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Surveillance Plan and Testing Preparedness against Myrtle Rust in Western Australia

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1. Executive Summary

Austropuccinia psidii (commonly known as myrtle rust) is a rust fungus with 524 hosts in the Myrtaceae family identified to date, which is native to Brazil and neighbouring countries, and was introduced to Australia in 2010.

The state of Western Australia is currently free of myrtle rust, despite having regions identified as climatically suitable for its establishment in previous modelling. Western Australia's flora is rich in Myrtaceae, with 1959 taxa – 84 of which are known to be susceptible to myrtle rust, but many more likely to be susceptible once the testing is undertaken or the pathogen enters the state.

The recognised pathways of introduction of myrtle rust to Western Australia are wind (including cyclonic winds) and the movement of people and their clothing via roads, airports, or ports. Animals are possible vectors of myrtle rust spores, but the travel distances needed to bring the rust to Western Australia from known infested sites precludes most species from being vectors into the state, with migratory birds being a notable exception. Finally, the high-risk pathway of nursery and cut flower trade has been regulated since 2011, with no Myrtaceous species movement from other States and territories into Western Australia.

Myrtle rust surveillance and detection has so far relied heavily on opportunistic visual inspections or surveillance efforts that are not nationally coordinated or formally structured. Detection of leaf lesions or sporulation implies that the disease is already established, and in such cases, eradication or control may be beyond reach. Molecular diagnostics of myrtle rust have to date focused on confirmation of visual diagnostics and identifying the strain of myrtle rust rather than being used as a surveillance tool.

Our research aims were to: develop a Surveillance Plan for Western Australia overlapping climate suitability, host species, and pathways; and to develop a forensic-level assay for the detection and identification of myrtle rust that could be used in eDNA sampling.

The Surveillance Plan was developed, including consultation with key stakeholders in the state, and includes the background information and practical guidance for officers to deploy it before the arrival of myrtle rust to the state. Within it are approaches for visual and eDNA surveillance, including a highly sensitive and fast molecular assay that can detect as few as 1-10 copies of DNA in a sample.

The Surveillance Plan and molecular assay assist Western Australia's preparedness against *Austropuccinia psidii* and, used in conjunction with the State's Contingency Plan, give the state an advantage for achieving early detection and rapid response against a pathogen that is a known risk to biodiversity, and consequently, to ecosystem processes.

2. Introduction

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 (Narouei-Khandan et al. 2020). The worldwide distribution of myrtle rust is shown in Figure 1.

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; Westaway 2016, 2018), while South Australia and Western Australia remain free from the rust (Berthon et al 2018). The presence records of myrtle rust in Australia are shown in Figure 2.

There is currently only one strain of *A. psidii* present within Western Pacific and Australasian regions as of June 2020, but other strains present in South and Central America and South Africa exist and seriously affect eucalyptus species (Makinson et al. 2020).

Myrtle rust is a known risk to biodiversity, bringing species to near extinction (Fensham et al. 2020). The risk to Western Australia should be considered in the context of the already existing threats of habitat destruction and fragmentation, *Phytophthora cinnamomic* dieback and climate change. Further, Myrtaceae species are key in the tree/canopy component of vegetations over the entire state (DPIRD 2021). Canopy decline could lead to landscape-scale ecosystem changes, such as an impact on trophic webs, changes in depth of water table and subsequent soil quality, and even changes to rainfall patterns.

