranthus melanostictus Craven & S.R.Jones	x		x	No		
Homoranthus montanus Craven & S.R.Jones	x		x	No		
Homoranthus papillatus Byrnes	x		x	No		
Homoranthus prolixus Craven & S.R.Jones	x		x	No		
Homoranthus virgatus A.Cunn. ex Schauer	x		x	No		
Kunzea pomifera F.Muell.		x	x	No		
Kunzea linearis (Kirk) de Lange & Toelken		x		No		
Kunzea robusta de Lange & Toelken		x		No		
Lenwebbia lasioclada (F.Muell.) N.Snow & Guymer	x		x	No		
Lenwebbia prominens N.Snow & Guymer	x		x	No		
Leptospermum barneyense A.R.Bean	x		x	No		
Leptospermum brachyandrum (F.Muell.) Druce	x		x	No		
Leptospermum continentale 'cv. Horizontalis'		x	x	No		
Leptospermum continentale Joy Thomps.	x		x	No		
Leptospermum deuense Joy Thomps.	x		x	No		
Leptospermum glaucescens Schauer		x	x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Leptospermum grandiflorum Lodd.		x	x	No		
Leptospermum juniperinum Sm.	x		x	No		
Leptospermum lanigerum (Aiton) Sm.		x	x	No		
Leptospermum liversidgei R.T.Baker & H.G.Sm.	x		x	No		
Leptospermum luehmannii F.M.Bailey	x		x	No		
Leptospermum morrisonii Joy Thomps.		x	x	No		
Leptospermum myrsinoides Schltdl.		x	x	No		
Leptospermum nitidum Hook.f.		x	x	No		
Leptospermum petersonii F.M.Bailey	x		x	No		
Leptospermum polygalifolium Salisb.		x	x	No		
Leptospermum riparium D.I.Morris		x	x	No		
Leptospermum rupestre Hook.f.		x	x	No		
Leptospermum scoparium J.R.Forst. & G.Forst.	x	x	x	No		
Leptospermum scoparium x L. macrocarpum		*x*	x	No		
Leptospermum semibaccatum Cheel	x		x	No		
Leptospermum spectabile Joy Thomps.	x		x	No		
Leptospermum trinervium (Sm.) Joy Thomps.	x	x	x	No		
Leptospermum whitei Cheel	x		x	No		
Leptospermum wooroonooran F.M.Bailey	x		x	No		
Leptospermum madidum subsp. s*ativum* A.R.Bean	x		x	No		
Lindsayomyrtus racemoides (Greves) Craven	x	x	x	No		
Lithomyrtus obtusa (Endl.) N.Snow & Guymer	x		x	No		
Lophomyrtus bullata Burret	x		x	No		
Lophomyrtus obcordata (Raoul) Burret	x			No		
Lophomyrtus × ralphii (Hook.f.) Burret (hybrid *L. bullata* × *L. obcordata*)	x		x	No		
Lophostemon suaveolens (Sol. ex Gaertn.) Peter G.Wilson & J.T.Waterh.	x	x	x	No		
Melaleuca alternifolia (Maiden & Betche) Cheel	x	x	x	No		
Melaleuca biconvexa Byrnes	x	x	x	No		
Melaleuca cajuputi subsp. *platyphylla* Barlow		x		No		
Melaleuca decora (Salisb.) Britten	x	x	x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Melaleuca ericifolia Sm.		x	x	No		
Melaleuca fluviatilis Barlow	x		x	No		
Melaleuca gibbosa Labill.		x	x	No		
Melaleuca gilesii (F.Muell.) Craven & R.D.Edwards	x		x	No		
Melaleuca howeana Cheel		x	x	No		
Melaleuca hypericifolia Sm.				No		
Melaleuca lophantha (Vent.) ined..	x		x	No		
Melaleuca nodosa (Sol. ex Gaertn.) Sm.	x		x	No		
Melaleuca pustulata Hook.f.		x	x	No		
Melaleuca quadrifida (R.Br.) Craven & R.D.Edwards	x	x	x	No		
Melaleuca quadrifida subsp. *aspera* (Turcz.) Craven & R.D.Edwards	x		x	No		
Melaleuca saligna Schauer	x		x	No		
Melaleuca sieberi Schauer	x		x	No		
Melaleuca squamea Labill.		x	x	No		
Melaleuca squarrosa Sm.		x	x	No		
Melaleuca styphelioides Sm.	x		x	No		
Melaleuca torulosa (Schauer) Craven & R.D.Edwards	x		x	No		
Melaleuca formosa (S.T.Blake) Craven	x		x	No		
Melaleuca linearifolia (Link) Craven		x	x	No		
Melaleuca linearis var. *acerosa* (Tausch) ined.	x		x	No		
Melaleuca linearis var. *linearis*	x		x	No		
Melaleuca pachyphylla (Cheel) Craven	x		x	No		
Melaleuca pallida (Bonpl.) Craven		x	x	No		
Melaleuca paludicola Craven	x		x	No		
Melaleuca polandii (F.M.Bailey) Craven	x		x	No		
Melaleuca virens Craven	x		x	No		
Metrosideros bartlettii J.W.Dawson	x			No		
Metrosideros bartlettii J.W.Dawson x *Metrosideros robusta* A.Cunn	x			No		
Metrosideros brevistylis J.W.Dawson	x			No		
Metrosideros carminea W.R.B.Oliv.	x		x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Metrosideros collina (J.R.Forst. & G.Forst.) A.Gray	x		x	No		
