ROSIANE FÁTIMA DE ALMEIDA

A NEW, HIGHLY AGGRESSIVE RACE OF *Austropuccinia psidii* INFECTS A WIDELY PLANTED, MYRTLE RUST-RESISTANT, EUCALYPT GENOTYPE IN BRAZIL

Dissertation submitted to the Plant Pathology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Acelino Couto Alfenas

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To GOD, who is good all the time...

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ABSTRACT

ALMEIDA, Rosiane Fátima de, M.Sc., Universidade Federal de Viçosa, July, 2021. **A new, highly aggressive race of *Austropuccinia psidii* infects a widely planted, myrtle rust-resistant, eucalypt genotype in Brazil**. Adviser: Acelino Couto Alfenas.

Myrtle rust (MR) caused by *Austropuccinia psidii* is one of the most important diseases affecting eucalypt (*Eucalyptus* spp.) plantations in Brazil. Over the years, selection and planting of MR-resistant clones has been the primary strategy for MR management. In May 2013, young trees of the GG100 hybrid (*E. grandis* × *E. urophylla*) clone – widely planted in Brazil and previously classified as resistant to MR – were infected by *A. psidii* in Minas Gerais, Brazil. In this study, artificial inoculations of a eucalypt clone set with differential reactions to *A. psidii* races were used to discover a new race of *A. psidii* (race 5) that was highly aggressive on the majority of eucalypt clones tested. In addition, only this new race successfully infected eucalypt 847 genotype, which was formerly classified as resistant to the four previously known races of *A. psidii*. Our findings demonstrate that this new *A. psidii* race is highly aggressive and capable of infecting a larger number of eucalypt genotypes compared to the previously known *A. psidii* races 1, 2, 3, and 4.

Keywords: *Puccinia psidii*. Physiological variability. *Eucalyptus*.

RESUMO

ALMEIDA, Rosiane Fátima de, M.Sc., Universidade Federal de Viçosa, julho de 2021. **Uma nova raça altamente agressiva de *Austropuccinia psidii* infecta um genótipo de eucalipto amplamente plantado e resistente à Ferrugem das Mirtáceas no Brasil**. Orientador: Acelino Couto Alfenas.

A Ferrugem das Mirtáceas (RM), causada por *Austropuccinia psidii*, é uma das doenças mais importantes que afetam as plantações de eucalipto (*Eucalyptus* spp.) no Brasil. Ao longo dos anos, a seleção e o plantio de clones resistentes a MR tem sido a principal estratégia para o manejo da doença. Em maio de 2013, árvores jovens do clone híbrido GG100 (*E. grandis* x *E. urophylla*) - amplamente plantado no Brasil e previamente classificado como resistente à MR - foram infectadas por *A. psidii* em Minas Gerais, Brasil. Neste estudo, inoculações artificiais de um conjunto de clones de eucalipto com reações diferenciais às raças de *A. psidii* foi usado para descobrir uma nova raça de *A. psidii* (raça 5) que foi altamente agressiva na maioria dos clones de eucalipto testados. Além disso, apenas esta nova raça infectou com sucesso o genótipo 847, que foi anteriormente classificado como resistente às quatro raças de *A. psidii* anteriormente conhecidas. Nossos resultados demonstram que esta nova raça de *A. psidii* é altamente agressiva e capaz de infectar um grande número de genótipos de eucalipto em comparação com as raças 1, 2, 3 e 4 de *A. psidii* anteriormente conhecidas.

Palavras-chave: *Puccinia psidii*. Variabilidade fisiológica. *Eucalyptus*.

SUMMARY

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A new, highly aggressive race of *Austropuccinia psidii* infects a widely planted, myrtle rust-resistant, eucalypt genotype in Brazil

A new, highly aggressive race of *Austropuccinia psidii* infects a widely planted, myrtle rust-resistant, eucalypt genotype in Brazil

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Running Head: A new race of *Austropuccinia psidii* infects eucalypt

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Abstract

Myrtle rust (MR) caused by *Austropuccinia psidii* is one of the most important diseases affecting eucalypt (*Eucalyptus* spp.) plantations in Brazil. Over the years, selection and planting of MR-resistant clones has been the primary strategy for MR management. In May 2013, young trees of the GG100 hybrid (*E. grandis* × *E. urophylla*) clone – widely planted in Brazil and previously classified as resistant to MR – were infected by *A. psidii* in Minas Gerais, Brazil. In this study, artificial inoculations of a eucalypt clone set with differential reactions to *A. psidii* races were used to discover a new race of *A. psidii* (race 5) that was highly aggressive on the majority of eucalypt clones tested. In addition, only this new race successfully infected eucalypt 847 genotype, which was formerly classified as resistant to the four previously known races of *A. psidii*. Our findings demonstrate that this new *A. psidii* race is highly aggressive and capable of infecting a larger number of eucalypt genotypes compared to the previously known *A. psidii* races 1, 2, 3, and 4.

