Australasian Plant Pathology (2026) 55:66
https://doi.org/10.1007/s13313-026-01145-7

ORIGINAL RESEARCH ARTICLE

The resistance of lemon myrtle (*Backhousia citriodora*) to myrtle rust (*Austropuccinia psidii*)

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Received: 9 April 2025 / Accepted: 31 March 2026

Abstract
Lemon myrtle (*Backhousia citriodora*, F. Muell.) is a native Australian myrtaceous species, highly valued for its unique, citral-rich essential oil. The development and success of this Australian industry has been in jeopardy since the detection of myrtle rust (*Austropuccinia psidii*) in Australia (April 2010). Since the initial invasion, it has spread across the major lemon myrtle growing regions in New South Wales and Queensland. The main commercial clones are highly susceptible to infection, with plantations experiencing up to 70% production losses. To identify resistance to *A. psidii* for industry use, disease assessments were conducted on germplasm originating from 13 Queensland provenances, established in two clonal field trials. Newly collected germplasm from the Ubobo provenance was also screened under controlled conditions. Infection caused symptoms ranging from small, restricted sori on a low percentage of leaves, to infection on up to 100% of leaves, with distorted shoots and severe dieback on infected stems. No fully resistant germplasm was identified, but significant differences in observed disease offer opportunities to select more resistant clones than those currently relied on by the industry. Germplasm from Eumundi, Noosa and Woondum (southern Queensland), and Silver Valley (northern Queensland), showed superior resistance to *A. psidii* across assessments and trial sites. Substantial variation in plant growth traits of importance to commercial production (e.g. canopy structure and foliage density) was observed among the provenances, families, and clones evaluated across the trials, suggesting the need to consider plant growth, biomass yield and essential oil properties when selecting industry-suitable germplasm. Implementing a breeding or hybridisation program should be a priority for the industry.

Keywords *Austropuccinia psidii* · *Backhousia citriodora* · Lemon myrtle · Myrtle rust · Resistance

Introduction

Austropuccinia psidii (formerly *Puccinia psidii*) is a fungal pathogen affecting plants in the Myrtaceae family (Beenken 2017; Winter 1884). In highly susceptible species, the disease causes extensive branch defoliation, dieback, stunted growth, as well as the death of mature plants (Carnegie et al. 2016; Pegg et al. 2014b, 2017). In April 2010, *A. psidii* was found in New South Wales (NSW), and this exotic pathogen has since become well established along the Australian east coast (Carnegie et al. 2010; Carnegie & Pegg 2018). *Austropuccinia psidii* is now proving a major threat to the expansion and success of the developing lemon myrtle (*Backhousia citriodora*, F. Muell.) industry, a tree species native to Queensland and grown commercially for its citral-rich essential oil. In natural ecosystems, the disease causes dieback and direct and indirect impacts on fecundity in numerous species, including several keystone species (Berthon et al. 2019; Carnegie et al. 2016; Fensham et al. 2021; Fernandez-Winzer et al. 2020; Pegg et al. 2014b). *Rhodamnia rubescens* and *Rhodomyrtus psidioides*, once considered common with a widespread distribution in NSW and Queensland, are now critically endangered, with localised extinctions already occurring due to the impacts of *A.*

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Published online: 14 April 2026

psidii (Carnegie et al. 2016; Carnegie & Lidbetter 2012). More recent surveys of rainforest Myrtaceae predicted the imminent extinction of 16 rainforest tree species in the wild due to myrtle rust within a generation (Fensham et al. 2021).

Many commercial plantations of lemon myrtle have been established using clonal material propagated from stem cuttings to ensure consistency in the essential oil properties and growth habit of trees (Chen 1997; House et al. 1996). These widely used commercial clones are highly susceptible to infection by *A. psidii*, and yield losses (leaf and stem) have been estimated at up to 70% in plantations in the absence of any disease management (Chudleigh et al. 2013; Entwistle 2015). While the infection is not known to alter the taste or consistency of lemon myrtle-derived products, it causes leaf loss, reduced leaf size and reduced tree vigour, and infected trees can take longer to reach harvestable size (Chudleigh et al. 2013; Entwistle 2015).

Before the introduction of *A. psidii*, diseases and pests were of minor concern in lemon myrtle production, enabling organic production systems for many growers (Chudleigh et al. 2013; Entwistle 2015). Given the severity of losses caused since the establishment of *A. psidii*, several systemic, curative and protectant fungicides have been registered for use in lemon myrtle plantations to control the rust (permit PER14643) (Australian Pesticides and Veterinary Medicines Authority 2015). Many growers have now foregone their organic status and apply fungicides to maintain production.

In a commercial setting, using resistant genotypes is considered the most economically viable and environmentally sustainable strategy for *A. psidii* management. Plant resistance to pathogens can generally be described as either qualitative (major gene/s) or quantitative (multiple genes with minor effects) (Zhang et al. 2013). Qualitative resistance typically occurs via elicitation of a defence response upon detection of effector proteins of the pathogen (Zhang et al. 2013). *Austropuccinia psidii* infection in a resistant genotype produces a hypersensitive reaction two days after infection, and this appears as flecks or necrotic lesions that do not sporulate (Junghans et al. 2003; Moon et al. 2007; Xavier et al. 2001). This is the response activated when the resistance genes of a plant are challenged by the avirulence genes of a pathogen (Flor 1971). Quantitative resistance, on the other hand, is phenotypically incomplete (i.e. partial resistance), so there is some infection and reproduction by the pathogen because each quantitative trait locus (QTL) contributes a slight reduction in infection (e.g. reduced infection rates, lesion development and sporulation) that hinders epidemic development (Niks et al. 2015). However, it is regarded as a more durable strategy because there is lower selection pressure against the pathogen whereby overcoming an individual quantitative resistance locus (QRL) is not hugely beneficial to the pathogen (Niks et al. 2015). With qualitative resistance, different genotypes in a population occur as obvious phenotypes, whereas with quantitative resistance this varies continuously between different phenotypes of the host population, from a slight to substantial reduction in pathogen growth (Ribeiro do Vale et al. 2001).

The successful identification and deployment of resistant lemon myrtle germplasm would reduce the need for fungicide applications, which may allow many growers to return to an organic production system. Deployment of disease resistant germplasm has been the principal strategy for managing *A. psidii* in other myrtaceous species, particularly those produced for human consumption, such as guava (*Psidium guajava*) (Ribeiro & Pommer 2004). In Australia and South America, the wide inter- and intra-specific variability for rust resistance have enabled the selection of superior germplasm for many commercially important Myrtaceae species, including *Corymbia*, *Eucalyptus* and *Melaleuca* (Lee et al. 2015; Pegg et al. 2014a; Zauza et al. 2010). As it stands, the search for resistant lemon myrtle germplasm remains in its infancy.

During 1995–97 a genebank planting of lemon myrtle germplasm was established in Beerburrum, Queensland using seed and coppice cuttings collected from all known provenances across the native range (Doran & House 1996; House et al. 1996). In 2012, after it became clear that the widely used commercial clones were highly susceptible to *A. psidii*, the genebank at Beerburrum (consisting of > 1,500 plants) was evaluated for any potential resistance to *A. psidii*, (Doran et al. 2012). In a subsequent study, 441 genotypes were propagated as coppice cuttings and screened for resistance to *A. psidii* under controlled conditions favourable for infection (Lee et al. 2016). This also revealed no qualitative resistance to the pathogen, though the authors noted that, due to lower disease observed in some families and provenances, it might be possible to select more quantitatively resistant clones than those currently used by the industry. As part of the study by Lee et al. (2016), the 441 genotypes tested under controlled conditions were also established in field trials at Dunoon, northern NSW and Traveston, southern Queensland. As no surviving plants from the Ubobo provenance were represented in the Beerburrum genebank, new genetic material was collected from Dawes National Park near Ubobo and screened in the glasshouse (Lee et al. 2016).

The overall objective of this study was to investigate the potential for disease resistance of lemon myrtle provenances, families and clones to the biotype of *A. psidii* established in Australia and provide options for future breeding programs or germplasm selection for plantation development. We first sought to assess the performance of different germplasm in field trials where plants were exposed to natural *A. psidii*

inoculum with repeated infection events. Disease severity was compared across the two trials that were in environmentally different locations. Controlled disease screening methods were also used to determine the susceptibility of recently collected germplasm from Ubobo and its potential for inclusion in any further selection or breeding program.

Materials and methods

Resistance of lemon myrtle provenances, families and clones to myrtle rust under field conditions at two sites.