Metrosideros collina (J.R.Forst. & G.Forst.) A.Gray x *Metrosideros excelsa* Sol. ex Gaertn.	x			No		
Metrosideros collina var. *thomasii*	x		x	No		
Metrosideros diffusa (G.Forst.) Sm.	x			No		
Metrosideros elegans (Montrouz.) Beauvis.	x			No		
Metrosideros excelsa Sol. ex Gaertn.	x	x	x	No		
Metrosideros excelsa Sol. ex Gaertn. x *Metrosideros kermadecensis* W.R.B.Oliv.	x			No		
Metrosideros excelsa Sol. ex Gaertn. x *Metrosideros robusta* A.Cunn	x			No		
Metrosideros fulgens Sol. ex Gaertn.	x			No		
Metrosideros kermadecensis W.R.B.Oliv.	x		x	No		
Metrosideros laurifolia Brongn. & Gris	x			No		
Metrosideros nervulosa C.Moore & F.Muell.		x	x	No		
Metrosideros nitida Brongn. & Gris	x			No		
Metrosideros operculata Labill.	x			No		
Metrosideros operculata Labill. var. *francii* J.W.Dawson	x			No		
Metrosideros operculata Labill. var. *operculata*	x			No		
Metrosideros perforata (J.R.Forst. & G.Forst.) Druce	x			No		
Metrosideros polymorpha Gaudich.	x			No		
Metrosideros punctata J.W.Dawson	x			No		
Metrosideros robusta A.Cunn.	x			No		
Metrosideros sclerocarpa J.W.Dawson		x	x	No		
Metrosideros vitiensis (A.Gray) Pillon	x		x	No		
Metrosideros collina var. villosa (L.f.) A.Gray	x		x	No		
Mitrantia bilocularis Peter G.Wilson & B.Hyland	x		x	No		
Myrcia splendens (Sw.) DC.				No		
Myrcia stenocarpa Krug & Urb.				No		
Myrcia xylopioides (Kunth) DC.				No		
Myrcianthes fragrans (Sw.) McVaugh		x		No		
Myrcianthes pungens (O.Berg) D.Legrand	x	x		No		
Myrrhinium atropurpureum var. octandrum Benth.	*x*	*x*		No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Myrtastrum rufopunctatum (Pancher ex Brongn. & Gris) Burret	x			No		
Myrtus communis L.	x	x	x	No		
Neofabricia myrtifolia (Gaertn.) Joy Thomps.	x	x	x	No		
Pilidiostigma glabrum Burret	x	x	x	No		
Pilidiostigma rhytispermum (F.Muell.) Burret	x		x	No		
Pilidiostigma tetramerum L.S.Sm.	x		x	No		
Pimenta dioica (L.) Merr.	x	x	x	No		
Pimenta racemosa (Mill.) J.W.Moore				No		
Plinia cauliflora (Mart.) Kausel	x	x	x	No		
Plinia edulis (Vell.) Sobral				No		
Psidium grandifolium Mart. ex DC.				No		
Rhodamnia acuminata C.T.White	x		x	No		
Rhodamnia angustifolia N.Snow & Guymer	x		x	No		
Rhodamnia arenaria N.Snow	x		x	No		
Rhodamnia argentea Benth.	x		x	No		
Rhodamnia australis A.J.Scott	x		x	No		
Rhodamnia blairiana F.Muell.	x		x	No		
Rhodamnia costata A.J.Scott	x		x	No		
Rhodamnia dumicola Guymer & Jessup	x		x	No		
Rhodamnia glabrescens Guymer & Jessup	x		x	No		
Rhodamnia glauca Blume	x		x	No		
Rhodamnia longisepala N.Snow & A.J.Ford	x		x	No		
Rhodamnia maideniana C.T.White	x		x	No		
Rhodamnia pauciovulata Guymer	x		x	No		
Rhodamnia rubescens (Benth.) Miq.	x	x	x	No		
Rhodamnia sessiliflora Benth.	x		x	No		
Rhodamnia whiteana Guymer & Jessup	x		x	No		
Rhodomyrtus effusa Guymer	x		x	No		
Rhodomyrtus macrocarpa Benth.	x		x	No		
Rhodomyrtus pervagata Guymer	x		x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Rhodomyrtus psidioides (G.Don) Benth.	x	x	x	No		
Rhodomyrtus sericea Burret	x		x	No		
Rhodomyrtus tomentosa (Aiton) Hassk.	x		x	No		
Rhodomyrtus trineura subsp. *capensis* Guymer	x		x	No		
Rhodomyrtus trineura (F.Muell.) Benth.	x		x	No		
Rhodomyrtus trineura var. *canescens* (C.T.White) A.J.Scott	x		x	No		
Ristantia pachysperma (F.M.Bailey) Peter G.Wilson & J.T.Waterh.	x		x	No		
Ristantia waterhousei Peter G.Wilson & B.Hyland	x		x	No		
Sannantha leratii (Schltr.) Peter G.Wilson	x			No		
Sannantha procera (J.W.Dawson) Peter G.Wilson	x			No		
Sphaerantia discolor Peter G.Wilson & B.Hyland	x		x	No		
Stereocaryum neocaledonicum (Brongn. & Gris) A.J.Scott	x			No		
Stockwellia quadrifida D.J.Carr, S.G.M.Carr & B.Hyland	x		x	No		
Syncarpia hillii F.M.Bailey		x	x	No		
Syzygium acre (Pancher ex Guillaumin) J.W.Dawson	x			No		
Syzygium alatoramulum B.Hyland	x	x	x	No		
Syzygium anisatum (Vickery) Craven & Biffin	x	x	x	No		
Syzygium apodophyllum (F.Muell.) B.Hyland	x		x	No		