Keywords: *Puccinia psidii*, rust pathogen race, physiological variability, genetic variability, *Eucalyptus*.

1 | INTRODUCTION

Austropuccinia psidii (G. Winter) Beenken, first reported as *Puccinia psidii* on guava (*Psidium guajava*) in Southern Brazil (Winter, 1884), is an obligate biotrophic pathogen that causes myrtle rust (MR) and threatens numerous Myrtaceae species around the world (Beresford et al., 2018; Carnegie et al., 2016; Pegg et al., 2017, 2014; Winzer et al., 2019). After the MR pathogen was first described in South America, it was found in North America and the Caribbean (Coutinho et al., 1998; MacLachlan, 1938; Marlatt and Kimbrough, 1979), and it has subsequently been found in other regions, including Asia (du Plessis et al., 2017; Kawanishi et al., 2009; McTaggart et al., 2016; Zhuang and Wei, 2011), Africa (Roux et al., 2013), and Oceania (Beresford et al., 2018;Carnegie et al., 2010; Winzer et al., 2018; Uchida et al., 2006). As *A. psidii* appeared in new global regions, its known host range has continued to expand, and it is now known to infect a wide range of host species in the Myrtaceae (Carnegie and Giblin, 2020; Pegg et al., 2017, 2014; Stewart et al., 2018).

Although *A. psidii* was first reported on eucalypt (*Eucalyptus* spp.) in Brazil during the early 20th century (Joffily, 1944), the first serious outbreak of MR was observed in *E. grandis* plantations of Espírito Santo, Brazil in the late 20th century (Ferreira, 1983, 1981). Subsequently, serious MR outbreaks have been reported sporadically in eucalypt plantations of Brazil. MR signs and symptoms depend on resistance/susceptibility reactions of the host, but may range from hypersensitivity reaction (HR) to small uredinia with few urediniospores or massive production of powdery, bright-yellow urediniospores associated with shoot dieback that may ultimately result in plant mortality (Alfenas et al., 2009; Coutinho et al., 1998). MR has significant economic importance in eucalypt plantations (Alfenas et al., 2009, 2003; Carvalho et al., 1998; Glen et al., 2007; Graça et al., 2011; Silva et al., 2017; Zauza et al., 2015), especially during the first year after planting when trees are 0.5- to 3.0-m tall (Zauza et al., 2010). In eucalypt plantations, MR can cause a 23 to 35% reduction in wood volume, as determined in a study conducted by the Company Votorantim Celulose e Papel covering five plantations of 6-year-old *E. grandis*, distributed in the regions of Guararema, São José dos Campos, and Taubaté in São Paulo state, Brazil (Santos et al., 2020a).

The inter- and intra-specific genetic variability among eucalypt species and hybrids allows selection of MR-resistant clones by artificial inoculations with *A. psidii* under controlled conditions (Assis and Mafia, 2007; Fonseca et al., 2010). Over recent decades, forest companies in Brazil have introduced screening for MR-resistance traits into their breeding programs. Planting of MR-resistant clones has been the most economical and efficacious measure for MR management in Brazilian eucalypt plantations (Alfenas et al., 2009; Miranda et al., 2013; Santos et al., 2020b; Silva et al., 2013). A major resistance gene, *Ppr1* (*P. psidii* resistance gene 1), was found in a family of *E. grandis* (Junghans et al., 2003a). However, according to the pattern of inheritance, slight changes in genetic structure of *A. psidii* populations may allow evolution of new races that overcome major gene resistance (MGR). For this reason, knowledge of physiologic and genetic variability within *A. psidii* populations is crucial to the selection of eucalypt clones with durable MR resistance. Unlike rust pathogens of cereal crops, relatively few physiological races of *A. psidii* have been described to date (Xavier, 2002; Graça et al., 2011).

A pioneering study on physiologic variability of *A. psidii* conducted by Xavier (2002), in which 21 *A. psidii* isolates from different hosts were used to inoculate eight eucalypt clones, identified three *A. psidii* races in Brazil. According to previous studies, *A. psidii* race 1 is considered the most common and widespread across Brazil, while *A. psidii* races 2 and 3 are apparently rare and capable of overcoming resistance of eucalypt clones 1205 (*E. grandis*) and 847 (*E. urophylla*), respectively (Xavier, 2002). These three *A. psidii* races were unable to infect eucalypt clones that carried *Ppr1* (Junghans et al., 2003a). Years later, *E. grandis* clone 6021, which was previously classified as resistant to *A. psidii* race 1, was found infected by a new race (*A. psidii* race 4) in Sao Paulo, Brazil (Graça et al., 2011). Artificial inoculations under controlled conditions showed that *A. psidii* race 4 was also able to infect a wide range of eucalypt clones previously shown to be resistant to race 1, including clone G21 (*E. grandis*) that contains *Ppr1* (Graça et al., 2011). Although *A. psidii* race 4 has been shown to be highly aggressive compared to the three previous races, it has not been detected in eucalypt plantations since it was first described in 2011.