Trial sites and experimental design Lemon myrtle germplasm from across the native range was assessed for resistance to *A. psidii* under conditions of natural infection at two field sites. As part of a previous study (Lee et al. 2016), two trials of lemon myrtle were established using germplasm originating from all known provenances throughout Queensland (excluding Ubobo), obtained via vegetative propagation of coppice cuttings from the Beerburrum genebank. The trials were established in the two climatically different areas of Dunoon, in northern NSW (−28.692, 153.294), and Traveston in southern Queensland (−26.335, 152.713) (Fig. 1). The Dunoon trial was planted in May 2014 on land owned by an industry representative (Australian Rainforest Products Pty Ltd) and consisted of 280 clones in 55 families from 11 provenances established in a randomised incomplete block design with three replicates (i.e. three ramets of each clone; $n=840$ ramets) (Table 1). The Traveston trial was planted in February 2015 on land owned by the Queensland Government and consisted of 404 clones in 58 families from 11 provenances established as in randomised incomplete block design with three replicates ($n=1{,}320$ ramets). Germplasm from all provenances was represented in each trial except for Ubobo, as there was no surviving germplasm at the Beerburrum site when the collections were undertaken, plus 18 clones with limited

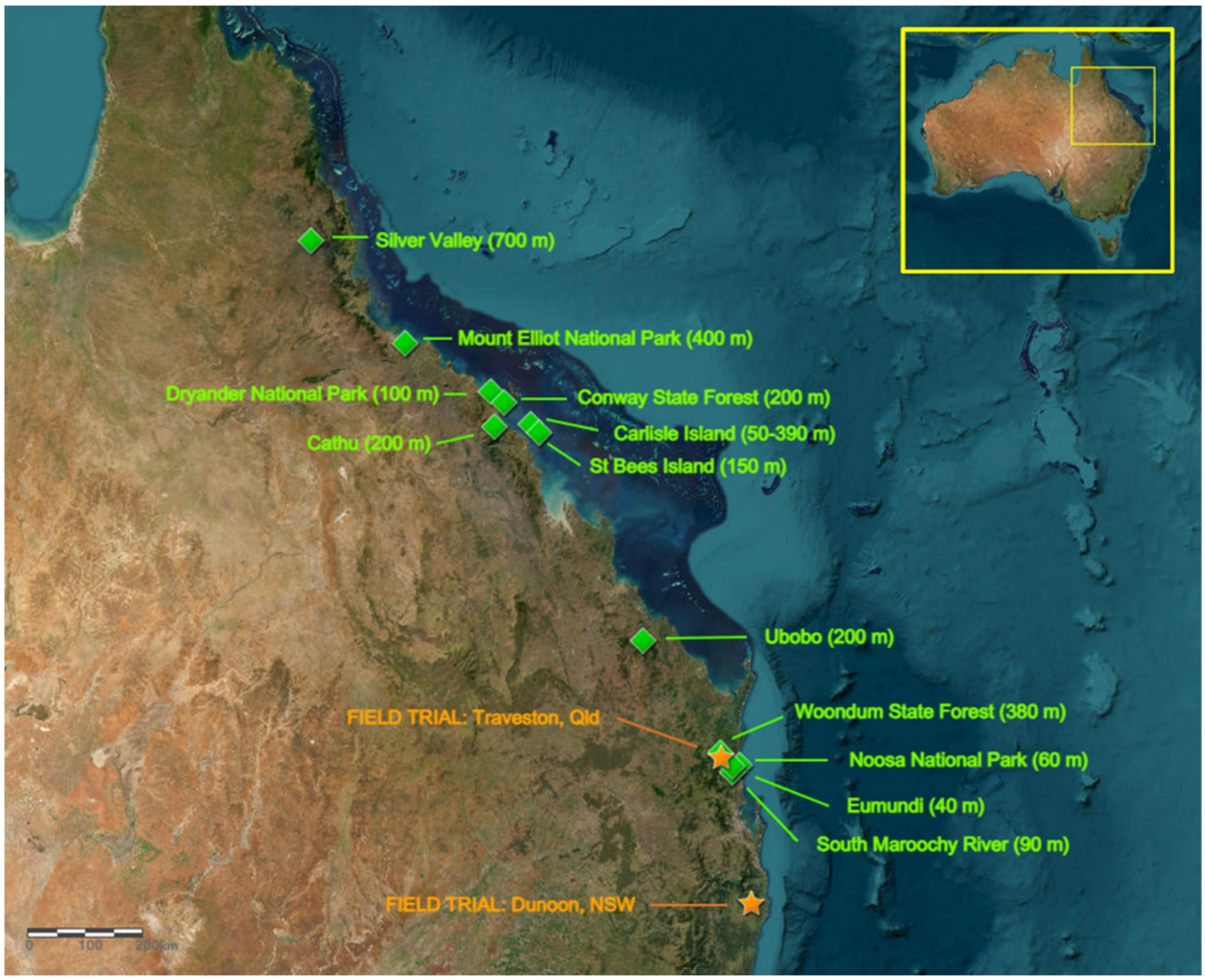

Fig. 1 Map of Queensland and northern New South Wales indicating locations and elevation of all known native populations of lemon myrtle (*Backhousia citriodora*) and field trial sites

Table 1 Lemon myrtle (*Backhousia citriodora*) germplasm tested across clonal trials in Dunoon, New South Wales and Traveston, Queensland (adapted from Doran & House 1996; Lee et al. 2016). The listed provenances cover the entire known native range of lemon myrtle

Provenance	Latitude[1]	Londitude[1]	Elevation (m)	Rainfall (mm/year)[2]	Families	Clones	Form
Carlisle Island	20° 47'	149° 17'	50–390	1,456	12	38	Mallee ≤ 4m; Tree ≤ 18 m
Cathu	20° 47'	148° 34'	200	1,253	2	10	Tree ≤ 20 m
Conway	20° 21'	148° 45'	200	1,408	9	37	Tree ≤ 20 m
Dryander	20° 16'	148° 34'	100	779	5	43	Tree ≤ 30 m
Eumundi	26° 30'	152° 59'	40	1,065	6	61	Tree ≤ 25 m
Maroochy	26° 34'	152° 48'	90	1,065	3	19	Tree ≤ 25 m
Mt Elliot	19° 25'	147° 00'	400	1,128	3	14	Tree ≤ 20 m
Noosa	26° 23'	153° 06'	60	1,692	2	19	Tree ≤ 25 m
Silver Valley	17° 32'	145° 19'	700	864	-	10	Mallee ≤ 6 m
St Bees Island	20° 56'	149° 26'	150	1,456	5	23	Tree ≤ 18 m
Ubobo	24° 27'	151° 11'	200	877	None	None	Tree ≤ 18 m
Woondum	26° 15'	152° 48'	380	986	11	98	Tree ≤ 30 m
Unknown clones			-	-	-	18	Various
Total					**58**	**418**	

[1] Latitude and longitude of original provenance locations

[2] Annual rainfall statistics from original provenance locations were obtained from the Australian Government Bureau of Meteorology (2018) using the closest weather station and calculated using the mean of all available years (all stations were within 40 km and had ≥ 15 years of available data)

Table 2 Rating system used to assess myrtle rust (*Austropuccinia psidii*) disease severity in lemon myrtle (*Backhousia citriodora*) germplasm

Score	Symptoms
1	No visible symptoms of infection
2	Minor infection: Lesions on expanding leaves small with 1–3 pustules per leaf; diseased area on leaves < 5%; and no evidence of stem infection
3	Minor to moderate infection: Lesions on expanding leaves generally small (occasionally large) with 3–6 pustules per leaf; diseased area on leaves 5–10%; and no evidence of stem infection
4	Moderate infection: Lesions on expanding leaves of various sizes with ≤ 15 per leaf; diseased area on leaves 10–25%; some evidence of stem and shoot infection; and minor leaf distortion
5	Moderate to severe infection: Lesions on expanding leaves of various sizes with 15–25 per leaf; diseased area on leaves 25–50%; obvious evidence of stem and shoot infection; and some leaf distortion and shoot death
6	Severe infection: Lesions on expanding leaves of various sizes with > 25 per leaf; diseased area on leaves 50–75% with leaf distortion; many stems and shoots infected; and shoot distortion and death common
7	Extreme infection: Lesions on expanding leaves of various sizes with > 25 per leaf; diseased area on leaves 75–100% with leaf distortion; many stems infected; all shoots infected and either distorted or dead and prominent dieback
Dead[1]	Whole plant dead (excluded from analysis)

[1]Dead plants were excluded as the cause of death may not be directly related to infection by *A. psidii*

pedigree information, known as the "unknown clones". The Dunoon trial site received supplementary irrigation during dry periods, whereas the Traveston site did not receive any supplemental watering.

Assessments and rating system To identify resistance and host response to repeated infection, the trials were evaluated for disease severity caused by *A. psidii* at the beginning of each season (i.e. summer, autumn, winter and spring), from December 2015 to December 2016 ($n = 5$ assessments). From the disease data, relative susceptibility/resistance was inferred after statistical analyses. Disease incidence (i.e. % of total leaves showing symptoms of infection) was recorded on a five-point (1–5) scale: (1) no leaves with symptoms; (2) 1–25% of leaves with symptoms; (3) 26–50% of leaves with symptoms; (4) 51–75% of leaves with symptoms; and (5) 76–100% of leaves with symptoms. The disease incidence data has not been presented as, particularly for the Dunoon site, most plants were category 4 or 5, which precluded meaningful comparisons between germplasm at the provenance, family and clone levels. Disease severity was determined using a seven-point (1–7) rating scale, which was based on previous assessment methods adopted to screen lemon myrtle germplasm (Doran et al. 2012; Lee et al. 2016) but optimised for this study (Table 2). The assessment method was developed following a review of published protocols and an initial survey of the trial at Dunoon, NSW (in September 2015). Also, disease severity could vary substantially within a single branch, so a rating system was needed at the whole plant level to accurately

screen large numbers of plants for resistance to *A. psidii* in the field.