Syzygium aqueum (Burm.f.) Alston	x		x	No		
Syzygium argyropedicum B.Hyland	x		x	No		
Syzygium armstrongii (Benth.) B.Hyland	x		x	No		
Syzygium australe (J.C.Wendl. ex Link) B.Hyland	x	x	x	No		
Syzygium australe (J.C.Wendl. ex Link) B.Hyland x *Syzygium paniculatum Gaertn.*	x			No		
Syzygium bamagense B.Hyland	x		x	No		
Syzygium banksii (Britten & S.Moore ex S.Moore) B.Hyland	x		x	No		
Syzygium baudouinii (Brongn. & Gris) N.Snow, Byng & J.W.Dawson	x			No		
Syzygium boonjee B.Hyland	x		x	No		
Syzygium brongniartii (Merr. & L.M.Perry) J.W.Dawson	x			No		
Syzygium buettnerianum (K.Schum.) Nied.	x		x	No		
Syzygium bungadinnia (F.M.Bailey) B.Hyland	x		x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Syzygium canicortex B.Hyland	x		x	No		
Syzygium claviflorum (Roxb.) Wall. ex A.M.Cowan & Cowan	x		x	No		
Syzygium coarctatum (Blume) Byng, N.Snow & Peter G.Wilson	x		x	No		
Syzygium cordatum Hochst. ex Krauss	x			No		
Syzygium cormiflorum (F.Muell.) B.Hyland	x		x	No		
Syzygium corynanthum (F.Muell.) L.A.S.Johnson	x		x	No		
Syzygium crebrinerve (C.T.White) L.A.S.Johnson	x		x	No		
Syzygium cryptophlebium (F.Muell.) Craven & Biffin	x		x	No		
Syzygium cumini (L.) Skeels	x	x	x	No		
Syzygium dansiei B.Hyland	x		x	No		
Syzygium endophloium B.Hyland	x		x	No		
Syzygium erythrocalyx (C.T.White) B.Hyland	x		x	No		
Syzygium erythrodoxum (S.Moore) B.Hyland	x		x	No		
Syzygium eucalyptoides (F.Muell.) B.Hyland	x		x	No		
Syzygium fibrosum (F.M.Bailey) T.G.Hartley & L.M.Perry		x	x	No		
Syzygium floribundum F.Muell.	x	x	x	No		
Syzygium forte (F.Muell.) B.Hyland subsp. *forte*	x		x	No		
Syzygium francisii (F.M.Bailey) L.A.S.Johnson		x	x	No		
Syzygium frutescens Brongn. & Gris	x			No		
Syzygium fullagarii (F.Muell.) Craven		x	x	No		
Syzygium glenum Craven	x		x	No		
Syzygium gracilipes (A.Gray) Merr. & L.M.Perry				No		
Syzygium grande (Wight) Walp.	x			No		
Syzygium graveolens (F.M.Bailey) Craven & Biffin	x		x	No		
Syzygium hedraiophyllum (F.Muell.) Craven & Biffin	x		x	No		
Syzygium hemilamprum (F.Muell.) Craven & Biffin	x		x	No		
Syzygium hodgkinsoniae (F.Muell.) L.A.S.Johnson	x		x	No		
Syzygium ingens (F.Muell. ex C.Moore) Craven & Biffin	x		x	No		
Syzygium kuranda (F.M.Bailey) B.Hyland	x		x	No		
Syzygium legatii Burtt Davy & Greenway in J.B.Davy				No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Syzygium longifolium (Brongn. & Gris) J.W.Dawson	x			No		
Syzygium luehmannii (F.Muell.) L.A.S.Johnson	x	x	x	No		
Syzygium macilwraithianum B.Hyland	x		x	No		
Syzygium macranthum Brongn. & Gris	x			No		
Syzygium maire (A.Cunn.) Sykes & Garn.-Jones	x			No		
Syzygium maraca Craven & Biffin	x		x	No		
Syzygium minutiflorum Miq.	x		x	No		
Syzygium moorei (F.Muell.) L.A.S.Johnson	x		x	No		
Syzygium mouanum Guillaumin	x			No		
Syzygium mulgraveanum (B.Hyland) Craven & Biffin				No		
Syzygium neoeugenioides N.Snow, Byng & J.W.Dawson	x			No		
Syzygium ngoyense (Schltr.) Guillaumin	x			No		
Syzygium oleosum (F.Muell.) B.Hyland	x	x	x	No		
Syzygium pancheri Brongn. & Gris	x			No		
Syzygium paniculatum Gaertn.	x		x	No		
Syzygium polyanthum (Wight) Walp	x		x	No		
Syzygium pseudofastigiatum B.Hyland	x		x	No		
Syzygium puberulum Merr. & L.M.Perry	x		x	No		
Syzygium resa (B.Hyland) Craven & Biffin	x		x	No		
Syzygium rubrimolle B.Hyland	x		x	No		
Syzygium samarangense (Blume) Merr. & L.M.Perry				No		
Syzygium sandwicense (A.Gray) Müll.Stuttg.	x			No		
Syzygium smithii (Poir.) Nied.	x	x	x	No		
Syzygium tierneyanum (F.Muell.) T.G.Hartley & L.M.Perry	x		x	No		
Syzygium unipunctatum (B.Hyland) Craven & Biffin				No		
Syzygium velarum B.Hyland	x		x	No		
Syzygium wagapense Brongn. & Gris	x			No		
Syzygium wilsonii (F.Muell.) B.Hyland	x		x	No		
Syzygium wilsonii (F.Muell.) B.Hyland x *Syzygium luehmannii* (F.Muell.) L.A.S.Johnson	x	x	x	No		
Syzygium xerampelinum B.Hyland	x		x	No		