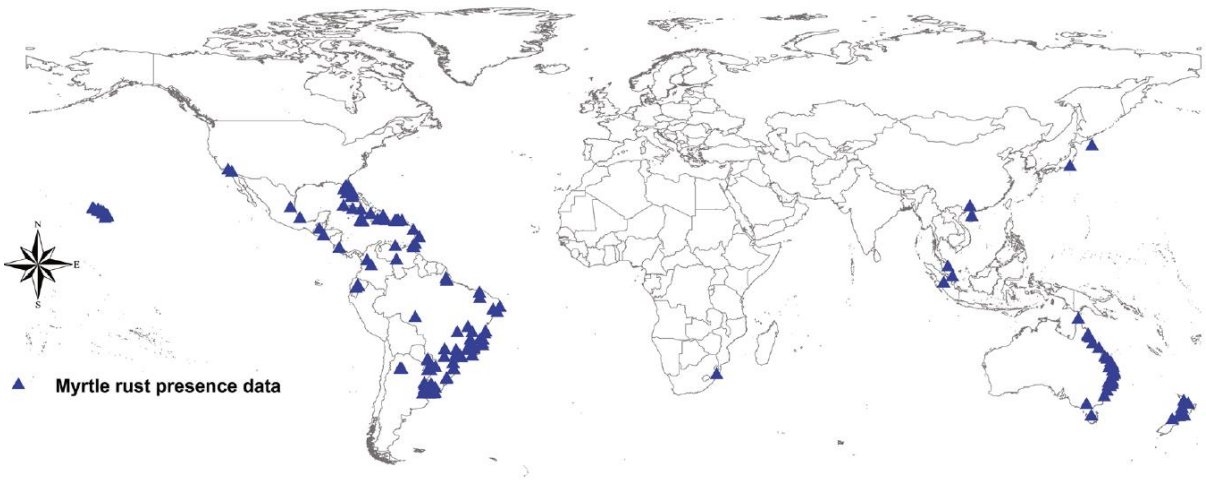

Figure 1: Global distribution of myrtle rust caused by *Austropuccinia psidii* (reproduced from Narouei-Khandan et al. 2020). Note that the Northern Territory records (since 2015) are not shown in the map.

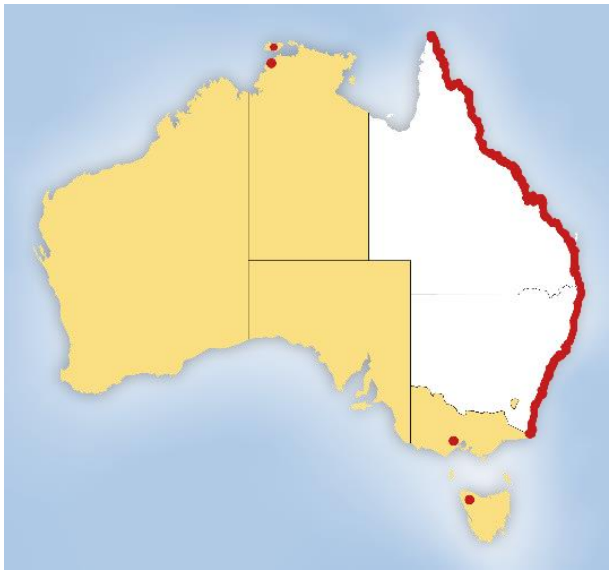

Figure 2: Australian distribution of myrtle rust in red (reproduced from DBCA 2022, adapted from Government of Queensland occurrence map).

Previous climatic suitability modelling

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).

Modelling developed in 2013 (Kriticos et al., Figure 3) used CLIMEX, a mechanistic niche model that is process-oriented and used to predict the likely distribution and abundance of a given species in relation to climate. At that time, however, the known occurrences of myrtle rust in Australia did not extend to the Northern Territory and to Tasmania, the first not identified in the model as having high suitability. As such, the output can be considered precise but outdated, and not sufficiently extensive.

In contrast, the modelling undertaken by Singh et al. in 2016 (Figure 4) used the Climatch tool, a simpler and more correlative approach, that incorporated data on Australian and overseas locations of myrtle rust to predict areas that are highly suitable climatically for this disease (Singh et al. 2016). Climatch uses a simple matching algorithm that correlates climatic conditions in locations where the species is present, with climatic conditions of the targeted area. Despite being run in 2016, after the records in the Norther Territory and Tasmania had been made, these were not included in the model, neither were records from Victoria, because it was believed at that time that outside of New South Wales and Queensland, myrtle rust did not occur outside nurseries and gardens (Singh et al. 2016).

Finally, in 2019 (Narouei-Khandan et al.), several models were run to assess the climatic suitability for myrtle rust in New Zealand: CLIMEX, MaxENT, and Multi-Model Frameworks (of which the best performing model was the Support Vector Machine model). In Australia, all models projected coastal areas in eastern Australia as suitable, which matched the 2018 known distribution of the pathogen, and small areas in Western Australia were projected as highly suitable by all three models where there have been no reports of the pathogen (Narouei-Khandan et al. 2019). The authors referred to publications and experts from which the occurrence data was compiled but did not list them, and it is unknown whether they included occurrences outside of Queensland and New South Wales.

Despite the infestations in the Northern Territory, Tasmania, and Victoria being less severe than those in Queensland and New South Wales, and somewhat restricted to nurseries and private gardens, they have also spread beyond metropolitan areas into public parks (Victoria State Government 2022) and into regional areas (Westaway 2018). One challenge encountered while undertaking this project was a lack of centralised databases and a scarcity of surveillance data on presence and absence of myrtle rust in each of the states. It is possible that the data exists and was unavailable to this project, but likely that it has not been compiled since myrtle rust was declared ineradicable from each state.