Assessments were made on each ramet so that analyses could be undertaken at the provenance, family, and clone level. At each assessment, new shoots, juvenile stems, expanding foliage, and the top three pairs of fully developed adult leaves were examined for symptoms of *A. psidii* infection. These included sori with yellow urediniospores on young shoots and expanding leaves, old brown non-sporulating pustules and necrotic lesions on older leaves, and stem dieback. Plants that died throughout the trial period were excluded from analysis as the cause of death could not be attributed to infection by *A. psidii*.

Plant canopy assessment As part of the final assessment, each ramet was given a foliage density score to estimate foliage yield. This was assessed using the method described by Frampton et al. (2001), which scores the foliage density of each tree by comparing its degree of silhouetting on a two-dimensional scale in comparison to a reference sheet (Frampton et al. 2001). The method was adapted for ease of use in the field and assessed based on the following four categories: (1) 1–25% foliage density; (2) 26–50% foliage density; (3) 51–75% foliage density; and (4) 76–100% foliage density. The presence of flowering at the final assessment was also recorded. Assessments to study the impact of repeated infection on plant growth in height were planned, but pruning activities by property owners (to prevent the plants from becoming "top-heavy" and falling over during the storm season) prevented accurate assessments. Data from the following assessments were used for analysis: (1) disease severity (1–7); (2) foliage density (1–4); and (3) proportion of flowering ramets (%).

Climatic conditions To evaluate whether the climatic conditions occurring at the two sites during the study period were conducive to disease development, daily rainfall, temperature and relative humidity (RH) readings were obtained for Dunoon (station number 58021 [Dunoon], −28.69, 153.32) and Traveston (station number 40206, −26.33, 152.79) from 1 October 2015 to 31 December 2016 (SILO Climate Data, Queensland Government, Department of Science, Information Technology and Innovation 2016). The climate measures were reported for each 3-month period leading up to an assessment.

Resistance of lemon myrtle clones from the Ubobo provenance under controlled conditions in the shade house

Germplasm selection and propagation To screen the newly acquired lemon myrtle germplasm from the Ubobo provenance for resistance to *A. psidii*, cuttings were propagated and once established, were inoculated under controlled conditions in the shade house. The germplasm was collected from the Ubobo provenance as part of a previous study (Lee et al. 2016), where samples were obtained from the root coppice of 19 naturally occurring lemon myrtle trees occurring along the Koolkurrum Creek near Dawes National Park, Ubobo (permit numbers TK13683813, WISP13683913 and TWB/40/2013). In February 2017, when sufficient new flush had been produced on the collected samples, juvenile tip cuttings were taken from all available material and set in the glasshouse at the DPI Gympie Research Centre, Queensland, following an established protocol for the species (Lee et al. 2016). Secateurs were used to take juvenile tip cuttings (typically 12 for each clone), which were placed into a solution of 0.1% chlorine for approximately one minute (to surface sterilise), then rinsed in fresh tap water to remove the chlorine. The bottom pairs of leaves were detached from each cutting, leaving only one or two pairs, which were then cut (using stainless steel scissors) to ~ 50% of their full size to reduce transpiration. The base of the cutting was then dipped in 3% IBA gel (a rooting hormone) and set in pre-labelled trays (93 cc hiko) with crackpot inserts. The cutting mix consisted of: (1) 75% coarse perlite and 25% composted pine bark fines (0–10 mm); (2) 12–14 month slow-release Osmocote (N-P-K, 17.9:0.8:7.3) at a rate of 4 kg/m^3; (3) gypsum at 1 kg/m^3; (4) Micromax at 1 kg/m^3; and (5) the granular wetting agent Hydroflow at 1 kg/m^3. In March 2017, additional cuttings were taken from material with low numbers of surviving cuttings and processed using the same procedure.

Environmental conditions and experimental design The cuttings were held in a temperature-controlled glasshouse under an irrigation misting regime of 10 s every 15 min. In July 2017, the cuttings were transferred to a shade house at the Ecosciences Precinct in Dutton Park, Queensland, where they were maintained under natural temperature and lighting, with overhead watering for twelve minutes, twice per day. In September 2017, each cutting was transplanted into a 42 mm^2 × 80 mm propagation tube containing Coco Pro Super Premium Potting Mix (Rocky Point Mulching) and tip pruned to encourage new growth. The cuttings were established in a randomised complete block design with eight replicates of 19 treatments and one replicate of each treatment per block (n = 152 ramets).

Inoculation procedure In November 2017, when the cuttings had produced sufficient new susceptible growth, they were inoculated following a previously described method (Pegg et al. 2014b). As studies have indicated that only a single strain of *A. psidii* is present in Australia (da S.

Machado et al. 2015; Sandhu et al. 2016), urediniospores collected from a naturally infected *Syzygium jambos* tree in Indooroopilly, Queensland, were used, rather than bulked inoculum built from single spore isolates. Urediniospores of *A. psidii* were collected by gently shaking the infected leaf material into a paper bag. The urediniospores were sieved to remove leaves and other unwanted material, dried in a desiccator for ~ 2 weeks and then stored for up to 12 months in Nunc™ CryoTube™ Vials at −80 °C. When needed, the urediniospores were warmed to room temperature, then a haemocytometer was used to prepare a 1×10^5 urediniospores/mL suspension in sterile distilled water containing Tween® 20 (Croda International PLC) at a rate of 2 drops/100 mL. The solution was gently mixed to ensure even dispersal. The plants were inoculated using a handheld spray gun (Iwata Studio series 1/6 hp; Gravity spray gun RG3, Portland, USA) that produces a fine mist (2.9 kPa pressure). The spore suspension was applied to both leaf surfaces, with runoff avoided. To ensure high RH was rapidly achieved and maintained, hot tap water (~ 60 °C) was poured into trays below the plants, then they were covered with large plastic bags. For 24 h post-inoculation, the plants were held under darkness at 18–20 °C in a controlled environment room (CER). After 24 h, the bags were removed, and the plants were returned to the shade house.

Disease assessments Symptom development was monitored daily with germplasm assessed 21 days post-inoculation using a 5-point scoring system based on lesion size, which was adapted from a previously described method (Junghans et al. 2003), and the percentage of total leaf area affected (Lee et al. 2016). Disease was evaluated on expanding leaves and new shoots. The 1–5 resistance scale was as follows: (1) no evidence of infection; (2) hypersensitive response; (3) < 0.8 mm diameter pustules; (4) 0.8–1.6 mm diameter pustules; and (5) > 1.6 mm diameter pustules. The inoculation and assessments were repeated in December 2017 to ensure that those showing a low infection level were not escapes or that low disease was not due to the stage of growth flush. *Syzygium jambos* plants were used as susceptible controls in each inoculation. Any cuttings lacking susceptible flush when inoculated were excluded from the analysis.

Statistical analyses

All analyses were undertaken using Minitab® 19th edition (version 19.2020.1 64-bit, Minitab, LLC in the United States). For data analysis from the field trials, due to the unequal sample sizes of the provenances, families and clones, and survival of ramets across the two sites, analyses at each level were carried out using a previously described approach (Lee et al. 2016). Differences were tested using linear mixed-effects models (LMM) by restricted maximum likelihood (REML), with replicate (nested within site) included as a random effect. Significant results were followed by the post hoc Fisher's Least Significant Difference (LSD) test to identify differences between means. This method was also used to analyse the data collected from screening the Ubobo germplasm in the shade house. Chi-squared analysis was used to test for significant differences in the survival and proportion of flowering germplasm between the field sites and regions of origin. For all analyses, $p < 0.05$ was considered significant. Data for the "unknown clones" was not included in the statistical analyses at the provenance or family level, given the lack of information about their origin, but were pooled and presented for comparison.

Results

Resistance of lemon myrtle provenances, families and clones to myrtle rust under field conditions at two sites.