It is important to note that, in this work, virulence is considered as the pathogen's ability to infect, reproduce, and cause damage in a given host, while aggressiveness is considered as a quantitative characteristic related to the amount of disease caused by a given isolate in a given period of time (Vanderplank, 1968). In addition, "race" term is used in the text to designate variants of a phytopathogenic species that cause disease in some cultivars of a host species but not in others (Amorim et al, 2018).

In May 2013, for the first time, young trees of the hybrid clone GG 100 (*E. grandis* × *E. urophylla*) were observed with typical MR signs and symptoms in Minas Gerais state, Brazil. This eucalypt hybrid clone has been widely planted across Southern Brazil, and it has previously displayed MR resistance to *A. psidii* races 1, 2, and 3 (Xavier, 2002). In this study, through artificial inoculation under controlled conditions, we tested the three following hypotheses: *i*) Eucalypt GG100 hybrid clone was misclassified as resistant to *A. psidii* race 1 in previous trials; *ii*) MR in GG100 is caused by *A. psidii* race 4; and *iii*) A new race of *A. psidii* has evolved that overcomes the MR resistance of GG100.

2 | MATERIALS AND METHODS

2.1 | Plant Material

A set of eucalypt clones capable of differentiating races of *A. psidii* was used for inoculation tests (Table 1). All clones were propagated by cuttings.

2.2 | Fungal Isolates and Urediniospores Multiplication

Three *A. psidii* isolates, representing UFV-2 (race 1), EUBA-1 (race 4), and EUMG-5 were tested in this study. UFV-2 was used as a positive control because it is the most widespread *A. psidii* race across Brazil. EUBA-1 was the last described race of *A. psidii* (Graça et al., 2011). *A. psidii* isolate EUMG-5 was originally collected from infected young trees of the GG100 hybrid (*E. grandis* × *E. urophylla*) clone in a commercial plantation of Guaxupé farm, in Ubá, Minas Gerais, Brazil (Geographic coordinates: S 20° 58’ 31.5’’ W 43° 05’ 18.8’’). Each fungal isolate was separately multiplied on young leaves of *Syzygium jambos* as previously described (Ruiz et al., 1989). To avoid cross contamination, *S. jambos* plants were inoculated with each *A. psidii* isolate on different days, and plants inoculated with each isolate were maintained in separate chambers. At 10 days post-inoculation, freshly produced urediniospores were collected in 1.5-mL Eppendorf® tubes and stored at -80 °C until used for inoculating the set of eucalypt clones with differential MR reactions. The isolates of *A. psidii* used in this study are maintained as a pure isolate collection at the Laboratory of Forest Pathology, Department of Plant Pathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais state, Brazil.

2.3 | Physiologic variability of *A. psidii* isolates through artificial inoculation assays

Rooted cuttings of eight eucalypt clones were transplanted into 2-L pots, containing MecPlant® (Telemaco Borba, PR, Brazil) substrate enriched with 26 g super simple phosphate (20% P_2O_5) and 12 g Osmocote® (19-6-10; ICL Specialty Fertilizer, Indaiatuba, SP, Brazil). During transplanting, each plant (one per pot) received 100 mL of monoammonium phosphate solution (52% P and 12% N). Plants were grown in a greenhouse with an average temperature of ca. 25 °C (± 5 °C) and natural light (ca. 12-h photoperiod). After 30-days growth, four newly formed and healthy leaves of each plant were marked and inoculated with 2 × 10^4 urediniospores mL^{-1} of each *A. psidii* isolate or mock inoculated as a negative control. The inoculum suspension, prepared with sterilized water with 0.05% Tween 20®, was homogenously sprayed on both adaxial and abaxial leaf surfaces using an electric compressor Jet Master 1/3 HP (Schulz, Joinville, SC, Brazil). To ensure efficiency of inoculation, three highly susceptible plants of *S. jambos* were randomly distributed among the eucalypt plants. Six plants of each clone were inoculated with each *A. psidii* isolate. As a negative control, six non-infected control plants were mock inoculated by spraying with only sterilized water containing 0.05% Tween 20® without urediniospores. All plants were incubated in a mist-irrigation chamber under dark conditions for 24 h, and then transferred to a growth chamber at 22 °C with a 12-h photoperiod and a light intensity of 130 µmol photons s^{-1} m^{-2} (Ruiz et al., 1989). To confirm the results of the first experiment, an additional inoculation assay was conducted for the *A. psidii* EUMG-5 isolate.