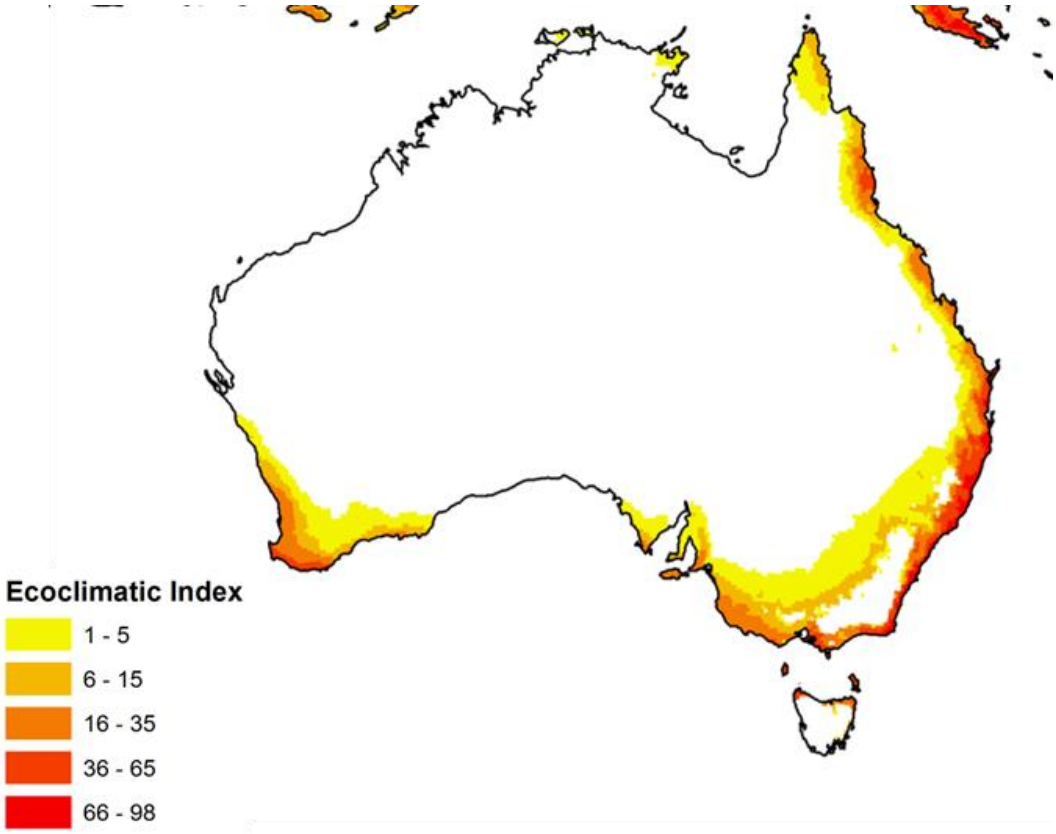

Figure 3: Climex model (modified from Kriticos et al. 2013) showing suitable climatic conditions for the occurrence of myrtle rust. Lowest ecoclimatic indices indicate lower suitability, and higher indices indicate higher suitability.