Plant survival, disease symptoms and flowering across sites Survival rates of the ramets at both sites was reasonably good, with 81% (683/840 ramets) at Dunoon and 96% (1,261/1,320 ramets) at Traveston alive at the first assessment in December 2015 and 79% (661/840 ramets) and 92% (1,211/1,320 ramets), respectively, alive at the final assessment in December 2016. Deaths that occurred throughout the trial period may not be directly related to infection by *A. psidii*. Symptoms of infection by *A. psidii* were recorded on every plant at both sites during at least one of the five assessments, indicating that all clones within the evaluated provenances and families showed a level of susceptibility to *A. psidii*. However, disease severity varied substantially between the tested provenances (Table 3), families (Table 4) and clones (Table 5), and between sites and assessments (Table 6). The impact ranged from small, restricted sori on a low percentage of leaves to symptoms on up to 100% of leaves, with distorted shoots and severe dieback on infected stems. No hypersensitive reaction, an indication of resistance, was observed during this study. At the final assessment in December 2016, 50% of the surviving ramets across both sites were flowering, but a significantly higher proportion of ramets were flowering at the Dunoon site than at Traveston (62% versus 43% respectively) ($\chi^2_{1,\ 1,815} = 59.63$, $p < 0.001$).

Resistance of provenances Mean disease severity (assessed on the 1–7 scale) across the five assessments was significantly different between the provenances ($F_{10,\ 1759.24} = 25.48$, $p < 0.001$) and between the sites ($F_{1,\ 4.58} = 290.98$, $p < 0.001$).

Table 3 Lemon myrtle (*Backhousia citriodora*) provenances assessed for myrtle rust (*Austropuccinia psidii*) resistance (means of all clones from a provenance) across field trials in Dunoon, New South Wales and Traveston, Queensland

Provenance [1]	Survival	Flowering	Foliage density [5]						Disease severity [5]					
			Dunoon			Traveston			Dunoon			Traveston		
	(%) [2]	(%) [3]	Mean	(±SE)		Mean	(±SE)		Mean	(±SE)		Mean	(±SE)	
Carlisle Island	93	64	2.30	(±0.13)	l	3.14	(±0.12)	f,g,i	4.29	(±0.10)	a,b	2.15	(±0.09)	g,h
Cathu	89	46	2.56	(±0.25)	j,k,l	3.31	(±0.17)	c,d,e,f,g,h	4.56	(±0.19)	a	2.22	(±0.13)	g,h,i
Conway	88	71	2.80	(±0.14)	i,j	3.55	(±0.12)	a,b,c	3.73	(±0.10)	d	2.05	(±0.09)	h,i,j
Dryander	84	52	2.44	(±0.13)	k,l	3.09	(±0.12)	f,g,i,j	4.05	(±0.10)	c	2.03	(±0.09)	h,i,j
Eumundi	90	47	3.32	(±0.12)	b,c,d,e,f,g,h	3.71	(±0.11)	a	3.28	(±0.09)	f	1.99	(±0.08)	i,j
Maroochy	85	27	2.67	(±0.18)	j,k	3.45	(±0.15)	b,c,d,e,h	3.98	(±0.14)	c,d	2.35	(±0.11)	g
Mt Elliot	88	49	2.43	(±0.18)	k,l	3.23	(±0.16)	e,f,g,h,i	4.03	(±0.14)	b,c	2.17	(±0.12)	g,h,i,j
Noosa	87	24	3.48	(±0.17)	a,b,c,e,f	3.75	(±0.14)	a	3.33	(±0.14)	e,f	2.02	(±0.10)	h,i,j
Silver Valley	85	68	3.28	(±0.19)	a,b,c,d,e,f,g,h	3.64	(±0.12)	a,b	3.37	(±0.15)	e,f	1.52	(±0.09)	k
St Bees Island	88	47	2.17	(±0.15)	l	2.99	(±0.14)	g,i,j	4.21	(±0.12)	a,b,c	2.05	(±0.10)	h,i,j
Woondum	84	38	3.14	(±0.12)	d,g,h	3.49	(±0.11)	b,c,d	3.46	(±0.09)	e	1.98	(±0.08)	j
Unknown clones	88	63	3.95	(±0.15)	-	3.87	(±0.13)	-	2.53	(±0.12)	-	1.77	(±0.10)	-

[1] Unknown clones not included in statistical comparison due to missing provenance data

[2] Survival calculated as proportion of ramets surviving to final assessment from total planted

[3] Flowering calculated as proportion of ramets flowering at final assessment from total surviving at final assessment

[4] Means followed by the same letter (a, b, c etc.) do not differ significantly in Fisher's LSD post hoc ($p < 0.05$)

[5] Cells highlighted in green indicate provenances that were significantly better than all others and cells highlighted in red indicate provenances that were significantly worse than all others

Table 4 Lemon myrtle (*Backhousia citriodora*) families assessed for myrtle rust (*Austropuccinia psidii*) resistance (means of all clones from a family) across field trials in Dunoon, New South Wales and Traveston, Queensland. Note: The unknown clones and the ramets from Silver Valley were not included in this analysis. Only families that were significantly higher or lower than all others for disease severity (based on Fisher's LSD post hoc pairwise comparisons) are listed. Means followed by the same letter (a or z) do not differ significantly in Fisher's LSD post hoc ($p<0.05$). The least and most resistant families are indicated by "a" and "z" respectively

Family	Provenance	Plants surviving (of planted)		Disease severity		
		Dunoon	Traveston	Mean	(±SE)	
1381	Carlisle Island	3/3	3/3	4.13	(±0.24)	a
1457	St Bees Island	3/3	3/3	3.85	(±0.24)	a
1459	St Bees Island	3/3	2/3	3.60	(±0.26)	a
1464	Conway	2/3	3/3	2.78	(±0.26)	z
1465	Woondum	-	3/3	2.77	(±0.34)	z
1345	Conway	-	2/3	2.77	(±0.41)	z
1337	Carlisle Island	2/3	3/3	2.66	(±0.26)	z
1467	Woondum	6/6	6/6	2.62	(±0.17)	z
1450	Carlisle Island	5/6	6/6	2.50	(±0.18)	z
1016	Eumundi	18/27	30/33	2.50	(±0.10)	z
1028	Woondum	21/30	28/33	2.48	(±0.10)	z
1026	Eumundi	13/18	33/33	2.47	(±0.10)	z
1010	Woondum	42/57	73/86	2.46	(±0.07)	z
1379	Carlisle Island	-	3/3	2.31	(±0.34)	z
1353	Conway	3/3	3/3	2.27	(±0.24)	z
1466	Woondum	6/6	6/6	2.27	(±0.17)	z
1468	Dryander	14/21	20/22	2.25	(±0.11)	z

The trial in Dunoon, NSW, had significantly higher average disease severity than the trial in Traveston, Queensland (3.84±0.08 versus 2.05±0.07 respectively; a difference of 1.80±0.11). There was also a significant site×provenance interaction ($F_{10,\ 1759.24}=11.58$, $p<0.001$). The Silver Valley germplasm planted at the Traveston site had significantly lower disease severity than all others (Table 3, Fig. 2). The highest disease severity was observed in clones from Cathu, Carlisle Island, and St Bees Island planted at the Dunoon site.

The survival of ramets to the end of the trial period ranged from 84–93%, depending on the provenance. Those originating from provenances in north and central coast Queensland were not significantly different to those from provenances in south coast Queensland (88% versus 86% respectively; $\chi^2_{1,\ 2,050}=1.17$, $p=0.279$). However, a significantly higher proportion of ramets originating from provenances in north and central coast Queensland were flowering, compared to those from south coast Queensland (60% versus 39% respectively; $\chi^2_{1,\ 1,719}=74.46$, $p<0.001$).

The mean foliage density of the ramets (assessed on a 1–4 scale) varied significantly between the provenances ($F_{10,\ 1689.20}=30.08$, $p<0.001$) and between sites ($F_{1,\ 4.44}=17.67$, $p<0.011$). Ramets at the Traveston site had a significantly higher foliage density than those at Dunoon (3.40±0.10 versus 2.78±0.11 respectively). There was also a significant site×provenance interaction ($F_{10,\ 1689.20}=3.03$, $p=0.001$), although there were minimal rank changes (Fig. 3). Those with the highest foliage density (and therefore lowest transparency) were clones from Conway and Eumundi planted at the Traveston site, and Noosa and Silver Valley planted at both sites. Although the unknown clones had the highest foliage density at both sites, but they were excluded from the statistical comparison due to lack of provenance information.

Resistance of families Disease severity varied significantly between the lemon myrtle germplasm at the family level ($F_{57,\ 1587.16}=10.33$, $p<0.001$) and between sites (Dunoon: 3.81±0.08 versus Traveston: 2.13±0.08; $F_{1,\ 4.11}=242.62$, $p<0.001$). The site×family interaction could not be statistically analysed as not all families were represented at both sites. However, the worst performing families, with the highest disease severity, were those from Carlisle Island (1381) and St Bees Island (1457 and 1459). The best performing families (with the lowest disease severity) were those from Carlisle Island (1337, 1379 and 1450), Conway (1345, 1353 and 1464), Dryander (1468), Eumundi (1016 and 1026) and Woondum (1010, 1028, 1465, 1466 and 1467) (Table 4).