2.4 | Disease severity evaluation

At 21 days after inoculation (dai), MR severity was evaluated by using a disease rating scale with the following four severity scores: S0 = immunity or hypersensitivity reaction (HR) – Immunity is when the reaction occurred so rapidly that no visual symptom can be observed at the macroscopic level, while HR is when necrotic spots are observed on the inoculated leaves due to the death of large groups of cells; S1 = small pustules (<0.8 mm in diameter); S2 = medium pustules (0.8 to 1.6 mm in diameter); and S3 = large pustules (>1.6 mm in diameter) and may have pustules on leaf petioles and young branches (Junghans et al., 2003b; Figure 1). The final severity score for each eucalypt clone was the maximum severity occurring among all plants (replicates) of each treatment (*A. psidii* isolate × eucalypt clone).

2.5 | Aggressiveness of *A. psidii* isolates on eucalypt clones

The aggressiveness of *A. psidii* isolates – UFV-2 (race 1), EUBA-1 (race 4), and EUMG-5 – was evaluated through percentage of infected leaf area (ILA) and number of urediniospores produced per cm^2 of leaf area, at 21 dai. ILA, which was determined for first two pairs of leaves previously marked on each plant, was calculated by analyzing digital photographs in the Quant® software (Vale et al., 2003). The number of urediniospores was determined for 24 samples per clone. To determine urediniospore number, the sample unit was made up of two representative, circular disks (1 cm in diameter), containing new pustules with an abundance of urediniospores, excised from each leaf previously used in the ILA evaluation. The pairs of leaf disks were placed in 2-mL microcentrifuge tubes containing distilled water and 2% Tween 20®. After mixing with a vortex mixer for 1 min, a 10-µL aliquot of the suspension was placed in a Neubauer chamber to quantify the number of urediniospores per mL. Three counts were performed per sample, totaling 72 counts per treatment (clone × *A. psidii* isolate or negative control).

Statistical analyses were performed by using the SISVAR program v. 5.7 (Ferreira, 2011). ILA and urediniospore number data were subjected to analysis of variance (ANOVA) and the means were compared by a Tukey test ($P \leq 0.05$).

3 | RESULTS

3.1 | Physiologic variability of *A. psidii* isolates on the eucalypt clones

On the eight eucalypt clones, inoculations of *A. psidii* isolate UFV-2 (race 1) resulted in S0 rust severity rating on six clones (847, 57 (=BA6021), 1205, G21, G26, and GG100) and an S3 rating on two clones (1183 and G45); whereas, EUBA-1 (race 4) resulted in an HR (S0) rating on two clones (847 and G26), an S0 rating on one clone (1205), an S1 rating on one clone (G21), an S2 rating on one clone (57 = BA6021), and an S3 rating on three clones (1183, G45, and GG100; Table 1). In contrast, two rounds of replicated inoculations with *A. psidii* isolate EUMG-5 (race 5) resulted in an S0 rating on only one eucalypt clone (1205), an S1 rating on three clones (847, G21, and G26), an S2 rating on one clone (57 = BA6021), and an S3 rating on three clones (1183, G45, and GG100; Table 1; Figure 1a-c). As expected, no MR was observed on mock-inoculated, negative control plants, and all *S. jambos* plants, which served as positive controls, were heavily infected by all *A. psidii* isolates.

TABLE 1 Physiologic variability of *Austropuccinia psidii* isolates on eucalypt clones on the basis of a rust severity scale

Clone	Genetic material	Fungal isolates and disease severity score[a]			
		UFV-2*	EUBA-1*	EUMG-5*	EUMG-5**
1183	*Eucalyptus urophylla*	S3	S3	S3	S3
847	*E. urophylla*	S0	HR	S1	S1
57 (=BA6021)	*E. grandis*	S0	S2	S2	S2
1205	*E. grandis*	S0	S0	S0	S0
G21	*E. grandis*	S0	S1	S1	S1
G26	*E. grandis*	S0	HR	S1	S1
G45	*E. grandis*	S3	S3	S3	S3
GG100	*E. grandis* x *E. urophylla*	S0	S3	S3	S3

[a] S0 and HR= negative interaction. S1, S2, and S3 = increasingly positive interaction (disease severity) (Junghans et al. 2003b).

* Results of the first inoculation experiment.