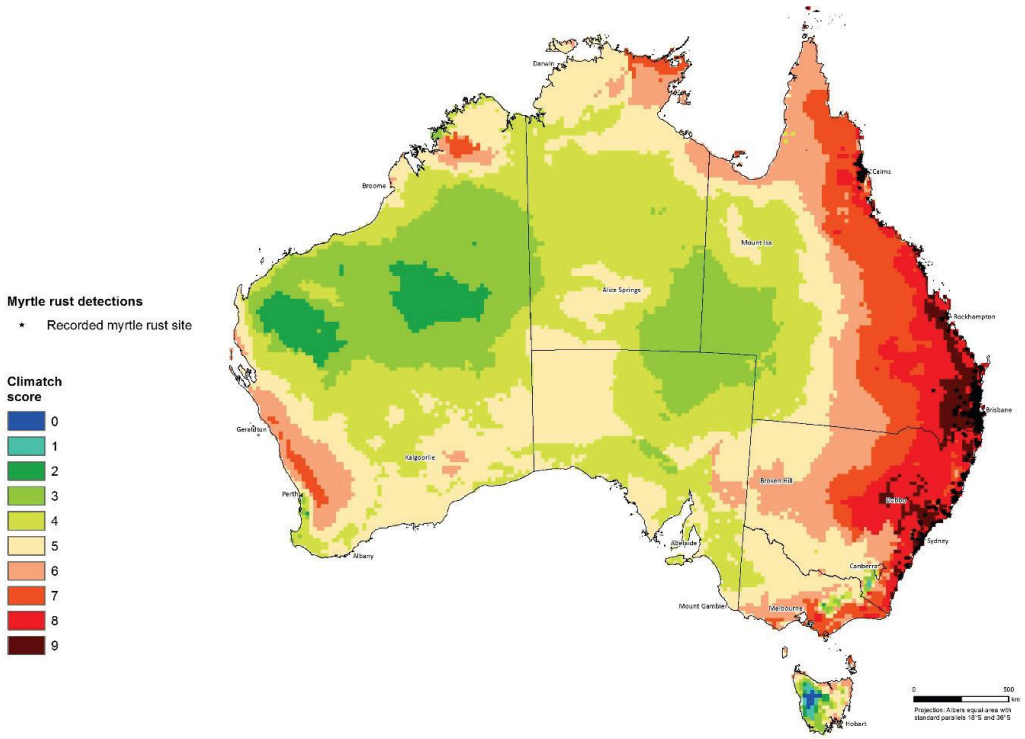

Figure 4: Climatch model (reproduced from Singh et al. 2016) showing areas considered climatically suitable (score 6 and higher) and highly climatically suitable (score 8 and higher) for the occurrence of myrtle rust.

Hosts in Western Australia

Western Australia has a very rich Myrtaceae flora, with 1959 Myrtaceae taxa (species, subspecies, varieties, and crosses), of which 1876 are native to the state (Florabase 2022). Of all Myrtaceae taxa, 139 are present in Beard's Northern Province, and 1535 are present in Beard's South-West Province (Florabase 2022).

Eighty-four taxa (including 4 Priority species and 17 alien species) which occur in Western Australia are known to be susceptible to myrtle rust based on the most recently compiled host list (Soewarto et al. 2019, Appendix 1 of the Enclosure). It is expected that many Western Australian endemic species could also be susceptible to myrtle rust but haven't been tested yet.

Pathways

The plant nursery trade was identified as a key pathway for myrtle rust, as nursery plants were amongst the initial detections in multiple states. Western Australia declared a ban on trade of myrtaceous species from other Australian mainland states and territories in February 2011 (McDonald 2011) and from Tasmania in 2015 (DPIRD 2015) to prevent the incursion of myrtle rust.

Pathways for myrtle rust are, however, not exclusive to nursery trade or soil. The spores can be transported by wind currents (CABI 2021 and references therein), people on their clothing and luggage, (CABI 2021 and references therein), and shipment cargo either containing myrtaceous goods or being shipped from ports where myrtle rust spores are present (Grgurinovic et al. 2006). In addition to these, animal activity is another recognised way in which long distance dispersal of rust pathogens can occur (Nagarajan & Singh 1990).

The prevalent winds in Australia are from west to east, although wind is one of the most highly variable meteorological elements, both in speed and direction, and is influenced by a wide range of factors, from large scale pressure patterns to the time of day and the nature of the surrounding terrain (BOM 2022).

Wind roses in Western Australia have a predominant west-to east direction and intensity, with some exceptions primarily along the coast (BOM 2022). Winds in the Northern Territory are also predominantly west-to-east, but it remains possible that non-cyclonic winds could move spores from the Northern Territory to the north of Western Australia. Wind parcels from southeast Asia have also been identified as a pathway of moderate risk for the introduction of pests and diseases to the northwest of Western Australia (Finlay, Weiss, and Blackett 2018). As myrtle rust has not been recorded in South Australia to date, we consider that non-cyclonic winds would be unlikely to carry spores from the Eastern States to the southwest of Western Australia.

Tropical cyclonic winds are considered another pathway for entry of *Austropuccinia psidii* spores into Western Australia. Cyclones originating in the tropics could carry spores from adjacent countries in the Timor Sea to the northwest of Western Australia or further south along the coast or inland, depending on cyclone path and intensity. Fifty-seven Tropical Cyclones occurred in the West region of the tropical north from January 2010 to April 2022 (BOM 2022). Cyclones have been linked to long-distance dispersal of rust pathogens (Nagarajan and Singh 1990), but the dispersal of *A. psidii*

specifically by cyclones has only been documented as probable in the literature (Lambert et al. 2018). However, Tropical Cyclones occur mostly from November to February, a time of year less suitable for establishment of the disease in the north of Western Australia.