Phenotypic disease expression of clones There were significant differences between the clones for disease severity ($F_{378,\ 1499.10}=4.78$, $p<0.001$) and disease severity was higher at Dunoon (3.73±0.08) than Traveston (2.03±0.07; $F_{1,\ 4.18}=249.00$, $p<0.001$). The site×clone interaction could not be statistically analysed as not all clones were represented at both sites. Of the 379 clones with at least one

Table 5 Lemon myrtle (*Backhousia citriodora*) clones assessed for myrtle rust (*Austropuccinia psidii*) resistance across field trials in Dunoon, New South Wales and Traveston, Queensland. Only clones that were significantly lower or higher for disease severity are listed

Clone	Family	Provenance	Plants surviving (of planted)		Disease severity		
			Dunoon	Traveston	Mean	(±SE)	
294	1468	Dryander	3/3	3/3	1.57	(±0.21)	a
394	1010	Woondum	3/3	6/6	1.86	(±0.18)	a
420	1010	Woondum	3/3	4/6	1.79	(±0.20)	a
219	UK15	Unknown	3/3	3/3	1.90	(±0.21)	a
206	UK2	Unknown	3/3	2/2	1.86	(±0.23)	a
310	1468	Dryander	1/3	3/3	2.05	(±0.26)	a
207	UK3	Unknown	3/3	6/6	2.02	(±0.18)	a
220	UK16	Unknown	3/3	3/3	1.87	(±0.21)	a
213	UK9	Unknown	3/3	3/3	2.10	(±0.21)	a
217	UK13	Unknown	3/3	3/3	1.86	(±0.21)	a
222	UK18	Unknown	2/3	-	1.91	(±0.36)	a
28	1028	Woondum	1/3	3/3	1.94	(±0.26)	a
55	1016	Eumundi	3/3	2/3	2.00	(±0.23)	a
291	1466	Woondum	3/3	3/3	2.03	(±0.21)	a
97	1011	Woondum	3/3	-	2.05	(±0.30)	a
361	1471	Dryander	3/3	3/3	4.18	(±0.21)	z
103	1014	Maroochy	-	3/3	3.95	(±0.30)	z
146	1356	Conway	2/3	3/3	4.01	(±0.23)	z
425	1413	Woondum	1/3	3/3	3.88	(±0.26)	z
301	1452	Carlisle	3/3	3/3	3.92	(±0.21)	z
324	1453	Carlisle	3/3	3/3	3.92	(±0.21)	z
343	1430	Woondum	3/3	2/3	3.96	(±0.23)	z
439	1424	Cathu	3/3	3/3	3.97	(±0.21)	z
381	1447	Carlisle	-	3/3	4.00	(±0.30)	z
428	1430	Woondum	3/3	2/3	4.12	(±0.23)	z
137	1381	Carlisle	3/3	3/3	4.13	(±0.21)	z
430	1430	Woondum	3/3	3/3	4.16	(±0.21)	z
429	1430	Woondum	1/3	3/3	4.17	(±0.26)	z
366	1471	Dryander	2/3	3/3	4.46	(±0.23)	z
176	1331	Dryander	3/3	-	4.52	(±0.30)	z

surviving ramet screened in at least one of the sites, there were 15 clones that had the highest disease severity: clones from Carlisle Island ($n=4$), Cathu ($n=1$), Conway ($n=1$), Dryander ($n=3$), Maroochy ($n=1$) and Woondum ($n=5$) (Table 5). The clones with the lowest disease severity originated from the provenances of Dryander ($n=2$), Eumundi ($n=1$), and Woondum ($n=5$), and several clones ($n=7$) whose family and provenance information is unknown.

Disease levels throughout the study Across all assessments and at all levels of analysis (i.e. provenance, family and clone), disease severity was consistently lower at the Traveston site than at Dunoon. Most plants (>80%) remained below severity level three, except during the fourth assessment (Fig. 4). At both sites, disease severity was highest in September (fourth assessment).

Environmental conditions Climatic conditions (e.g. rainfall, days of rain, temperature, and RH) at the two sites varied substantially in the quarter leading up to the first assessment and throughout the study period (Fig. 5, Table 6). The total quarterly rainfall (mm) and days of rain were typically higher in Dunoon than in Traveston. From October 2015 (two months before the first assessment that was carried out) until December 2016 (month of final assessment), the Dunoon trial site received 360 mm more rainfall than Traveston (1,654 mm versus 1,295 mm respectively). Rainfall fell over 218 days at Dunoon versus 129 days at Traveston. The highest rainfall (mm) and days of rain occurred in the January-March 2016 quarter at both sites, although a similar level of rainfall was also recorded for the April-June 2016 quarter at Dunoon.

Resistance of lemon myrtle clones from the Ubobo provenance under controlled conditions in the shade house.

Of the 152 cuttings from Ubobo, 11 had died before inoculations were carried out and were therefore excluded from the analysis. All tested Ubobo clones were highly susceptible to *A. psidii*, with large sori (pustules) and urediniospores observed on new shoots and expanding leaves, and several

Table 6 Myrtle rust (*Austropuccinia psidii*) disease severity (evaluated on a 1–7 scale) in lemon myrtle (*Backhousia citriodora*) germplasm assessed across field trials in Dunoon, New South Wales and Traveston, Queensland, mean daily temperature and humidity records, and total rainfall (mm) and days of rain per assessment quarter

Site & assessment	Mean daily temperature		Mean daily relative humidity		Quarterly rainfall		Severity 1–7 scale	
	Min	Max	Min[1]	Max[1]	Total	Days of rain		
	(°C)	(°C)	(%)	(%)	(mm)	(*n*)	Mean	(±SE)
Dunoon, New South Wales								
Oct-Dec, 2015	15.9	27.0	54.8	98.0	364.8	50	3.26	(±1.10)
Jan-Mar, 2016	18.5	28.2	58.8	98.4	414.0	54	3.71	(±0.96)
Apr-Jun, 2016	12.2	23.6	54.0	96.6	413.6	41	4.25	(±1.19)
Jul-Sep, 2016	9.7	21.5	50.6	94.7	283.2	39	4.53	(±1.25)
Oct-Dec, 2016	14.9	28.3	46.5	96.4	178.8	34	2.49	(±1.18)
Total	**14.2**	**25.7**	**52.9**	**96.8**	**1,654.4**	**218**		
Traveston, Queensland								
Oct-Dec, 2015	16.7	27.0	54.9	96.7	271.4	23	1.66	(±0.59)
Jan-Mar, 2016	19.3	28.5	58.7	97.3	339.8	34	1.59	(±0.60)
Apr-Jun, 2016	13.3	24.1	55.6	96.7	245.4	24	1.85	(±0.81)
Jul-Sep, 2016	10.6	22.1	52.2	95.3	191.9	27	2.82	(±1.07)
Oct-Dec, 2016	15.9	28.3	46.4	92.9	246.2	21	1.92	(±0.70)
Total	**15.2**	**26.0**	**53.5**	**95.8**	**1,294.7**	**129**		

[1] "Relative humidity minimum" is estimated relative humidity at maximum temperature (i.e. daytime) and "relative humidity maximum" is estimated relative humidity at minimum temperature (i.e. overnight). Both sites experienced daytime maximum temperatures of≥30 °C. The greatest number of days where this occurred was in the October-December 2016 quarter (27 days at both sites), followed by the January-March 2016 quarter (Dunoon: 20 days; Traveston 23 days) and the October-December 2015 quarter (Dunoon: 18 days; Traveston: 15 days). Neither site recorded temperatures of≥30 °C in the July–September 2016 quarter, and Dunoon recorded this for only two days in the April-June 2016 quarter (Traveston: 0 days)