** Results of the second inoculation assay: re-inoculation of EUMG-5 (*A. psidii* race 5).

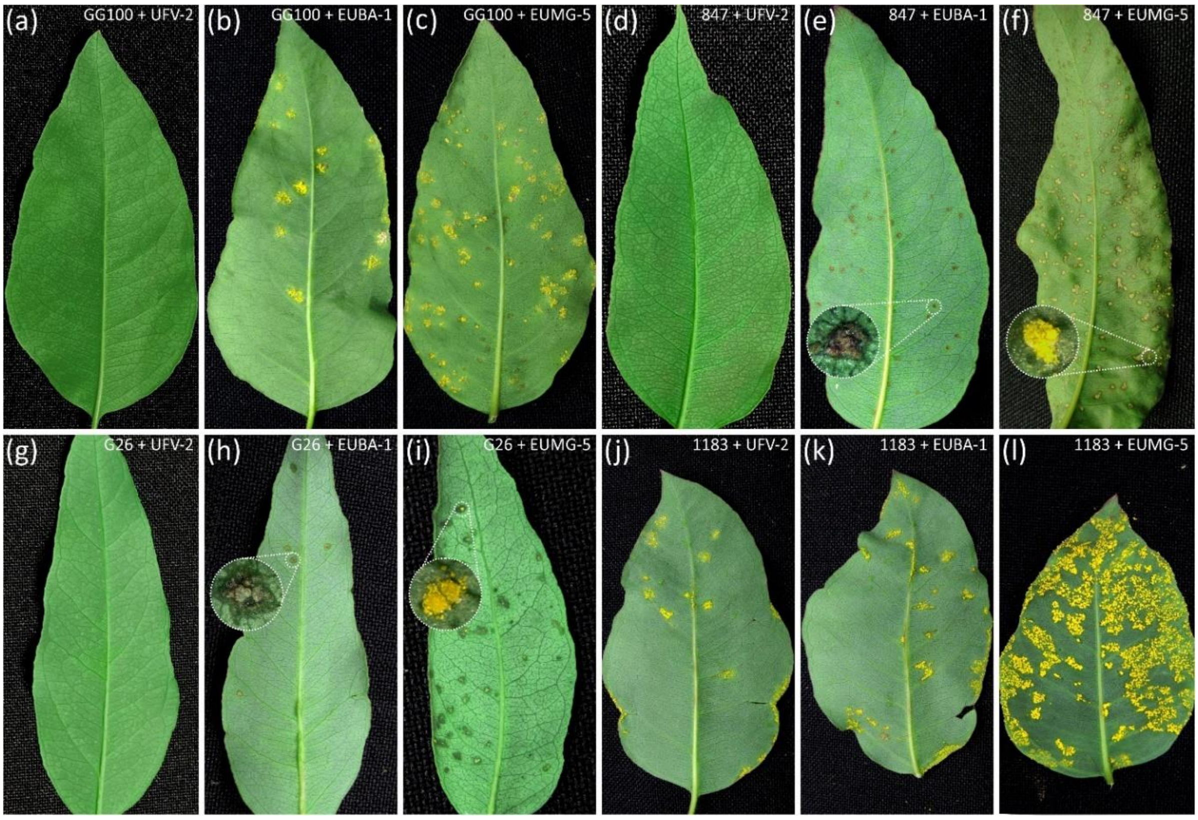

FIGURE 1 Signs and symptoms of myrtle rust on the leaves of eucalypt clones (GG100, 847, G26, and 1183) at 21 days after inoculation with *Austropuccinia psidii* isolates [UFV-2 (race 1), EUBA-1 (race 4), and EUMG-5 (race 5)]. (a, d, g) Disease severity rating S0: no symptoms of infection. (b, c, j, k, l) Disease severity rating S3: uredinia with abundant production of urediniospores – higher urediniospore production and higher percentage of infected leaf area in (c) and (l). (e, h) HR: hypersensitive reaction exhibiting death of cells in detail, but no urediniospore formation, which is also recognized as disease severity rating S0. (f, i) Disease severity rating S1: small uredinia with urediniospore formation in detail.

Although EUBA-1 (*A. psidii* race 4) and EUMG-5 isolates successfully infected a wide variety of eucalypt clones, in this study, only the EUMG-5 isolate was able to infect eucalypt clones 847 and G26 (Table 1; Figure 1). In these two specific eucalypt clones (847 and G26), the *A. psidii* EUBA-1 (race 4) isolate caused only HR (Figure 1e, h), while the EUMG-5 isolate induced small uredinia, containing urediniospores (Figure 1f, i).

3.2 | Aggressiveness of *A. psidii* isolates on the eucalypt clones

ANOVA of ILA and urediniospore production data showed a significant effect ($P<0.0001$) for the interaction among clones and isolates (Table 2). Therefore, a differential response to *A. psidii* infection was observed among eucalypt clones, while *A. psidii* isolates also displayed different aggressiveness levels.

