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ORIGINAL RESEARCH ARTICLE

Threatened and Priority listed *Melaleuca* species from Western Australia display high susceptibility to *Austropuccinia psidii* in controlled inoculations

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Abstract
Austropuccinia psidii causes rust disease on species within the family Myrtaceae. It was first detected in Australia in 2010, with the first detection in Western Australia in 2022. While species within the genus *Melaleuca* from eastern Australia show variable responses to the pathogen, little is known of the response of species from Western Australia. This study established that 13 previously unscreened species of *Melaleuca*, including Threatened and Priority listed species that were grown from seeds sourced from Western Australian populations, were susceptible four months post-germination to the pandemic strain of the pathogen. The proportion of highly susceptible plants within a single species ranged from 2 to 94%, with several species displaying highly variable levels of resistance to *A. psidii*. These results highlight the importance of disease screening and may direct conservation efforts.

Keywords Myrtle rust · Rust resistance · Conservation · Myrtaceae

Introduction

Austropuccinia psidii, formerly *Puccinia psidii* (Beenken 2017), is a rust fungus and the causal agent of the disease myrtle rust that impacts species within the family Myrtaceae. Originating in South America, the first detection of the pathogen in Australia was in 2010 and it has since spread to all states and territories except South Australia (Carnegie et al. 2010; Carnegie and Lidbetter 2012; Westaway 2016; Department of Natural Resources and Environment Tasmania 2020; Agriculture Victoria 2022; The Department of Primary Industries and Regional Development 2022a). The most recent new detection within Australia was in the Kimberley region of Western Australia (WA), where infection was observed on two *Melaleuca* species near the Northern Territory border (The Department of Primary Industries and Regional Development 2022b).

Austropuccinia psidii infects the young and expanding tissues of susceptible hosts, including the leaves, stems, petioles, and reproductive and seed-bearing structures. In susceptible species, yellow urediniospores appear on the infected surfaces, which may be followed by other symptoms such as leaf distortion and defoliation (Pegg et al. 2014). In species with no resistance to *A. psidii*, repeated infections may lead to tree death as a result of defoliation, and impact reproduction through infection of reproductive and seed-bearing structures (Carnegie et al. 2016). In Australia, *A. psidii* has caused the near extinction of several rainforest understory species including *Rhodamnia rubescens* and *Rhodomyrtus psidioides* (Pegg et al. 2014; Carnegie et al. 2016; Carnegie and Pegg 2018; Fensham and Radford-Smith 2021; Environment Protection and Biodiversity Conservation Act 1999 1999), and could be potentially devastating for other keystone species including *Melaleuca quinquenervia* (Pegg et al. 2018).

Melaleuca is the third largest genus within the family Myrtaceae, comprising over 200 species (Ryan 2016) that are adapted to a range of habitats (Naidu et al. 2000). Although well adapted, changing conditions as a result of

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climate change are contributing to the decline of *Melaleuca* species in Australia (Saintilan et al. 2019). An increased threat is placed on these species by *A. psidii*, with several *Melaleuca* species found to be highly susceptible to the pathogen under field conditions and in controlled inoculations (Carnegie and Lidbetter 2012; Morin et al. 2012; Pegg et al. 2014, 2018; Berthon et al. 2019; Martino et al. 2022). Modelling predicts changes in the geographic range suiting the pathogen as a consequence of climate change, with increased suitability in areas of NSW, TAS, VIC, and WA (Berthon et al. 2018). With the increasing severity of weather events associated with a changing climate predicted to disrupt native ecosystems (Trisos et al. 2020), pathogens such as *A. psidii* pose an additional threat to plant populations within these systems.