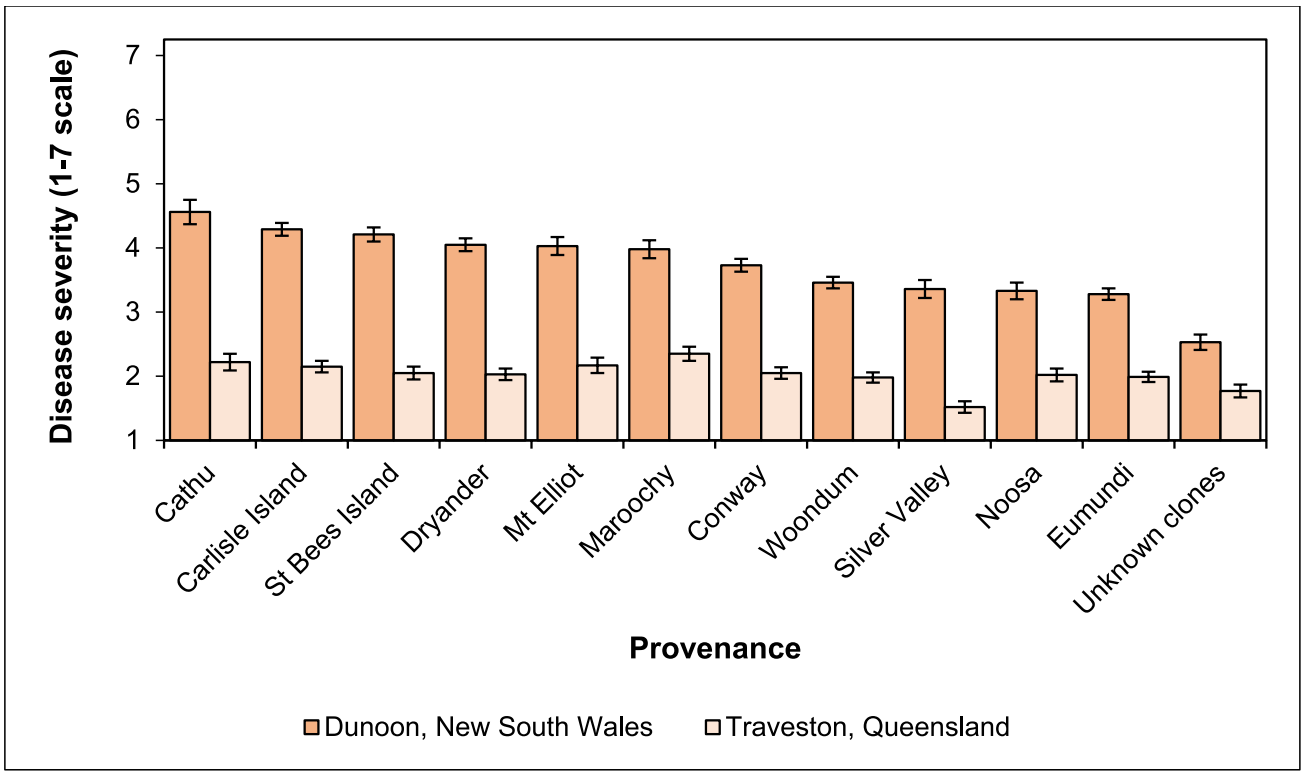

Fig. 2 Myrtle rust (*Austropuccinia psidii*) mean disease severity (evaluated on a 1–7 scale) in lemon myrtle (*Backhousia citriodora*) provenances assessed across field trials in Dunoon, New South Wales and Traveston, Queensland. Data sorted by decreasing disease severity at Dunoon

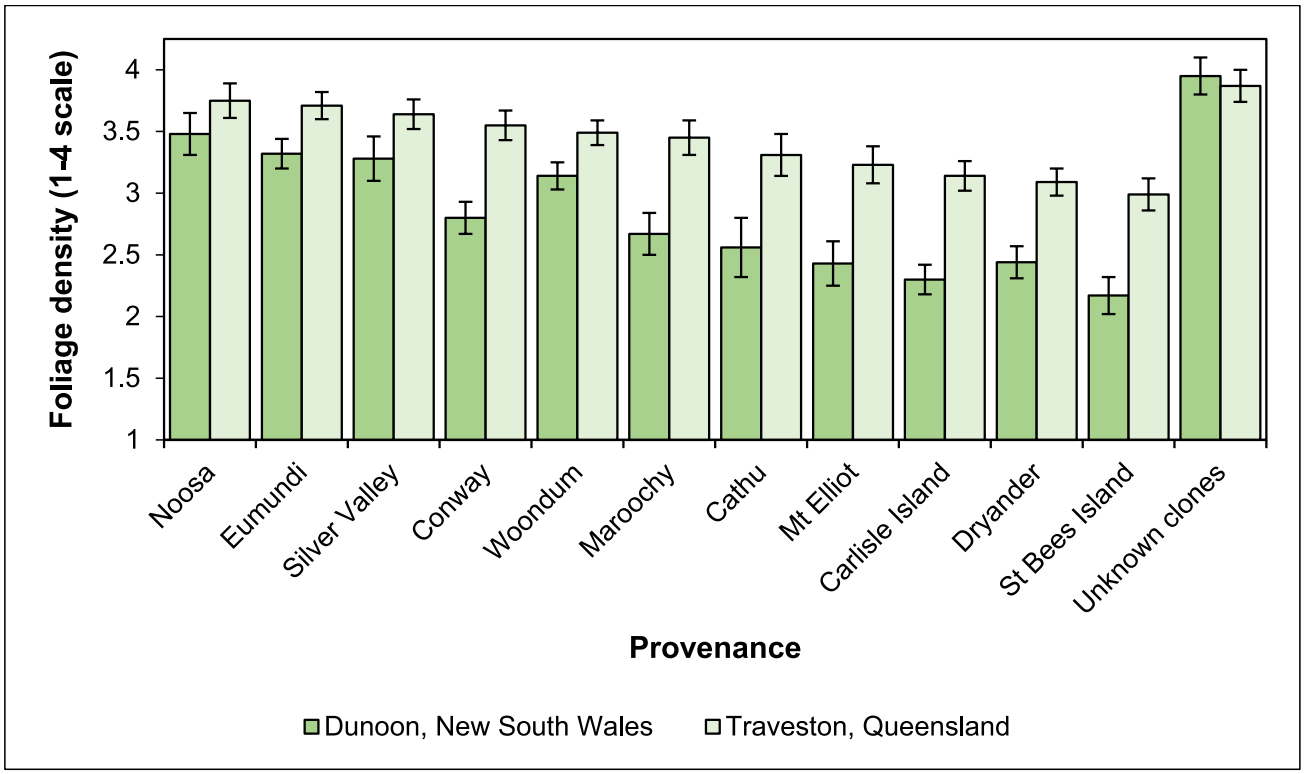

Fig. 3 Mean foliage density (evaluated on a 1–4 scale) of lemon myrtle (*Backhousia citriodora*) provenances assessed across field trials in Dunoon, New South Wales and Traveston, Queensland. Data sorted by decreasing foliage density at Traveston

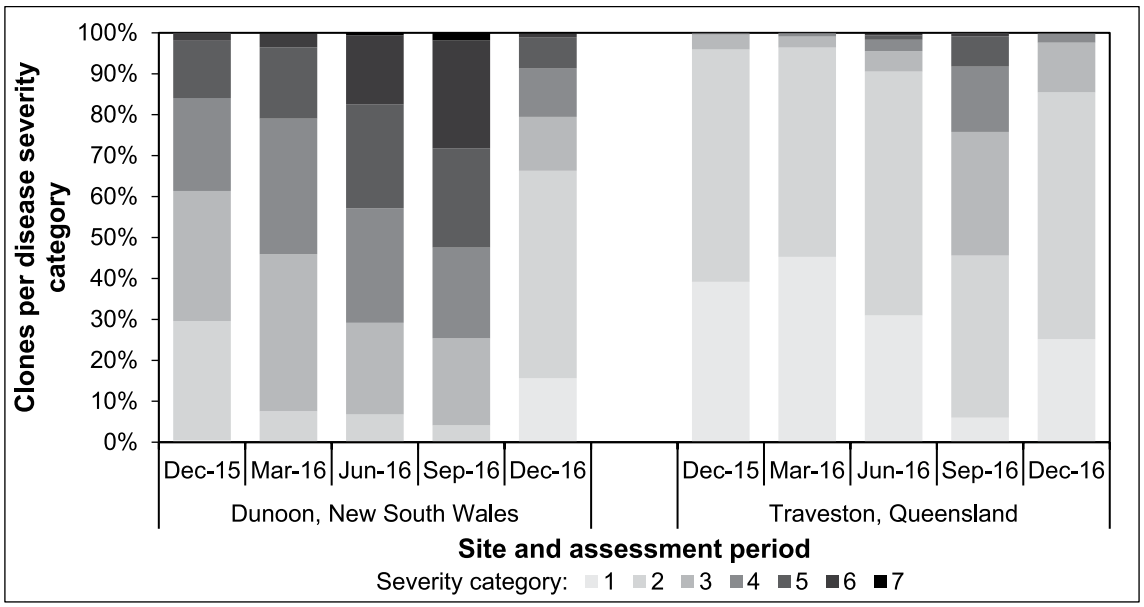

Fig. 4 Proportion of lemon myrtle (*Backhousia citriodora*) clones in each disease severity category (evaluated on a 1–7 scale) when assessed for resistance to myrtle rust (*Austropuccinia psidii*) in Dunoon, New South Wales and Traveston, Queensland from December 2015 to December 2016. Colours from white to black indicate increasing disease severity categories