TABLE 2 Analysis of variance (ANOVA) of the percentage of the infected leaf area (ILA) and urediniospore number by *Austropuccinia psidii* isolates on eucalypt clones at 21 days after inoculation

	Source	*F* value	*P* value[a]
ILA	Clones	31.800	0.0000
	Isolates	67.653	0.0000
	Clones × isolates	20.042	0.0000
	CV[b] (%)	118.78	
Urediniospore	Clones	32.473	0.0000
	Isolates	42.611	0.0000
	Clones × isolates	9.800	0.0000
	CV[b] (%)	109.63	

[a] *P* value lower than 0.0001 is considered as significant.

[b] Generic coefficient of variation.

The *A. psidii* UFV-2 (race 1) isolate only infected eucalypt clones 1183 and G45, exhibiting <2% ILA (Figure 2a). Although EUBA-1 (race 4) isolate was able to infect a wide variety of eucalypt clones, it also resulted in <2% ILA (Figure 2a). No significant difference was observed for ILA between UFV-2 and EUBA-1 isolates (Figure 2a). In contrast, EUMG-5 isolate showed higher values of ILA, ranging from 0.03 to 15.13%, on eucalypt clones G26, 847, G21, GG100, 57 (= BA6021), G45, and 1183 (Figure 2a). In the second inoculation assay, EUMG-5 isolate exhibited even higher ILA values, reaching 21.23% on eucalypt clone 1183 (Figure 2b).