WA is rich in *Melaleuca* species, with the greatest diversity and highest level of endemism located within the South-West region of the state in which approximately 72 *Melaleuca* species occur per 100 km^2 and endemism scores are up to 9.9 (Brophy et al. 2013). Many of these species are valued for their important ecological, cultural, and economic roles (Brophy et al. 2013). In the absence of myrtle rust in large parts of WA, the vulnerability of many endemic *Melaleuca* species is unknown. With the arrival of *A. psidii* into WA and evidence for high susceptibility of several *Melaleuca* species (Martino et al. 2022), there is an urgent need to expand current disease screening of WA species to aid pre-emptive conservation and monitoring efforts. Additionally, if highly resistant individuals are identified within species, they may be useful in future conservation breeding strategies. Both the screening of species and identification of resistance are key objectives of the National Action Plan (Makinson et al. 2020). Here, the responses of 13 previously untested *Melaleuca* species to controlled inoculation of *A. psidii* were investigated. Using seed sourced from populations in areas climatically suited to *A. psidii* (Berthon et al. 2018; Narouei-Khandan et al. 2020), the aim was to determine the risk the pathogen may pose in the natural environment.

Materials and methods

Species selection for initial myrtle rust screening

To conduct an initial risk assessment of the impact *A. psidii* may have in WA, the response of endemic *Melaleuca* species from selected populations was determined. *Melaleuca* seed was obtained from the Department of Biodiversity, Conservation and Attractions (DBCA) Kings Park and Kensington seed banks. For many species, their natural range spans large geographic distances (Fig. 1A & B), therefore seed collections were conducted by DBCA staff in areas deemed as highest suitability for myrtle rust establishment. For each species, seed was collected from multiple parent trees in a single population following Florabank guidelines (Commander 2021) with co-ordinates obtained and mapped (Fig. 2A–C).

