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

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Abstract

Austropuccinia psidii causes rust disease on species within the family Myrtaceae and was first detected in Northern 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 species that were grown from seeds sourced from Western Australian populations were susceptible to the pandemic strain of the pathogen.

Keywords

Austropuccinia psidii - myrtle rust – Western Australia - *Melaleuca* species

Austropuccinia psidii, formerly *Puccinia psidii* (Beenken 2017), is the causal agent of the disease myrtle rust on many species within the family Myrtaceae. Since the first detection of the pathogen in Australia in 2010, *A. psidii* has spread to all states and territories aside from 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 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).

Melaleuca is the third largest genus within the Myrtaceae, comprising over 200 species (Ryan 2016). Although adapted to a range of habitats, changing conditions as a result of 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).

WA is rich in *Melaleuca* species, with the greatest diversity and highest level of endemism located within the South-West region of the state (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 the

pathogen in the many parts of WA, the vulnerability of many *Melaleuca* species remains unknown. With the arrival of *A. psidii* into WA and high susceptibility of several *Melaleuca* species, there is an urgent need to expand current disease screening of WA species to aid pre-emptive conservation and monitoring efforts.

To determine the susceptibility of selected *Melaleuca* species from WA in response to *A. psidii*, seed was obtained from the Department of Biodiversity, Conservation and Attractions (DBCA) Kings Park and Kensington seed banks. Seed was obtained for species listed under the Biodiversity Conservation (BC) Act (2016) as Threatened, including critically endangered, endangered, or vulnerable species (*Biodiversity Conservation Act* 2016 (WA) 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 include; *Melaleuca dempta*, *Melaleuca incana* ssp. *gingilup*, *Melaleuca penicula*, *Melaleuca similis*, and *Melaleuca sophisma*. Seed was also obtained for the Threatened (endangered) listed species *Melaleuca* sp. *Wanneroo*. Seed was also obtained from species listed as Not Threatened (*Biodiversity Conservation Act* 2016 (WA) s 19) and included *Melaleuca acutifolia*, *Melaleuca argentea*, *Melaleuca cajuputi* ssp. *cajuputi*, *Melaleuca fulgens* ssp. *fulgens*, *Melaleuca lanceolata*, *Melaleuca lateralis*, and *Melaleuca viminea* ssp. *appressa*. For each species, seed was collected from multiple trees with co-ordinates obtained and mapped (Figure 1).

Figure 1. Seed collection sites by coordinate or nearest town for (A) *Melaleuca cajuputi* ssp. *cajuputi*, (B) *Melaleuca cardiophylla* (brown), *Melaleuca argentea* (red), *Melaleuca acutifolia* (plum), (C) *Melaleuca fulgens* ssp. *fulgens* (mustard), *Melaleuca* sp. *Wanneroo* (light green), *Melaleuca incana* ssp. *gingilup* (yellow), *Melaleuca penicula* (dark green), *Melaleuca sophisma* (light blue), *Melaleuca lateralis* (green), *Melaleuca similis* (purple), *Melaleuca viminea* ssp. *appressa* (dark blue), *Melaleuca dempta* (orange), and *Melaleuca lanceolata* (olive). Seed was collected from multiple parents at each site. Image generated in Google My Maps and viewable at https://tinyurl.com/zvffxccd.

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 set at 24°C/20°C day-time/night-time temperature on a 12 hour cycle. Germinated seedlings were 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.

For all species, we inoculated seedlings approximately four months post germination at the Plant Breeding Institute at the University of Sydney (Cobbitty, NSW) alongside four highly susceptible *Syzygium jambos* plants as positive controls. Approximately 50 mg of *A. psidii* urediniospores from a glasshouse increased single pustule isolate (accession 622) (Sandhu and Park 2013) was added to 50 mL of Isopar® for a final concentration of 1 mg spores/mL. Seedlings were inoculated with the suspension using an aerosol sprayer and relocated to a humid incubation chamber for 24 hours at 20°C. After incubation, seedlings 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. As inoculations were carried out in winter under shorter day-length conditions, plants were left for 16 days prior to scoring to allow for development of plant disease symptoms.

Within 16 days post inoculation, symptoms had developed on the highly susceptible control plants (Figure 2 A-B). Plants were scored using the system developed by Morin et al. (2012), ranging from completely resistant (score 1) to highly susceptible (score 5). The proportion of highly susceptible plants within a single species ranged from 2% – 94% with over half of the species displaying over 50% of total plants highly susceptible to *A. psidii* (Table 1). The highest level of susceptibility was observed in *M. lateralis* with 94% of plants scored as highly susceptible and 6% presenting with no observable symptoms (Table 1). The lowest level of susceptibility was recorded in *M. sophisma*, with 2% of plants scored as highly susceptible and the remaining 98% presenting with no observable symptoms (Table 1). Urediniospores were observed on the leaves of all highly susceptible plants (Figure 2 C-O), as well as infection on stems and petioles on all species aside from *M. sophisma* and *M. incana* ssp. *gingilup*.

Figure 2. Representative highly susceptible disease symptoms on (A – B) *Syzygium jambos* positive control, (C) *Melaleuca acutifolia*, (D) *Melaleuca argentea*, (E) *Melaleuca cajuputi* ssp. *cajuputi*, (F) *Melaleuca dempta*, (G) *Melaleuca fulgens* ssp. *fulgens*, (H) *Melaleuca incana* ssp. *gingilup*, (I) *Melaleuca lanceolata*, (J) *Melaleuca lateralis*, (K) *Melaleuca penicula*, (L) *Melaleuca similis*, (M) *Melaleuca sophisma*, (N) *Melaleuca* sp. *Wanneroo*, and (O) *Melaleuca viminea* ssp. *appressa*. Scale = 0.5 cm.S

110 Table 1. Disease scoring, based on Morin et al. (2012), of controlled inoculation of *Austropuccinia psidii* of
111 Threatened (*Biodiversity Conservation Act* 2016 (WA) s 19) and Priority (Department of Biodiversity,
112 Conservation and Attractions 2017) listed *Melaleuca* species included total number of plants scored and the
113 percentage of plants observed in each disease scoring category.

			Disease Score (% of Total Plants)				
Species name	**Listing under Biodiversity Conservation Act 2016 or DBCA Priority Flora List**	**Total Number of Plants Scored**	**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

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

118

119 Here, we investigated the extent of rust susceptibility in 13 previously unscreened *Melaleuca* species
120 from a range of geographic locations in Western Australia in response to *A. psidii*, revealing varying
121 levels of susceptibility between species. Correlating disease responses in our greenhouse seedling
122 tests with field response will be important in refining risk to these species. We were encouraged by

the presence of some resistant individuals within some vulnerable species. These 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 may inform disease surveillance in the natural environment and direct conservation efforts such as seed collection.

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Conflict of interest

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

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