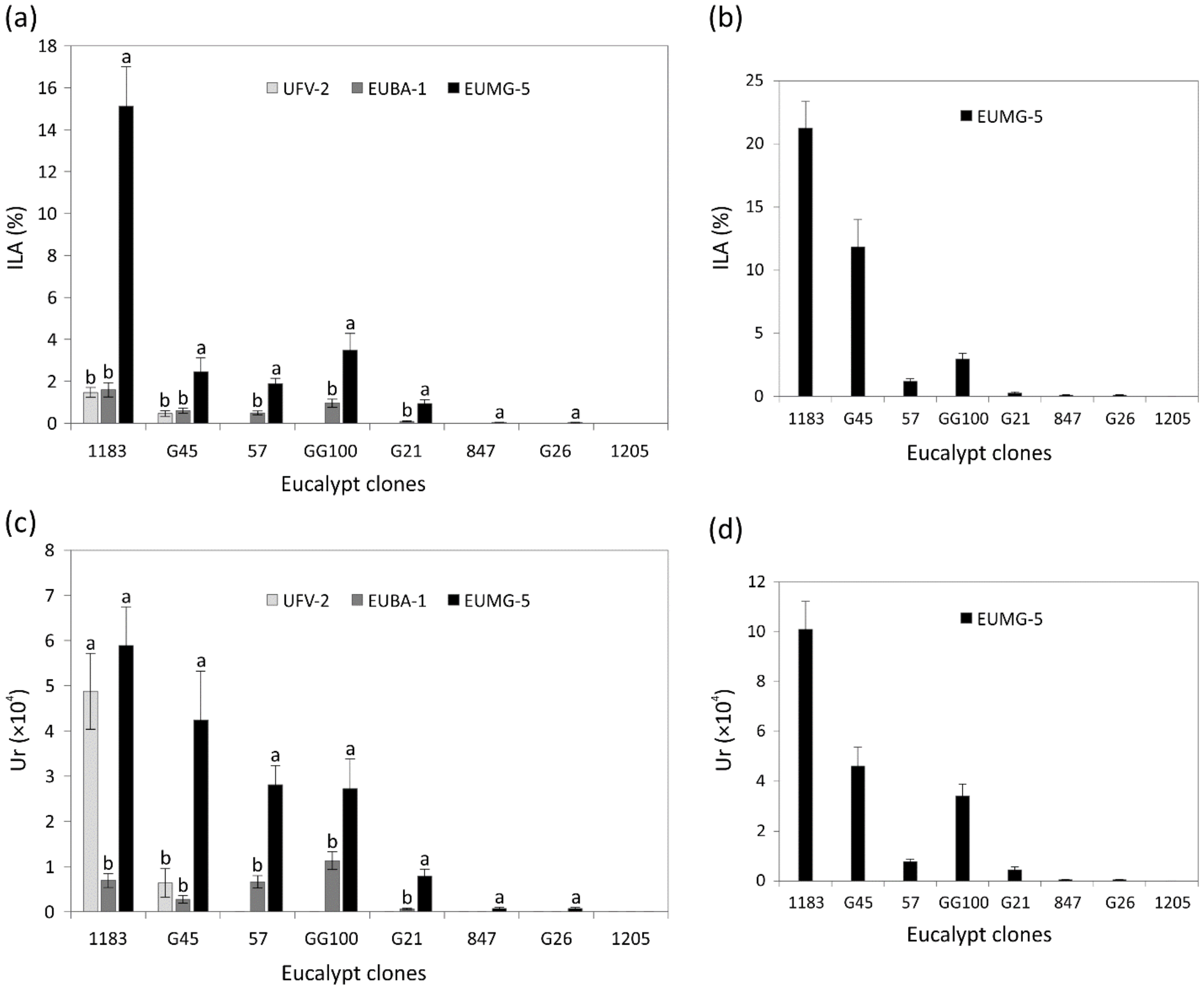

FIGURE 2 Average of percentage of leaf infected area (ILA) and urediniospore (Ur) production on the eucalypt clones at 21 days after inoculation with *Austropuccinia psidii* isolates [UFV-2 (race 1), EUBA-1 (race 4), and EUMG-5 (race 5)]. (a, c) Results of the first inoculation assay. Letters compare the *A. psidii* isolates within each eucalypt clone: Means followed by different lowercase letters indicate statistically significant (P<0.05) differences by a Tukey-adjusted test. (b, d) Results of the second inoculation assay: re-inoculation of EUMG-5 (*A. psidii* race 5). The error bars correspond to the standard error.

For urediniospore production, higher values were found on eucalypt clone 1183 inoculated with *A. psidii* UFV-2 and EUMG-5 isolates, with 4.9 and 5.9 $\times 10^4$ cm^{-2}, respectively (Figure 2c). For the majority of infected eucalypt clones, including clone GG100, *A. psidii* EUMG-5 isolate exhibited the highest urediniospore quantity compared to other two *A. psidii* isolates (Figure 2c), and these results were confirmed in the second inoculation assay with EUMG-5 isolate (Figure 2d). *A. psidii* EUMG-5 isolate is considered as the most aggressive because it induced the highest percentage of ILA and the highest urediniospore production, especially on eucalypt clones 1183 (Figure 1c).

4 | DISCUSSION

In this study, a new isolate of *A. psidii* (EUMG-5), which was found infecting eucalypt trees of clone GG100, was compared with the most widely spread (isolate UFV-2; race 1) and the most recently reported (isolate EUBA-1; race 4) races of *A. psidii* in Brazil. Through artificial inoculations of *A. psidii* isolates on a set of eucalypt clones with differential MR-resistance reactions, we established that the EUMG-5 isolate – denominated here as race 5 – is a new race of the MR pathogen. Our results show that the clone GG100 was susceptible to both the new race 5 (EUMA-5) of *A. psidii* and race 4 (EUBA-1), previously described by Graça et al. (2011) (Table 1). This clone was not used in the studies by Graça et al. (2011). Although the EUMG-5 isolate was significantly more aggressive than the EUBA-1 isolate in this study (Figure 2), discrimination of the new race of *A. psidii* (race 5) is based on the differential reaction of eucalypt clone 847 that produced S1 severity responses when inoculated with the EUMG-5 isolate, which differed from previous differential screening results (Junghans et al. 2003b; Graça et al., 2011). Similarly, in this study, only the isolate EUMG-5 (race 5) infected clone G26, resulting in the formation of easily visualized small uredinia (S1 severity response). This clone was immune (S0) or showed Hypersensitivity Reaction (HR) when inoculated with UFV-2 (race 1) and EUBA-1 (race 4) isolates, respectively. However, according Graça et al. (2011), clone G26 is susceptible to race 4. In this case, the physiological condition of the clone G26 seedlings used in the present study may have negatively influenced the infective process.

Four races of *A. psidii* have been previously reported in Brazil (Graça et al., 2011; Xavier, 2002). Since *A. psidii* race 1 (e.g., UFV-2) is the most widespread across eucalypt plantations in Brazil, it has been widely used for the selection of MR-resistant, eucalypt clones (unpublished data by Alfenas, A. C., DFP/UFV). Over recent years, extensive planting of eucalypt clones with genetic resistance to *A. psidii* race 1 likely exerted selection pressure in the pathogen population, resulting in the emergence of highly aggressive *A. psidii* genotypes, capable of infecting eucalypt clones previously classified as MR resistant. Eucalypt GG100 clone is a hybrid that originated from a controlled cross between *E. grandis* from Atherton, Queensland, Australia and *E. urophylla* from East Timor that was released to the Brazilian forestry market in 1998 (João Flávio, Eldorado Brasil, personal communication, 2020). Eucalypt GG100 clone has been widely grown in several regions of Brazil where it displayed MR resistance for approximately 15 years, until May 2013 – when it has been found infected by *A. psidii*, which is described herein as race 5. Interestingly, eucalypt clone 57 (= BA6021) was widely planted in Brazil after it had been classified as resistant to *A. psidii* race 1, but its

MR resistance was subsequently overcome by *A. psidii* race 4 after ca. 19 years of planting in Brazil (Graça et al., 2011). Based on this observation, it can be inferred that 15-19 years is sufficient for a new *A. psidii* race to arise in eucalypt plantations of Brazil.