Taxa*	Natural infection*	Artificial infection*	Recorded as infected in Australia*	Occurs in WA^	Region^	WA Status (Priority, Alien)^
Thaleropia queenslandica (L.S.Sm.) Peter G.Wilson	x		x	No		
Thryptomene calycina (Lindl.) Stapf	x	x	x	No		
Tristania neriifolia (Sims) R.Br.	x	x	x	No		
Tristaniopsis callobuxus Brongn. & Gris	x			No		
Tristaniopsis collina Peter G.Wilson & J.T.Waterh.	x		x	No		
Tristaniopsis exiliflora (F.Muell.) Peter G.Wilson & J.T.Waterh.	x		x	No		
Tristaniopsis glauca Brongn. & Gris	x			No		
Tristaniopsis guillainii Vieill. ex Brongn. & Gris	x			No		
Tristaniopsis laurina (Sm.) Peter G.Wilson & J.T.Waterh.	x	x	x	No		
Tristaniopsis polyandra (Guillaumin) Peter G.Wilson & J.T.Waterh.	x			No		
Tristaniopsis reticulata J.W.Dawson	x			No		
Ugni molinae Turcz.	x		x	No		
Uromyrtus australis A.J.Scott	x		x	No		
Uromyrtus emarginata (Pancher ex Baker f.) Burret	x			No		
Uromyrtus lamingtonensis N.Snow & Guymer D	x		x	No		
Uromyrtus metrosideros (F.M.Bailey) A.J.Scott	x		x	No		
Uromyrtus tenella N.Snow & Guymer	x		x	No		
Xanthostemon aurantiacus (Brongn. & Gris) Schltr.	x			No		
Xanthostemon chrysanthus (F.Muell.) Benth.	x	x	x	No		
Xanthostemon formosus Peter G.Wilson	x		x	No		
Xanthostemon fruticosus Peter G.Wilson & Co	x		x	No		
Xanthostemon graniticus Peter G.Wilson	x		x	No		
Xanthostemon laurinus (Pamp.) Guillaumin	x			No		
Xanthostemon oppositifolius F.M.Bail	x		x	No		
Xanthostemon vieillardii (Brongn. & Gris) Nied.	x			No		
Xanthostemon youngii C.T.White & W.D.Francis	x		x	No		