Wind modelling such as CSIRO's Tool for Assessing Pest and Pathogen Aerial Spread, or TAPPAS can model dispersion with precision, but was not sought during this project as the knowledge on occurrence and biology of myrtle rust is known to be out of date and would require a significantly larger undertaking to fill that gap.

The movement of people (and their clothing) can also represent a pathway for introduction of *A. psidii* spores into Western Australia. Myrtle rust spores have been detected on people's clothing even when they were wearing zip-up overalls (Holliday et al. 2013). Pathways of entry of people into Western Australia are:

- roads, primarily the two sealed interstate roads: Eyre Highway, connecting Adelaide to Perth, and Victoria Highway, connecting the Kimberley region with Darwin
- airports: Perth, Broome, Busselton have interstate direct flights
- ports, primarily those for passenger travel: Broome, Exmouth, Port Hedland, Geraldton, Fremantle, Bunbury, Busselton, Augusta, Albany, Esperance (noting that contaminated freight could arrive at any port depending on the origin of the goods and stop points along the journey).

The inadvertent transport of spores by animals is also possible, with birds being capable of the longest-distance transport, followed by vertebrates and invertebrates. The East Asia/Australasia Flyway connects Arctic Russia and North America to the southern limits of Australia and New Zealand (BirdLife International 2022). Western Australia is directly connected to flight routes to South Australia and Southeast Asia, and indirectly connected to flight routes to the eastern states and further East Asia regions (BirdLife International 2022). Birds are, therefore, considered a potential vector for the spores, although causation remains to be empirically determined.

Terrestrial vertebrates can have large home-ranges in the order of 550 km in less than a year for emus *Dromaius novaehollandiae* and 200-300 km for red kangaroos *Macropus rufus* (Dickman et al. 1994), but movement from infested sites outside of the state into Western Australia would only be realistic in the Northern Territory and Kimberley boundary, where wind and human pathways are also being considered.

Myrtle rust testing

Molecular diagnosis of *Austropuccinia psidii* was published with focus on "a quarantine threat to Australian eucalypt and Myrtaceae biodiversity" in 2008 (Langrell, Glen and Alfenas). The authors designed a species-specific nested PCR based assay for the detection of *A. psidii*, intended for possible use in biosecurity and quarantine screening. Testing was collaborative and undertaken both in Perth and Hobart, Australia; and in Brazil (following AQIS restrictions), and used infected leaves, stems, shoots, and fruit material obtained from a diverse range of samples between 1999 and 2004

from Brazil, including typical and atypical *A. psidii* infections (Langrell, Glen and Alfenas 2008). The test relied on the development of two primer sets targeting the entire rRNA internal transcribed spacer (ITS) one and two and the 5.8S rRNA gene. The ITS region showed interspecific sequence divergence between *A. psidii* and other *Puccinia* rust species. Six putative primers were identified, of which two (Ppsi1, Ppsi6) were used in the nested PCR as 'first-round/outer' and then two more (Ppsi2 and Ppsi4) were chosen for 'second-round/inner' (nested); primer sets Ppsi3 and Ppsi5 were not pursued due to failure in performing at higher annealing temperatures. The authors reached detection down to 1-2 urediniospores in the presence of pollen or leaf tissues and primer sets used in the nested PCR assay were found to exhibit complete specificity to *A. psidii* (Langrell, Glen and Alfenas 2008).

Real-time PCR assays for the detection of *Austropuccinia psidii* have previously been developed by Baskarathevan et al. (2016) in an approach that focused on the development and validation of three novel real-time polymerase chain reaction (qPCR) assays (PpsiITS1, PpsiITS2, and PpsiBT1) which utilised ribosomal DNA and b-tubulin gene sequences – all three of the assays shown to detect *A. psidii*. Species-specific primers and Taqman© probes were developed from 46 complete internal transcribed spacer (ITS), and the ITS qPCR assays were shown to be 100 times more sensitive than the b-tubulin qPCR, as expected (Baskarathevan et al. 2016). PpsiITS1 showed greater analytical sensitivity when compared to the other assays and was nominated to be the best-performing qPCR assay, and overall, Baskarathevan et al. (2016) reported their work as the first qPCR assay developed for *A. psidii* as a rapid and sensitive detection tool.