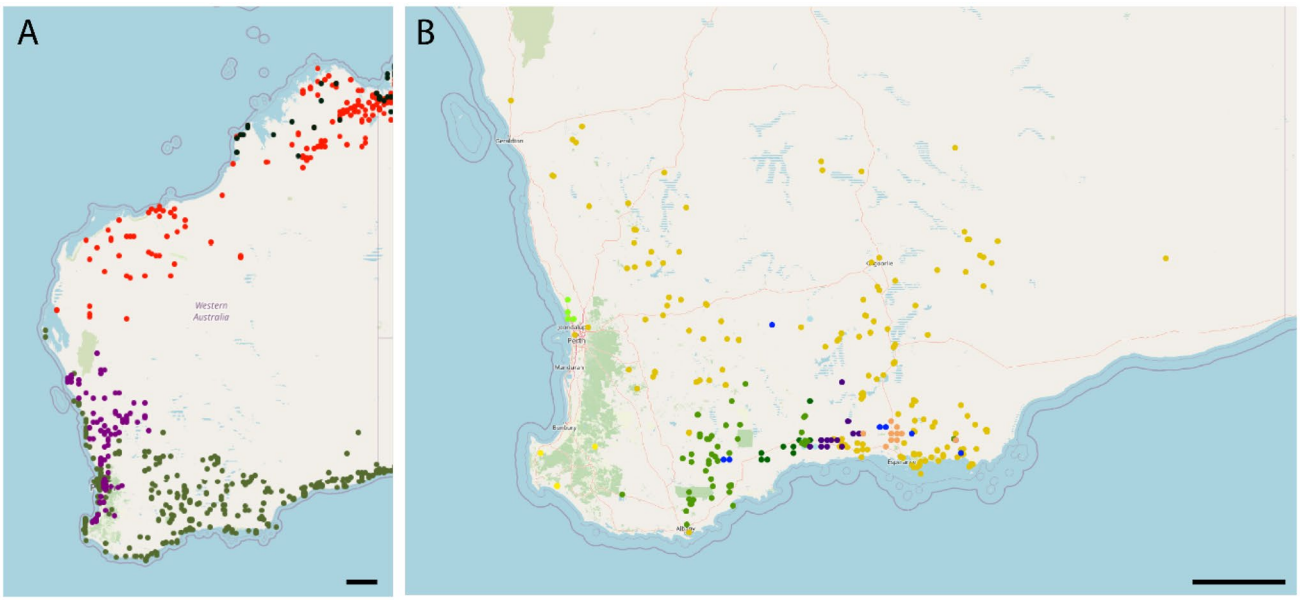

Fig. 1 Geographic distribution of species selected for seed collection. **A** *Melaleuca cajuputi* ssp. *cajuputi* (black), *M. argentea* (red), *M. acutifolia* (purple), *M. lanceolata* (olive) **B** *M. fulgens* ssp. *fulgens* (mustard), *M.* sp. *Wanneroo* (light green), *M. incana* ssp. *gingilup* (yellow), *M. penicula* (dark green), *M. sophisma* (light blue), *M. lateralis* (green), *M. similis* (purple), *M. viminea* ssp. *appressa* (dark blue), *M. dempta* (orange). Scale bar = approximately 150 km. Image generated using Atlas of Living Australia's Spatial Portal (Belbin 2011) and is viewable at https://tinyurl.com/3cu5f5ue

Seed was obtained for species listed under the Biodiversity Conservation (BC) Act 2016 (2016) as Threatened, including critically endangered, endangered, or vulnerable species (Biodiversity Conservation Act 2016 (WA) (2016) s 19), and species listed as Priority on DBCA's priority flora list (Department of Biodiversity, Conservation and Attractions 2017). While not designated under the BC Act, Priority listed species may be threatened but lack sufficient survey data to list under the Act. Seed from Priority listed species for this work included *M. dempta*, *M. incana* ssp. *gingilup*, *M. penicula*, *M. similis*, and *M. sophisma*. Seed was also obtained for the Threatened (endangered) listed species *M.* sp. *Wanneroo*. Seed was also obtained from species listed as Not Threatened (Biodiversity Conservation Act 2016 (WA) (2016) s 19) and included *M. acutifolia*, *M. argentea*, *M. cajuputi* ssp. *cajuputi*, *M. fulgens* ssp. *fulgens*, *M. lanceolata*, *M. lateralis*, and *M. viminea* ssp. *appressa*.

Seed germination and plant growth

Seeds were sown into perforated trays containing a mix of 2:1:1 peat, coconut coir, and perlite supplemented with Osmocote® Native Controlled Release Fertiliser then covered with a fine coating of vermiculite. Perforated trays were placed into solid trays filled with 1 cm of water every 3–4 days allowing for periods of drying to promote root growth. Seeds were germinated under natural light in a climate-controlled greenhouse at the Plant Breeding Institute at the University of Sydney (Cobbitty, NSW) set at 24 °C/20 °C day-time/night-time temperature on a 12 h cycle. Germinated seedlings were

Fig. 2 Seed collection sites by coordinate or nearest town. **A** *Melaleuca cajuputi* ssp. *cajuputi*, **B** *M. argentea* (red), *M. acutifolia* (plum), **C** *M. fulgens* ssp. *fulgens* (mustard), *M.* sp. *Wanneroo* (light green), *M. incana* ssp. *gingilup* (yellow), *M. penicula* (dark green), *M. sophisma* (light blue), *M. lateralis* (green), *M. similis* (purple), *M. viminea* ssp. *appressa* (dark blue), *M. dempta* (orange), and *M. lanceolata* (olive). Seed was collected from multiple parents at each site. Scale bar = approximately 150 km. Image generated in Google My Maps and interactive map is viewable at https://tinyurl.com/zvffxccd

transplanted into 85 mL pots (5 cm diameter and depth) containing a mix of 2:1:1 Osmocote® Native Premium Potting Mix, peat, and perlite supplemented with Osmocote® Native Controlled Release Fertiliser then placed on capillary mats. Seedlings were grown under the same light and temperature conditions as for germination, and fertilised once a month with Osmocote® Native Controlled Release Fertiliser.