Appendix 2: *Austropuccinia psidii* risk mapping for Western Australia.

Myrtle rust (*Austropuccinia psidii*) is known to affect 524 species in the Myrtaceae family (Soewarto et al. 2019). Eighty-four of the species in the host list are present in Western Australia as native or alien species (Florabase 2021), with additional cultivated species not listed but expected to occur in the state (such as *Psidium guajava; P. guineense; P. cattleyanum;* and *Syzygium jambos*).

A map was produced with the occurrence of known hosts in Western Australia (Figure A1), based on the intersection of the known host list (Soewarto et al. 2019) and online data available (GBIF 2021). It is highly likely that other Western Australian Myrtaceae species are also susceptible, but have not yet been tested, therefore, a comprehensive map of all Myrtaceae species in Western Australia has been reproduced here (Figure A2, Florabase 2021).

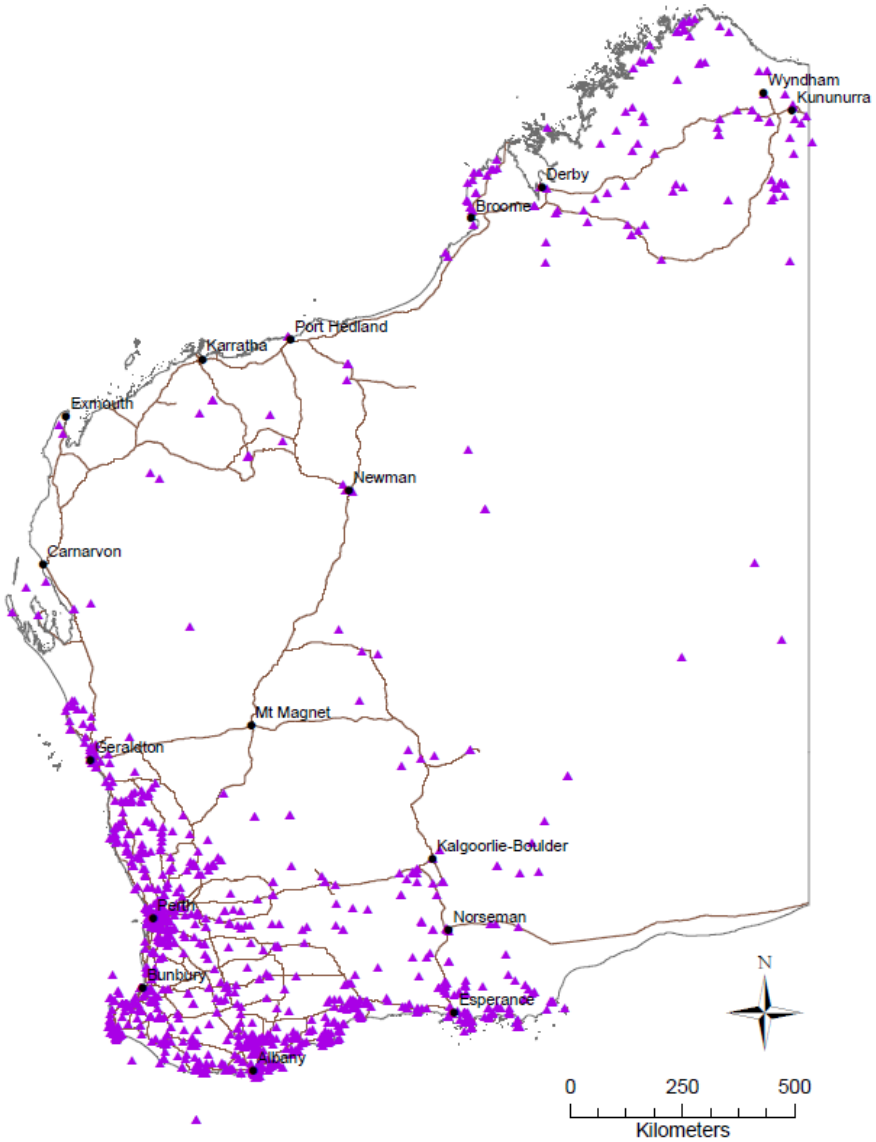

Figure A2.1: Occurrence of known hosts of myrtle rust in Western Australia. Map created with species data extracted from GBIF (2021), and not considered a complete representation of true occurrences.

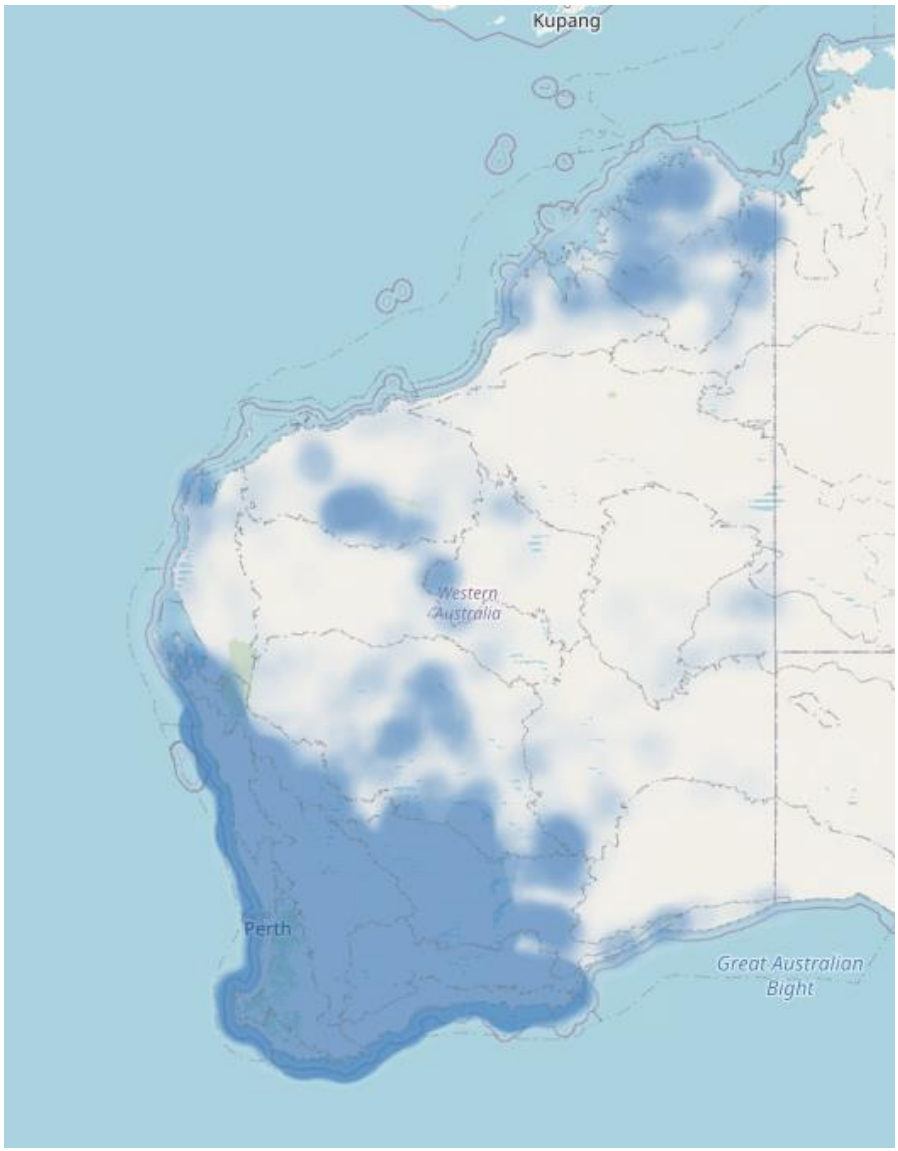

Figure A2.2: Occurrence of all Myrtaceae species in Western Australia. Map reproduced from Florabase (2021; Leaflet | Map data @ OpenStreetMap contributors).