The following year, Bini et al. (2017) authored a paper on Development of a quantitative real-time PCR assay using SYBR Green for early detection and quantification of *Austropuccinia psidii* in *Eucalyptus grandis*, finding their developed assay to be able to detect and quantify asymptomatic and symptomatic leaves of *E. grandis* under natural infection conditions in Brazil, stating it could be an important tool for monitoring the rust disease and its dispersion. Bini et al. (2017) developed three sets of primers based on the *A. psidii* intergenic space region (IGS) present in ribosomal DNA, beta-tubulin and elongation factor genes, and the sensitivity and specificity of the primer sets were achieved by PCR, obtaining approximately 200-bp amplicons. The GS7/IGS9 primers resulted in 100-fold higher sensitivity than that displayed by BTub1/BTub3 and EF5/EF2 primers, and the highest detection efficiency reported was of 0.5 pg of *A. psidii* DNA (Bini et al. 2017).

These three studies are just examples of the breath of molecular testing developed to diagnose *A. psidii*, but all of the tests known to us to date from the literature and from personal communication aim to diagnose or test plant material (generally containing pustules or spores), while no tests have been developed with the sensitivity to capture the very low concentrations of DNA present in environmental samples where surveillance is aimed at early detection.

Surveillance worldwide

Surveillance is a fundamental component of any plant biosecurity system, as knowledge of pest status is the basis for managing risk, and surveillance systems help inform decisions that impact on market access, management of established pests and responses to exotic plant pest incursions (Anderson et al. 2017). Surveillance also enables early detection, which allows for the most cost-

effective management of a biosecurity organism and leads to the least amount of damage to the environment and industry, when prevention if incursion is not possible (Welsh et al. 2017).

Myrtle rust was considered as 'probably present' in Australia for months or years before being detected in the cut flower industry (Carnegie and Cooper, 2011). To our knowledge, no Surveillance Plans have been developed and implemented for targeted structured myrtle rust surveillance in Australia or worldwide. Opportunistic surveillance and delineation surveillance have been adopted in Australia (e.g., NSW Government 2011, Carnegie and Nahrung 2019, McDonald 2012), New Zealand (Biosecurity New Zealand 2022, Biological Heritage 2022), and Hawai'i (Anderson 2012).

However, Australia's national response to myrtle rust has not been, nor is currently nationally coordinated (Makinson, Pegg and Carnegie 2020). The 'General Surveillance Criteria for Freedom from Myrtle Rust' pointed out that regarding passive surveillance and active surveillance, "nothing [was] in place specifically for myrtle rust" (National Management Group 2010); and the responsibility to "establish and maintain some targeted surveillance in non-infected areas to detect the spread of Myrtle rust to new areas early enough to implement effective disease management measures" was placed on States and territories, industry, clearly stating that "the Commonwealth is not proposing to invest in this area" (Plant Health Australia 2011).

A Coordinated, Risk-Based, National Forest Biosecurity Surveillance Program for Australian Forests has been called for (Carnegie and Nahrung 2019, Carnegie et al. 2022). For Western Australia, implementing a Surveillance Plan prior to the incursion of myrtle rust could lead to rapid response following a positive detection, and would represent a significant advantage in attempting eradication from the state.

3. Aim

In order to strengthen the Western Australian biosecurity capability against myrtle rust, this project was developed with engagement and support from the Department of Primary Industries and Regional Development (DPIRD) and the Department of Biodiversity Conservation and Attractions (DBCA) to:

(1) Develop a surveillance plan specific to Western Australia for *A. psidii*, taking into consideration the key recognized pathways (winds, cyclones, and human movement); predictive modelling of climate suitability; and presence of host species.

(2) Develop an assay of forensic-level sensitivity and equip a laboratory with the materials needed for local, rapid testing using molecular techniques.

4. Process

The authors of this study were in close discussions with key stakeholders and possible contributors to the project from its inception. Initial engagement with the Western Australian Department of Biosecurity, Conservation and Attractions (DBCA), and the Department of Primary Industries and Regional Development (DPIRD) in October 2020, directed the authors to fill knowledge gaps and create a surveillance plan that could be implemented by one or both departments and their collaborators upon its completion.

Surveillance Plan

Approximately one year into the project, Dr Mariana Campos (originally with Murdoch University, then CSIRO) joined the Myrtle Rust working group and maintained the liaison with other stakeholders, including South Coast NRM, Plant Health Australia, Plantation Industry Pest Management Group, Nursery & Garden Industry Western Australia (NGIWA), and Queensland Department of Agriculture and Fisheries (QDAF).