Plant inoculations

For all species, plants were inoculated at approximately four months post germination alongside four highly susceptible *Syzygium jambos* plants as positive controls. Owing to space limitations, inoculations were carried out in two batches of equal size, following the method of Sandhu and Park (2013). Approximately 50 mg of *A. psidii* urediniospores from a greenhouse increased single pustule isolate (accession 622, Pandemic strain) (Sandhu and Park 2013) was added to 50 mL of ASCC Isopar® L for a final concentration of 1 mg spores/mL. Plants were inoculated with the suspension using an aerosol sprayer and relocated to a dark humid incubation chamber for 24 h at 20 °C. After incubation, the plants were transferred to a greenhouse with the temperature set to 24 °C/20 °C day-time/night-time temperature on a 12-hour cycle under natural light.

Disease response scoring

Host response to *A. psidii* inoculation was scored visually using a 1–5 scoring system based on Morin et al. (2012) and adapted for disease scoring on *Melaleuca* species (this study) where 1 indicates completely resistant or no visible response and 5 indicates highly susceptible (Table 1). *Syzygium jambos* was scored as score 5 for both inoculation batches indicating successful inoculation as it's known to be highly susceptible. As inoculations were carried out in winter under shorter day-length conditions, disease symptoms were slower to develop than in a previous screening study (Martino et al. 2022). Plants were left for 16 days prior to scoring to allow for complete development of plant disease symptoms.

Results

Within 16 days post inoculation, symptoms had developed on the highly susceptible *S. jambos* control plants (Fig. 3A-B). Using the adapted *Melaleuca* scoring system (this study), resistant and susceptible plants were identified across and within *Melaleuca* species screened (Supplementary Figs. 1–13). Abundant urediniospore production was observed on the leaves of all highly susceptible plants

Table 1 Disease scale adapted from Morin et al. (2012) used to score *Melaleuca* species for their response to *Austropuccinia psidii* in controlled inoculations. Scoring was based on the disease symptoms on *M. quinquenervia* scored at 14-days post inoculation with greenhouse increased single pustule isolate (accession 622, Pandemic strain) of *A. psidii* urediniospores (Sandhu and Park 2013)

Infection Score	Disease Rating	Infection symptoms based on Morin et al. (2012)	Infection symptoms adapted from *Melaleuca quinquenervia* for other *Melaleuca* sp.	Representative leaf image
1	Completely Resistant	No visible symptoms attributable to rust infection	No visible symptoms attributable to rust infection	
2	Highly Resistant	Chlorotic, purplish, or necrotic spots or blotches	Chlorotic or necrotic spots or blotches	
3	Low Susceptibility	Purplish or necrotic flecks with underdeveloped uredinia. Pin sized uredinia, limited sporulation	Necrotic flecks with limited sporulation	
4	Moderate Susceptibility	Fully developed uredinia with or without purplish halos that cover less than 25% of the leaf and abundant sporulation	Abundant sporulation with necrotic halos. Spores may appear on leaves, stems, and/or petioles	
5	High Susceptibility	Fully developed uredinia with or without purplish halos that cover more than 25% of the leaf and abundant sporulation	Abundant sporulation with no visible necrosis. Spores may appear on leaves, stems, and/or petioles	

Fig. 3 Representative highly susceptible (score 5) disease symptoms on **A** – **B** *Syzygium jambos* positive control, **C** *Melaleuca acutifolia*, **D** *M. argentea*, **E** *M. cajuputi* ssp. *cajuputi*, **F** *M. dempta*, **G** *M. fulgens* ssp. *fulgens*, **H** *M. incana* ssp. *gingilup*, **I** *M. lanceolata*, **J** *M. lateralis*, **K** *M. penicula*, **L** *M. similis*, **M** *M. sophisma*, **N** *M.* sp. *Wanneroo*, and **O** *M. viminea* ssp. *appressa*. Scale bar = 0.5 cm