Vegetation descriptions at the association level (National Vegetation Information Systems Level 5) are available as a publicly available map with associated features table (DPIRD 2021). The dominant species of each strata in the vegetation description were compared against the species list which are known to be susceptible to myrtle rust (Soewarto et al. 2019) to create a risk map of the vegetation associations which have susceptible dominant species (Figure A3). These are considered areas that would have significant ecological and landscape changes were myrtle rust to occur.

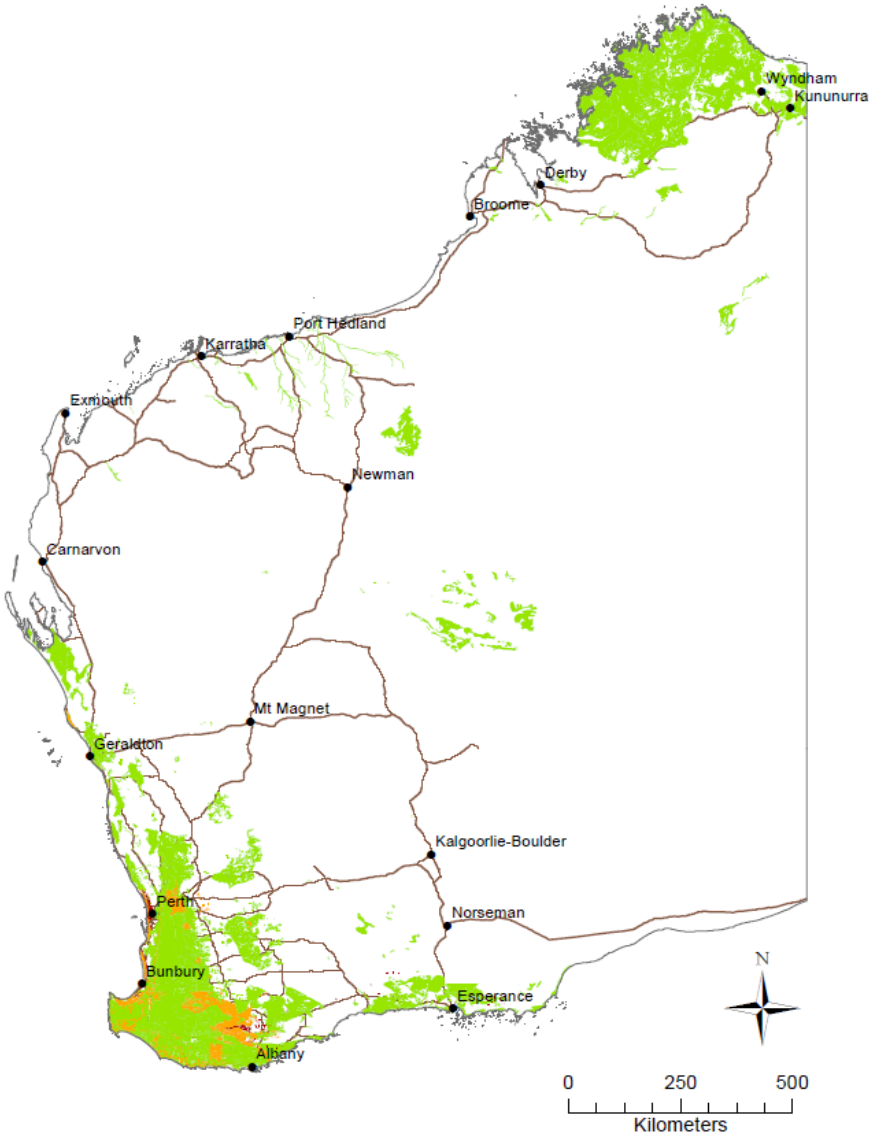

Figure A2.3: Western Australian vegetation with one (green), two (orange) or three (red) susceptible species listed in the vegetation associations descriptions (DPIRD 2021).

Appendix 3: Genetic testing for *Austropuccinia psidii* (myrtle rust).

Testing for single cell levels of *Austropuccinia psidii*, myrtle rust, was developed to detect invisible (pre-symptom) traces of DNA. The test uses three targets that are specific to *A. psidii*, meaning the test will only work with that species of rust. The test provides confidence and confirmation of *A. psidii* through the combination of the specific targets. If any one target were to cross react with a non-target DNA, no result would be obtained, all three targets are required for a positive result.