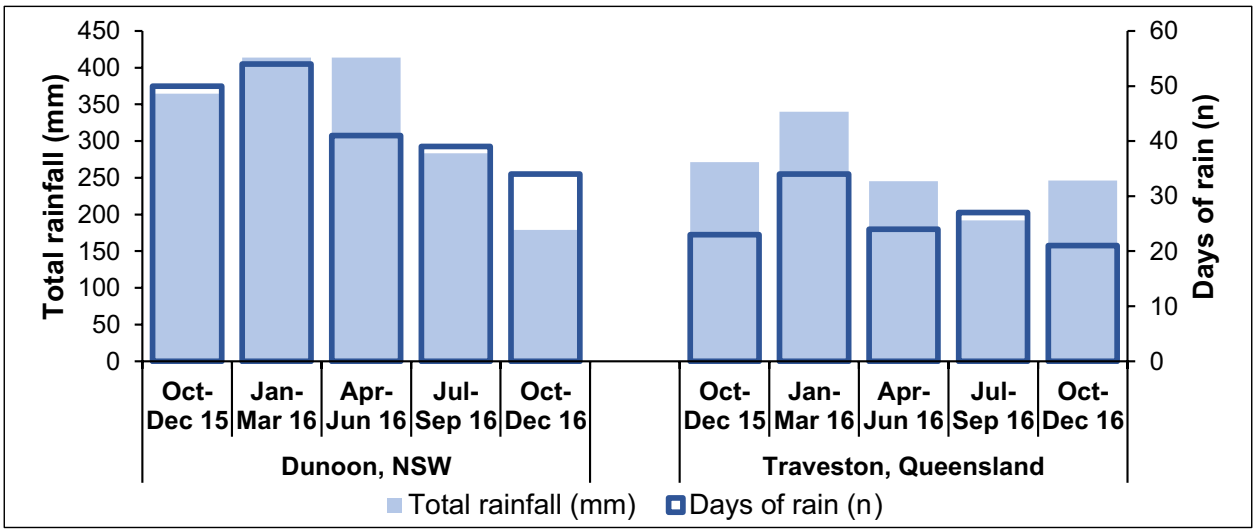

Fig. 5 Total rainfall (mm) and days of rain per 3-month period from October 2015 to December 2016 in Dunoon, New South Wales and Traveston, Queensland

Fig. 6 Myrtle rust (*Austropuccinia psidii*) disease symptoms observed in lemon myrtle (*Backhousia citriodora*) germplasm from the Ubobo provenance following inoculation under controlled conditions: large lesions with uredinia and urediniospores on young expanding foliage and developing stems approximately 14 days post-inoculation (left) and restricted red-brown coloured lesions on older leaves approximately six weeks post-inoculation (right)

cuttings had stem infection and dieback (Fig. 6). No clones were identified as having qualitative resistance, but there were significant differences in their disease susceptibility based on the Junghans scale ($F_{18, 122}=2.01$, $p=0.014$) and in their proportion of leaf area (%) with disease symptoms ($F_{18, 122}=1.97$, $p=0.017$), indicating quantitative resistance (Table 7). On the Junghans scale, all clones were in categories 4 (31% of ramets) or 5 (69% of ramets) and 15 of the 19 tested clones had disease symptoms covering > 50% of their leaf area (the remainder had > 40%).

Discussion

Lemon myrtle clones from all known provenances throughout Queensland (excluding Ubobo) were screened for resistance to the pandemic biotype of *A. psidii* under field conditions at two sites. Assessments of the germplasm established in the field trials at Dunoon, NSW and Traveston, Queensland, could not identify qualitative resistance to *A. psidii* at a provenance, family or clonal level. These results were consistent with what was previously reported when the Beerburrum genebank was assessed (Doran et al. 2012) and when germplasm was screened following inoculation under controlled conditions (Lee et al. 2016). Based on the glasshouse and field assessments carried out thus far, it seems that all tested germplasm is susceptible to *A. psidii*, but some clones have greater apparent resistance to the disease.

While the primary goal of this study was to identify clones with higher resistance to *A. psidii* than those currently used by the industry, analyses were also undertaken at the family and provenance levels. Germplasm from north and south coast Queensland provenances generally showed lower disease severity than those from central coast Queensland provenances. Clones from Eumundi, Noosa and Woondum (several of the southernmost provenances in the known native distribution) and Silver Valley (the northernmost provenance in the known native distribution) consistently displayed lower disease at each assessment, similar

Table 7 Disease susceptibility using Junghans scale and leaf area with disease symptoms observed on lemon myrtle (*Backhousia citriodora*) germplasm from Ubobo screened for resistance to myrtle rust (*Austropuccinia psidii*) after inoculation under controlled conditions. Data sorted by decreasing disease severity

Clone	Ramets	Junghans scale (1–5)[1]			Leaf area with symptoms (%)[1]		
ID	*n*	Mean	(±SE)	Grouping	Mean	(±SE)	Grouping
2568	7	5.00	(±0.17)	a	59.3	(±7.2)	a,b,c,d
2569	5	5.00	(±0.20)	a	69	(±8.5)	a,b
2582	7	5.00	(±0.17)	a	67.1	(±7.2)	a,b
2587	6	5.00	(±0.18)	a	72.5	(±7.7)	a,b
2585	8	4.88	(±0.15)	a,b	68.1	(±6.7)	a,b
2571	8	4.88	(±0.15)	a,b	63.1	(±6.7)	a,b,c
2576	8	4.88	(±0.15)	a,b	76.3	(±6.7)	a
2579	8	4.75	(±0.15)	a,b,c	55.6	(±6.7)	b,c,d
2572	7	4.71	(±0.17)	a,b,c	61.4	(±7.2)	a,b,c
2573	8	4.63	(±0.15)	a,b,c,d	55.6	(±6.7)	b,c,d
2580	8	4.63	(±0.15)	a,b,c,d	58.1	(±6.7)	a,b,c,d
2581	8	4.63	(±0.15)	a,b,c,d	61.3	(±6.7)	a,b,c
2570	8	4.63	(±0.15)	a,b,c,d	58.8	(±6.7)	a,b,c,d
2578	8	4.63	(±0.15)	a,b,c,d	46.9	(±6.7)	c,d
2586	7	4.57	(±0.17)	a,b,c,d	54.3	(±7.2)	b,c,d
2577	8	4.50	(±0.15)	b,c,d	45	(±6.7)	c,d
2574	8	4.38	(±0.15)	c,d	52.5	(±6.7)	b,c,d
2583	6	4.33	(±0.18)	c,d	44.2	(±7.7)	c,d
2584	8	4.25	(±0.15)	d	40.6	(±6.7)	d

[1]Means followed by the same letter (a, b, c etc.) do not differ significantly in Fisher's LSD post hoc ($p < 0.05$)

to that reported previously (Doran et al. 2012; Lee et al. 2016). Germplasm from Silver Valley, the most northern provenance and from an area of relatively low annual rainfall, had the lowest overall disease severity across both sites. The "unknown clones" from provenances in south coast Queensland also had low disease severity at Traveston and Dunoon, similar to that reported from Beerburrum (Doran et al. 2012; Lee et al. 2016).

There is some, albeit conflicting, evidence to suggest that the resistance of several myrtaceous species to *A. psidii* is associated with the provenance origin. For example, in tea tree (*Melaleuca alternifolia*), which like lemon myrtle is native to subtropical regions of Australia and grown for its essential oil, significant differences for resistance to *A. psidii* were detected at the region, provenance and family levels (Shepherd et al. 2015). Germplasm from upland provenances generally had a consistently higher proportion of individuals with apparent resistance than those from coastal provenances (Shepherd et al. 2015). Screening under controlled conditions revealed inland provenances of *Eucalyptus cloeziana* had higher resistance to *A. psidii* and thicker leaves (in the cuticle, epidermis and palisade layers) than coastal provenances (Lee et al. 2015). However, when spotted gum (*Corymbia* spp.) species were screened for resistance to *A. psidii* under controlled conditions, there was no correlation between the climatic conditions (i.e. rainfall, temperature and altitude) at the provenance of origin and resistance (Pegg et al. 2014a). There was also no significant correlation between climatic conditions and the susceptibility of Tasmanian provenances of *E. globulus* and *E. obliqua* to *A. psidii* (Yong et al. 2019). Although, for *E. globulus*, there was a trend of increased resistance from the south-eastern to north-eastern regions. Further research is needed to examine the factors driving the higher resistance to *A. psidii* of lemon myrtle germplasm from north and south coast Queensland. Testing the association between provenance climate variables and resistance may also be beneficial.

It is possible that germplasm from Silver Valley has adaptations for low rainfall/resource environments, which may be associated with the lower disease observed in this study. The Silver Valley provenance is the most northerly naturally occurring population of lemon myrtle. It also occurs at the highest elevation (700 m) by a substantial margin (+300 m) and receives the second-lowest annual rainfall (864 mm). During the field assessments carried out in this study and the previous glasshouse trial (Lee et al. 2016), germplasm from Silver Valley was noted to have smaller and thicker leaves than those from other provenances (although it was not explicitly quantified). According to Freeman et al. (2019), in the case of native pathogens, host populations from areas with environmental conditions conducive to disease outbreaks are typically less susceptible due to pathogen-imposed selection. Whereas in the case of exotic pathogens, host populations from areas favourable for the pathogen are typically more susceptible (Freeman et al. 2019). Understanding the cause-and-effect mechanisms of this is relevant

for future selection programs and research investigating the association between adaptations for lower rainfall/resource environments and resistance to *A. psidii* is justified.