(Fig. 3C-O), as well as infection on stems and petioles on all species except for *M. sophisma* and *M. incana* ssp. *gingilup*. The proportion of highly susceptible plants (score 5) within a single species ranged from 2% for *M. sophisma*, to 94% for *M. lateralis*. Eight species were rated as either completely resistant (score 1) or mostly moderately to highly susceptible (score 4 or 5), with no plants within these species rated as score 2 or 3 (Table 2). For *M. lateralis*, 94% of plants were rated as highly susceptible with the remaining 6% free of disease symptoms. Only 2% of *M. sophisma* plants were rated as highly susceptible with the remaining 98% having no observable symptoms (Table 2). Only three of the 13 species tested — *M. argentea*, *M. cajupti* spp. *cajupti*, and *M. incana* ssp. *gingilup* — had representative plants from each disease score (Table 2).

Discussion

In our study, the host response to *A. psidii* in 13 previously unscreened *Melaleuca* species from a range of geographic locations in Western Australia was investigated, revealing varying proportions of highly susceptible plants within and between species. The broad-leaved paperbark species included in this study, *M. cajuputi* ssp. *cajuputi* and *M. argentea*, both displayed variable responses to *A. psidii* with plants displaying symptoms in each disease scoring category from completely resistant to highly susceptible. This has previously been shown for other broad-leaved species including *M. quinquenervia*, *M. viridiflora*, and *M. leucadendra* (Pegg et al. 2018; Martino et al. 2022). Pegg et al. (2018) assessed the proportion of resistant *M. viridiflora* from two

Table 2 Disease scoring, based on Morin et al. (2012) and adapted for *Melaleuca* species (this study), of controlled inoculation of *Austropuccinia psidii* of Threatened (*Biodiversity Conservation Act* 2016 (WA) s 19) and Priority (Department of Biodiversity, Conservation and Attractions 2017) listed *Melaleuca* species included total number of plants scored and the percentage of plants observed in each disease scoring category. Plants were grown from seed collected from the Department of Biodiversity, Conservation and Attractions Kings Park and Kensington seed banks. Seed was collected from multiple, between 10 and 15, parent trees in a single population following Florabank guidelines (Commander 2021)

Species name	Listing under Biodiversity Conservation Act 2016 or DBCA Priority Flora List	Total Number of Plants Scored	Disease Score (% of Total Plants)				
			1	2	3	4	5
Melaleuca acutifolia	NT	20	10	0	0	20	70
Melaleuca argentea	NT	52	44	23	4	4	25
Melaleuca cajuputi ssp. *cajuputi*	NT	54	28	15	13	35	9
Melaleuca dempta	P3	11	9	0	0	0	91
Melaleuca fulgens ssp. *fulgens*	NT	27	18	0	18	7	57
Melaleuca incana ssp. *gingilup*	P2	41	32	5	5	19	39
Melaleuca lanceolata	NT	27	15	0	18	26	41
Melaleuca lateralis	NT	31	6	0	0	0	94
Melaleuca penicula	P4	32	66	0	0	6	28
Melaleuca similis	P1	45	24	0	0	0	76
Melaleuca sophisma	P1	55	98	0	0	0	2
Melaleuca sp. *Wanneroo*	EN	80	14	0	0	0	86
Melaleuca viminea ssp. *appressa*	P2	25	28	0	0	4	68

EN Endangered (Threatened species considered to be "facing a very high risk of extinction in the wild in the near future, as determined in accordance with criteria set out in the ministerial guidelines"), *NT* Not Threatened, *P1–3* Priority 1–3 (Poorly-known species with conservation threat highest for Priority 1), *P4* Priority 4 (Rare, Near Threatened and other species in need of monitoring)

provenances in WA determining 22–23% of seedlings were resistant to *A. psidii*. The same study assessed *M. leucadendra* seedlings from three provenances in WA determining 1–53% resistant seedlings, while a separate study assessing a population from the Wunaamin Conservation Park in WA determined 30% of plants to be resistant to *A. psidii* (Martino et al. 2022). These results indicate variability in host response to the pathogen between populations of broad-leaved paperbarks. As the *M. cajuputi* ssp. *cajuputi* and *M. argentea* screened in this study were grown from seed collected from a single provenance, further detailed studies should be conducted to determine variation in host response between populations. Such information would be valuable in understanding the threat that *A. psidii* poses to individual populations across the geographic range if it were to establish in these areas. Additional studies may also shed light on the forces driving potential differences in disease response between populations. All myrtle rust studies using broad-leaved paperbark to date indicate that these species may be useful for differential pathotype trials, particularly if changes to pathogen populations emerge (McTaggart et al. 2020).