The assay is performed using real-time PCR (QPCR) meaning that time from sampling to result is minimized. There were several reports of using QPCR for *A. psidii* detection, but none have the sensitivity or speed of the current developed assay. The three previous studies which use QPCR for *A. psidii* use a technique called SYBR Green, which uses two targets for detection. It is less sensitive, less specific, and prone to errors. The current assay uses a QPCR technique called TaqMan, which is more sensitive and specific and uses an additional target for greater accuracy.

Primers were used that target the beta-tubulin (BTub) gene of *A. psidii* which were originally developed by Bini *et al.* (2018). These primers were searched against the NCBI database using BLAST, which searches for close matches to the query sequences. A new *A. psidii* whole genome shotgun sequence was identified (Tobias - CACRXL010000018). By comparing the BTub primers against the sequence, the targeted amplified genomic sequence (the DNA between the two primers) was determined and based on that sequence a new marker was developed. An internal positive control sample was purchased from ThermoFisher to include with the test.

Additionally, a synthetic standard was developed (Conte 2018). This standard contains the primers and probe sequences and acts as a control to show the test is working, as well as to provide accurate quantification of the sample. The standard has interspaced synthetic DNA that means that it cannot be confused with a real *A. psidii* sequence, if a suspected contamination event occurs. The standard is also accurately quantified down to the single copy, so can be used as an absolute quantification indicator for *A. psidii*. The primer, probe and standard details can be found in Table A3.1.

Testing shows a good detection limit with all 10 copy samples showing a product, but only 25% of 1 copy samples showing amplification (Figure A3.1). With further optimisation, the 1 copy samples should show a product in at least 50% of the reactions (due to variation in distribution of the DNA in a fluid). The standard curve (Figure A3.2) shows a reaction efficiency of 97% (anything above 80% is considered a good assay and 100% indicates a doubling of product at every cycle). The internal positive control (Figure A3.3) is showing amplification in all samples at approximately the same rate, indicating that no inhibition is shown from any level of DNA tested.

Table A3.1: The Primers, probes and standards for myrtle rust testing.

Primer/Probe	Sequence	Source
BTub1 (forward)	GGACTCTGTTTTAGATGTCGTC	Bini (2018)
BTub3 (reverse)	TTGATGGACTGATAGGGTAGCG	Bini (2018)
A. psidii 637 P Probe	/56-FAM/ACCTTCGGG/ZEN/GATGGAACAAC/3IABkFQ/	This study
Synthetic Standard	tgcatgatctacgtgcgtcacatgcagtacTTGATGGACTGATAGGGTAGCGtagtaatgcagacac ttgcggtccatcACCTTCGGGGATGGAACAACgctgtcagcactactaacttgcggtcagtgactgat gctcagtgagttactacgcagtcactcatatctggtgatacatgaacagatccgtgcaccgtcacacttgcggtc catcGCTGAGGGCTGTGATTGTCTacttgatgaGACGACATCTAAAACAGAGTCCcactag ctcagattcagtagaccgctgttg	This study
Probe 2 Not ordered	GCTGAGGGCTGTGATTGTCT – not tested	This study

Notes: 56-FAM – 5’ 6-FAM™; ZEN – Internal ZEN™; 3IABkFQ – 3’ Iowa Black® FQ; Yellow – primers; Green - A. psidii 637 P; Red – potential other probe site.

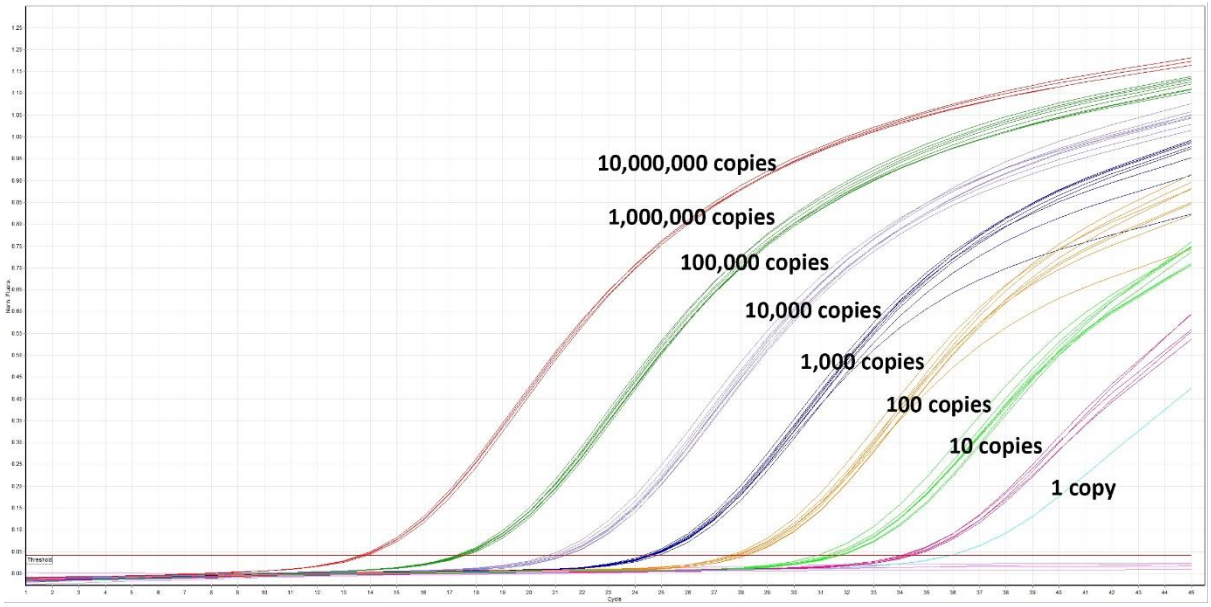

Figure A3.1: The detection limit of the assay.