Much of the information sought was not available in the peer-reviewed or grey literature, and as such, much of the engagement was made through phone calls and emails to people known to have been involved with myrtle rust surveillance and diagnostics, primarily in Australia and New Zealand. The development of the surveillance plan was undertaken using a template Surveillance Plan from DPIRD and comments were sought from the group after a presentation of the draft plan and its submission to the Myrtle Rust working group.

We thank the Working group and in particular Laura Fagan (DPIRD), Justin Bellanger (South Coast NRM), and Simon McKirdy (Murdoch University) for the comments on the Surveillance Plan. We also acknowledge the contributions of Emer O'Gara (DBCA), Kylie Ireland (DPIRD), Darryl Hardie (DPIRD), Sonia Broughton (DPIRD), Angus Carnegie (NSW Department of Primary Industries), Geoff Pegg (Queensland Department of Agriculture and Fisheries), Louise Morin (CSIRO), Hank Bower (Lord Howe Island), Michael Robinson (Australian Plant Biosecurity Science Foundation) and Claire Stringer (Ministry for Primary Industries New Zealand).

Molecular assay and testing preparedness

The assay and testing preparedness were developed by Dr Shane Tobe (Murdoch University) with assistance from Ms Erin Whiteman (Murdoch University). Following an extensive literature review (of which only highlights were presented in the Introduction section of this report), testing for single cell levels of *Austropuccinia psidii* was developed to detect invisible (pre-symptom) traces of DNA.

Three targets that are specific to *A. psidii* were selected, so that the test would only work with that species of rust (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 was developed using a real-time PCR (qPCR) platform, in order to minimise the time from sampling to result and allowing for quantification of target DNA detected. The assay was developed with the qPCR technique 'Taqman'©, which is more sensitive and specific than SYBR Green© and uses an additional target for greater accuracy.

Primers were used that target the beta-tubulin (BTub) gene of *A. psidii,* 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 1:.

Table 1: Primers, probes, and standards for myrtle rust (*Austropuccinia psidii)* 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.

5. Achievements, Impacts and Outcomes

A surveillance plan (Enclosure 1) was developed through extensive literature review and stakeholder engagement. The resulting approach of the Surveillance Plan is to use a combination of opportunistic and targeted surveillance, as well as eDNA surveillance. The sampling methodology and timing were decided against information on modelled climate suitability, host species distribution, and potential pathways of introduction of spores, noting that all three parameters are to some extent incomplete or out of date.

The Surveillance Plan has been received by the Myrtle Rust Working Group and DPIRD, and is interlinked with the Contingency Plan and the Memorandum of Understanding (unpublished, confidential documents) and together, represent an advanced level of preparedness for Western Australia against myrtle rust.

The Surveillance Plan includes crucial information for the prioritisation of surveillance efforts, such as a map of distribution of known host species for the state and a map of vegetation types where a known host is a dominant plant of that vegetation. Tables were produced indicating the most climatically suitable seasons to survey each of the regions, and appropriate timing for detection of spores through eDNA for each of the pathways and locations.

The molecular test developed within this project is highly sensitive and accurate, and greatly improves on existing methods of detection. The significance is that the method can be used not only to confirm a suspect myrtle rust detection, but rather, to detect as few as 1 to 10 copies of DNA from a sample: in short, suitable for eDNA testing. The test is also upward of 2.5 hours faster than that of Bini et al. (2018), taking only 13 minutes of cycling (total run being 75 minutes. The added accuracy of having the three specific targets makes the developed test superior in accurate detection *A. psidii*.

The benchtop-tested assay is underway to being tested in the field, with an upcoming project in collaboration with CSIRO Health & Biosecurity, Queensland under development to determine the sampling boundaries of detection and best collection techniques in the field.

Murdoch University is equipped to analyse up to 500 samples, which will cover the field trials and future samples collected by DPIRD and DBCA. Beyond that, ongoing discussions will determine whether eDNA surveillance will be undertaken in parallel with opportunistic and targeted visual surveillance.

There have been to date four outputs to this project: the Surveillance Plan (enclosed), released to the Western Australian Myrtle Rust Working Group; this report; delivery of a pre-recorded talk on this project at the Myrtle Rust National Symposium (23-25 March 2021); and an oral presentation at the Biosecurity Symposium (3-5 May 2022).

Further developments with in-field testing of the essay in Queensland are expected to lead to a peer-reviewed publication in the next year.