Unlike the broad-leaved paperbarks, most remaining species tested in this study displayed little variability in response to the pathogen. This difference may be explained by the geographic distribution differences of these species. For the broad-leaved paperbark species screened in this and in previous studies, populations are numerous and distributed broadly across large geographic regions of Australia (Brophy et al. 2013). This distribution pattern is also true for *M. fulgens* ssp. *fulgens* and *M. lanceolata* (Western Australian Herbarium 2023), which both display similar variability in response to the pathogen as the broad-leaved species. Conversely for *M. dempta*, *M. penicula*, *M. similis*, *M. sophisma*, *M.* sp. *Wanneroo*, and *M. viminea* ssp. *appressa*, where populations are geographically sparse (Western Australian Herbarium 2023), all displayed low variability in pathogen response. As *Melaleuca* species are predominantly outcrossing (Quang Tan 2008; Baskorowati et al. 2010; Brophy et al. 2013; Kartikawati et al. 2021), these differences may be the result of reductions in gene flow within small, isolated populations, leading to reduced genetic diversity within populations as determined within isolated populations of *Lychnis alpina* (Carlsson-Granér and Thrall 2002). Based on our results, future studies assessing vulnerability of *Melaleuca* species in WA should encompass wider population assessment.

Of particular interest is the high proportion of resistant *M. sophisma* plants observed, with only 2% of all plants tested being susceptible to *A. psidii*. The remaining 98%

of plants displayed no observable symptoms, potentially indicating preformed resistance mechanisms. This has been reported in other Myrtaceae species inoculated with *A. psidii*, including several *Eucalyptus* species (Dos Santos et al. 2019). In species with no observable symptoms post-inoculation, *A. psidii* was not detected within leaf tissues as determined by qPCR (Dos Santos et al. 2019). The results indicated that the leaves were not colonised by the pathogen, with the tested hypothesis that chemical compounds within cuticular waxes provide preformed resistance in these species (Dos Santos et al. 2019). Leaf epidermal appendages have also been implicated in contributing to responses to the pathogen with studies correlating rust susceptibility with increased trichome density (Wang et al. 2020; Varma et al. 2023). Here, the suggestion is that trichomes facilitate increased adherence of spores to the leaf surface. The lack of a hypersensitive response, a well characterised defence response indicating genetically controlled resistance (Mur et al. 2008), in 98% of the *M. sophisma* plants may indicate the inability of *A. psidii* urediniospores to penetrate and colonise the leaves of this species. Penetration and colonisation processes are influenced by structural and biochemical leaf properties such as the presence of trichomes and cuticular wax thickness and chemical composition (Dos Santos et al. 2019). As many of these species remain poorly characterised, histological analyses during rust infection may shed light on preformed resistance mechanisms on these species.

Correlating disease responses in our greenhouse tests with field responses will be important in defining potential risks to these species. It was encouraging to observe the presence of some resistant individuals within some of the Priority listed species. The results highlight the importance of continued disease screening to determine the vulnerability of individual Myrtaceae species to *A. psidii*. The identification of species with high susceptibility to the pathogen will be useful to inform disease surveillance in the natural environment, and to direct conservation efforts such as seed and ex-situ collection (Council of Heads of Australian Botanic Gardens and Botanic Gardens Australia and New Zealand 2023).

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Declarations

Conflict of interest The authors declare no conflict of interest in the reporting of these results.

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