Another factor that needs consideration is the influence of ontogenic or physiological aging on susceptibility and response to disease. The resistance of the Silver Valley germplasm and the unknown clones to *A. psidii* may be associated with their being derived from tissue of greater ontogenic or physiological age. The Silver Valley germplasm established in the Beerburrum genebank, and subsequently used to establish the field trials, was sourced from established trees of unknown age, not grown from seed like the other tested provenances. Furthermore, the "unknown clones" in the Beerburrum genebank, which lack pedigree information but are known to have been derived from coppice cuttings of the seed grown material from Eumundi, Maroochy, Noosa and Woondum already established at the same site (Chen 1997), have also shown only low disease. In the study by Shepherd et al. (2015), the authors reported that clones planted adjacent to their seedling trial, which were derived from mature native trees, were more resistant to *A. psidii*. This was despite them being planted at about the same time and being exposed to comparable environmental conditions. The concept of ontogenic age as a means of increased resistance to *A. psidii*, as well as differences in host phenology, warrants further investigation.

Multiple disease assessments conducted at different times of the year enabled susceptibility to be evaluated under different environmental conditions, increasing the likelihood of disease development and subsequent identification of any disease resistant germplasm. At both sites, disease severity was greatest at the September 2016 assessment. During the summer quarter, both sites had numerous days with temperatures ≥ 30 °C (Dunoon: 20; Traveston: 23); the effect these high temperatures have on lemon myrtle growth and its influence on observed disease symptoms and the severity of symptoms or impact has not been reported. The cooler temperatures occurring during the July–September 2016 quarter (autumn/winter period) led to a greater number of days with conditions highly conducive to disease development and no days at either site with temperatures of ≥ 30 °C. Furthermore, the high levels of rainfall and days of rain experienced during the January-March 2016 period, which typically supports plant growth, may have reduced the airborne inoculum load (Tessmann et al. 2001; Zauza et al. 2015). A greater understanding of the disease epidemiology and host–pathogen interactions would be valuable.

In this study, there was a significant genotype × environment interaction, supporting the notion that germplasm selection should not be undertaken at a single site with the expectation that it will perform similarly in other areas. Disease severity was significantly higher at the Dunoon site across all assessments, which resulted in rank changes among the provenances. *Backhousia citriodora* is a rainforest species naturally occurring in areas with > 800 mm annual rainfall (Doran & House 1996). Both sites received > 1,200 mm across the 15-month period reported for the study, which fell over 130 days at Dunoon and 124 days at Traveston. However, rainfall was the only source of moisture at Traveston, but the Dunoon site was irrigated and received supplementary water during dry periods. So, irrespective of the other environmental conditions at the two sites, the lower levels of rainfall that occurred in Traveston were not as conducive to host growth as that occurring in Dunoon, which are likely to have influenced the level of infection by *A. psidii* given the need for new active growth to be present for infection to occur.

Environmental conditions can influence the expression of genetic variation for rust resistance, leading to rank changes at the provenance, family, or clone level, particularly if the environmental conditions are not conducive for host growth. For example, when screening commercial chrysanthemum species for resistance to white rust (caused by *Puccinia horiana*), the results ranged from immune to highly susceptible, and while they were similar across the two trials, two populations of *Chrysanthemum indicum* had inconsistencies where each had high apparent resistance in one trial but was highly susceptible in the other (Zeng et al. 2013). Although in that study, the inoculum consisted of teliospores collected from diseased cv. Jinba plants in the first instance, and diseased cv. Iwanohakusen in the second. When screening *Eucalyptus grandis* germplasm for resistance to *A. psidii*, trials involving multiple sites enabled more accurate quantification of genotype × environment interactions and their impact on germplasm selection for commercial production (da Silva et al. 2019a, 2019b; Miranda et al. 2013; Silva et al. 2013). For accurate resistance screening to be carried out in the field, it is vital that the inoculum load is high, and the environmental conditions support host growth, pathogen infection and disease development.

The results of this study support previous reports that lemon myrtle expresses much lower observed disease symptoms when planted in drier, irrigated areas, where the impact of *A. psidii* is likely to be lower due to less conducive environmental conditions for infection (Lee et al. 2016). Disease severity at the Traveston site was significantly lower than that of Dunoon, despite both sites being surrounded by susceptible myrtaceous species (and therefore presumably a high airborne inoculum load). Severe *A. psidii* infection and decline of the highly susceptible scrub turpentine (*Rhodamnia rubescens*) has been reported from the Traveston site (Carnegie et al. 2016). Several studies using climatic mapping software to forecast the spread of *A. psidii* in Australia have indicated that the climatic conditions in northern and

inland areas may be less conducive to disease development, and the pathogen will primarily remain restricted to tropical and subtropical regions along the eastern and southern coasts (Berthon et al. 2018; Booth et al. 2000; Booth & Jovanovic 2012; Kriticos et al. 2013).

Analyses at the provenance, family, and clone levels from the two clonal plantings showed significant differences between the respective groups, but sample sizes varied considerably. For example, at the family level, disease assessments were carried out on 3–143 ramets (depending on the family). In some cases, the ramets were only assessed at the Traveston site, which experienced significantly lower disease. In other cases, only one surviving ramet of each clone could be assessed at each site. While REML analyses can account for unequal samples sizes, as well as fixed and random factors (Gleeson & Cullis 1987; Virk et al. 2009), care should be taken to ensure that further testing is undertaken on any superior germplasm identified before there is mass propagation for industry deployment in commercial plantations. Nevertheless, given the significant differences in disease severity between the tested germplasm, it should be possible to select clones that are more resistant to the disease than the highly susceptible ones that are currently used in commercial plantations. Given that the tested germplasm originates from 13 distinct provenances in environmentally variable areas within Queensland, some clones showing higher levels of susceptibility to *A. psidii* may also be better adapted to commercial production in drier areas.

This is the first time that germplasm from the Ubobo provenance has been screened for resistance to *A. psidii*. Inoculation under controlled conditions revealed that the newly collected germplasm from the Ubobo provenance is highly susceptible to the biotype of *A. psidii* established in Australia. However, these results should be confirmed with field testing, as material from St Bees Island, for example, appears to be more resistant in the field than it does in glasshouse assessments (Lee et al. 2016).

Further testing is needed to determine whether the superior clones identified in this study also meet the industry requirements for growth traits, essential oil characteristics, and amenability to regular machine harvesting. The between-tree competition that occurs in single tree plots such as these (where an individual could be surrounded by highly susceptible or highly resistant clones) do not facilitate accurate indications of productivity in a commercial setting where genetically similar trees are planted together in blocks (Callister et al. 2013). A selection of the superior germplasm identified in this study should be established in replicated block plantings across multiple sites with contrasting environmental conditions. In addition, controlled commercial practices (e.g. irrigation, regular fertilisation, weeding and harvesting) should be carried out to evaluate how these practices affect myrtle rust development, lemon myrtle biomass production, and essential oil properties.

The selection of resistant germplasm is complicated by the presence of multiple strains of *A. psidii*. As is common in many plant pathogenic rust fungi, *A. psidii* strains exist that can overcome the resistance. For example, *E. grandis* clones carrying the *Ppr-1* gene and recognised as resistant to *A. psidii* race-1 were susceptible to other strains (Graça et al. 2011). In 2016, a genotype of *A. psidii* that is different from that currently established in Australia was discovered infecting a *B. citriodora* plant in South Africa (Roux et al. 2016). That study confirmed that *B. citriodora* germplasm is susceptible to more than one strain of *A. psidii*, which is why quantitative resistance may be superior, and this should be considered when selecting superior germplasm for commercial planting. Ideally, the germplasm should also be screened against other biotypes of *A. psidii* distributed elsewhere internationally, reducing risk in the case of future *A. psidii* incursions.

Acknowledgements This research was supported by the Plant Biosecurity Cooperative Research Centre. The authors would like to sincerely thank Gary Mazzorana, Scott Powell and the staff at Australian Rainforest Products Pty Ltd for their continued support in maintaining the clonal planting in Dunoon (New South Wales) and staff at the Department of Primary Industries (Gympie office) for maintaining the clonal planting in Traveston (Queensland). The authors would like to thank F. S. Bonora, M. Neilsen and J. Oostenbrink for their assistance with field assessments, sample collection and propagation training. We would also like to acknowledge the previous research undertaken by J. Doran, D. Lea and D. Bush, which was the basis for this study.

Authors' contributions Conceptualization: Emily K. Lancaster, David J. Lee and Geoffrey S. Pegg; Methodology: Emily K. Lancaster and David J. Lee; Formal analysis and investigation: Emily K. Lancaster, David J. Lee and Geoffrey S. Pegg; Writing—original draft preparation: Emily K. Lancaster; Writing—review and editing: André Drenth, R. Andrew Hayes, David J. Lee and Geoffrey S. Pegg; Funding acquisition: André Drenth, David J. Lee and Geoffrey S. Pegg; Resources: David J. Lee and Geoffrey S. Pegg; Supervision: André Drenth, R. Andrew Hayes, David J. Lee and Geoffrey S. Pegg.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. This research was supported by the Plant Biosecurity Cooperative Research Centre.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not applicable.

Competing interest The authors have no relevant financial or non-financial interests to disclose.

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