The impacts of this work have already been reflected in both the researchers' portfolio and on the plant surveillance network in Western Australia. The funding provided by APBSF for this seminal work has provided both Dr Campos and Dr Tobe with opportunities for further collaborations and funding for other projects with myrtle rust and with eDNA surveillance. For the state of Western Australia, this project represents, to the best of the Working Group's knowledge, the first surveillance plan for the state developed prior to arrival of the invasive organism. It represents, as such, a step change in preparedness.

6. Discussion and Conclusion

Previous modelling of climatic suitability for myrtle rust in Western Australia indicates that the state has regions where the pathogen may get established. The available models differ from each other in the myrtle rust occurrence data that was put into them and on the parameters used for the modelling itself. It is then unsurprising that the models result in different regions highlighted as suitable for the establishment of myrtle rust; one model highlighting only the southwest of Western Australia (Kriticos et al. 2013) and the other highlighting the Swan Coastal Plain and a portion of the Kimberley region in the north of the state (Singh et al. 2016). Narouei-Khandan et al. (2019) developed consensus models for myrtle rust worldwide and ran Multi-Model Frameworks, which did not increase the areas considered as climatically suitable for myrtle rust. We, on the other hand, chose to take the precautionary approach and have added the regions considered as suitable in each of the first two models. Considering that myrtle rust has established in the Northern Territory (where models showed either no or low climatic suitability), it would be a worthwhile effort to re-run models of climatic suitability, and until those are updated, to expand the surveillance area as much as practically feasible to allow for early detection and rapid response.

Even with climatic suitability, it is unknown at this stage how big a threat myrtle rust would pose to Western Australian vegetation, as there is restricted information on the phenology of most species. Myrtle rust affects predominantly growing tissues, which means that the plant's phenology must line up with the appropriate season for the rust to successfully infest. Although this might be an argument to reduce the perceived threat, it must be counteracted by three factors: firstly, that we are under a rapidly changing climate, and that this relationship is not a static one. Secondly, that spore persistence might not affect mature plants but would likely cause damage to seedlings. Finally, that increasing inoculum pressure may change the spread and severity of the disease and infection of less susceptible species (Pegg et al. 2017, Ireland and Pegg 2020). Within this framework, we again hinge on the precautionary principle and believe in prevention as the best approach.

Throughout our Surveillance Plan, we have made the distinction between detecting myrtle rust symptoms on plants though visual surveillance and detecting spores through a genetic assay. Visual (targeted or opportunistic) surveillance is crucial in the areas where there is an overlap between higher climatic susceptibility, presence of known host species, and where suitable pathways have been identified. These efforts are, however, very resource-intensive and as such, not likely to cover vast areas in the state. The eDNA approach developed is proposed in the surveillance plan as a complementary approach that can expand the search area and detect spores (not disease) and function as a triage mechanism – any positive samples, if present, would enable the responsible agencies to direct human surveillance to the area; and would also allow for an examination of the pathways through which the spores might be entering the state, hopefully leading to an evaluation of controls applicable to that pathway.

The eDNA test is, therefore, not a replacement to human surveillance, but rather, an alternative that can increase the state's toolkit used in surveillance. The assay also allows for detection of 1 to 10 copies of DNA in samples, meaning exogenous DNA or spores can be detected before any symptoms are visible in plants.

The Surveillance Plan has been developed in consultation with key stakeholders in the state and is based on the latest science available. In conjunction with the Contingency Plan and the Memorandum of Understanding of the Myrtle Rust Working Group (confidential documents), it represents a proactive line of prevention against this pathogen that has caused immense ecological, cultural, and economical damage elsewhere in Australia and the world.

Finally, this project aids in addressing action 5.2.2 of the National Myrtle Rust Action Plan (Makinson, Pegg and Carnegie 2020), which called for 'Ongoing review and identification of potential risk pathways for entry of Myrtle Rust to Western Australia and South Australia", in particular through creation of measures for prevention and early detection.

7. Recommendations

The Surveillance Plan and eDNA assay developed through this project are a significant step forward in early detection and rapid response for the state of Western Australia. In order for them to be implemented, we recommend:

- undertaking further work to determine the boundaries of detection of the assay in-field, and
- continuing engagement with the government organisations responsible for surveillance to maximise the likelihood of implementation of the Surveillance Plan with the inclusion of eDNA surveillance.

We also recommend a national approach to surveillance data gathering and sharing to maintain up-to-date risk assessments and to enable best prevention and control measures. The adoption of the Myrtle Rust Action Plan (Makinson, Pegg and Carnegie 2020) is highly recommended.

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9. Enclosure

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