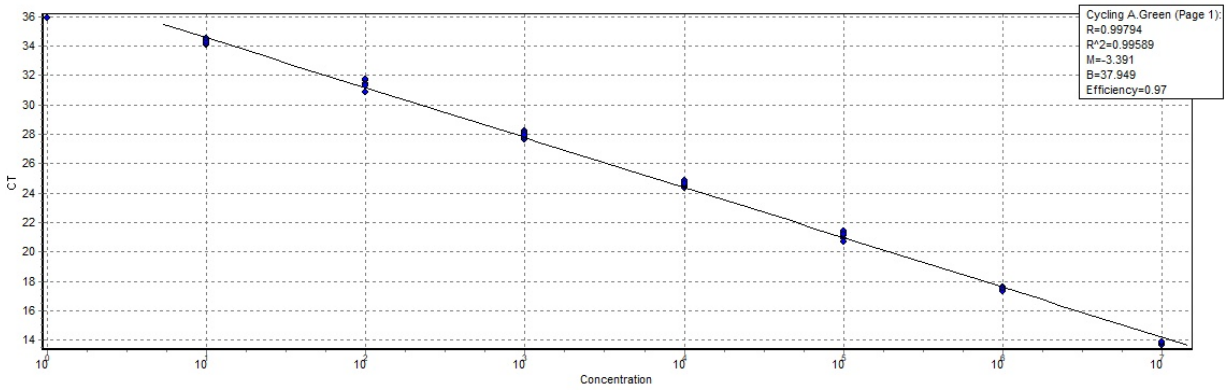

Figure A3.2:P Standard Curve.

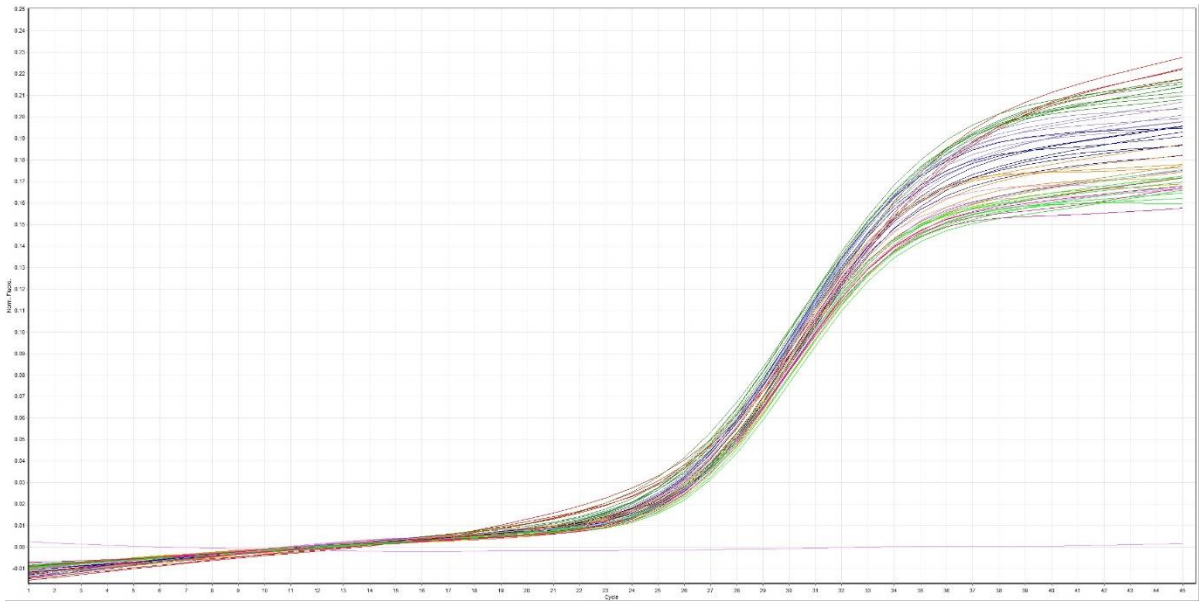

Figure A3.3: The internal positive control (IPC).

The developed test is rapid, taking only 13 minutes of cycling (although the total run takes 75 minutes), whereas the Bini test took 60 minutes to cycle, making the overall test take upward, or over, 2.5 hours. The added accuracy of having the three specific targets makes the developed test superior in accurate detection *A. psidii*. The sensitivity is several magnitudes greater than previous assays (although they only listed the sensitivity as a fraction of weight, which is not an accurate method).

Knowing how much DNA is in the sample can advise on containment or eradication strategies. There is currently no indication of the relationship between spore loads of *A. psidii* and infection rates, but the developed assay should be able to answer that with further study.

The next steps in validation will be to send the developed assay to laboratories in Queensland, where there are significant *A. psidii* infections. Pre-made master mix and standard can be shipped to those labs relatively easily. Following interlaboratory testing the next steps will be to test environmental DNA mass screening strategies, but more funding will be required to carry out this aspect, with cooperation from researchers in the areas where *A. psidii* is active.