In Brazilian eucalypt plantations, *A. psidii* populations exhibited highly clonal structures (Graça et al., 2013), which is characteristic of populations with high asexual reproduction (Arnaud-Haond et al., 2007; Goyeau et al., 2007). In addition, asexual spores (urediniospores) are commonly observed in the field, while teliospores and basidiospores are rarely observed on eucalypt commercial plantations (unpublished observations, A. C. Alfenas). Recent studies have tried to elucidate the role of basidiospores in *A. psidii* infection of Myrtaceae (McTaggart et al., 2018; Morin et al., 2014). No signs of penetration of plant cells by *A. psidii* basidiospores were observed on *Agonis flexuosa* and *Syzygium jambos* (Morin et al., 2014). Whilst *A. psidii* basidiospores were demonstrated to infect *S. jambos* plants and result in sori that were a product of recombination (McTaggart et al., 2018), no unequivocal evidence has yet shown that sexual recombination occurs on eucalypt in Brazil. In theory, an *A. psidii* sexual cycle that is absent or rare on eucalypt (Graça et al., 2013; Yong et al., 2019) would extend the time period needed to generate new *A. psidii* races in populations that occur in commercial eucalypt plantations. Like other rust fungi, the majority of *A. psidii* genome comprises repetitive sequences, such as transposable elements (TEs; Unpublished data by Santos, S.A., DFP/UFV; Tobias et al., 2020). TEs play multiple roles in genome plasticity, pathogenicity, and evolution in fungal phytopathogens (Razali et al., 2019). Therefore, it can be hypothesized that the high proportion of TEs in the *A. psidii* genome could increase the likelihood of genetic rearrangements, such as deletions, duplications, inversions, and translocations through homologous recombination and alternative transposition (e.g., Gray et al., 2000).

In previous studies, eucalypt G26 and 847 clones/genotypes were classified as resistant to both *A. psidii* races 1 and 4 (Graça et al., 2011; Xavier, 2002). The G26 clone/genotype is homozygous at the *Ppr1* locus (Junghans et al., 2003a). Our study also shows that eucalypt G26 clone is not infected by *A. psidii* races 1 and 4. However, small uredinia with urediniospores (disease severity score: S1) were observed when eucalypt G26 clone was inoculated with *A. psidii* EUMG-5 that belongs to race 5, identified in this study (Figure 1i). Hence, this is the second report of a breakdown of MR resistance in a eucalypt clone/genotype homozygous for *Ppr1* (Graça et al., 2011). MR resistance linkage to *Ppr1* is considered as qualitative or complete, and as such, it is considered as an MGR gene; however, Junghans et al. (2003a) found evidence of a more complex pattern of inheritance. A high number of MR-susceptible plants was observed in a eucalypt family derived from a genotype that carried *Ppr1*, which suggested that segregation of MR resistance genes did not fit a simple Mendelian model (Junghans et al., 2003a). Years later, many quantitative trait loci (QTL) were mapped in *Eucalyptus* spp. and *Corymbia* spp., indicating that genetic control of rust resistance is complex and may depend on many other minor-effect genes (Alves et al., 2012; Butler et al., 2019, 2016; Lima et al., 2011; Rosado et al., 2010). In addition, transcriptomics analysis of a *E. grandis* genotype revealed a constitutive overexpression of several genes related to MR resistance (Santos et al., 2020b). Unlike HR, which is characteristic of MGR associated with major-effect genes, quantitative resistance controlled by minor-effect genes may result in slight MR signs and symptoms, such as an S1 rating with small uredinia (< 0.8 mm diameter). Eucalypt clones G26 and 847 were infected by *A. psidii* race 5, which resulted in a S1 disease severity rating in our experiments. It should be noted, however, that eucalypt plants with S1 disease severity are considered as MR resistant, since small uredinia are believed to have no significant epidemiological impact in the field (Junghans et al. 2003b). Of further note is that MR signs and symptoms have not been yet observed in the field with either eucalypt clone, G26 or 847.

In our experiments, we used the same differential set of eucalypt clones as previous studies for identification of *A. psidii* races (Graça et al., 2011; Xavier, 2002). Here, three pathogen isolates [UFV-2 (race 1), EUBA-1 (race 4), and EUMG-5 (race 5)] displayed the expected differential interactions with the set of inoculated eucalypt clones, but only EUMG-5, which is characterized as a new *A. psidii* race (race 5), successfully infected eucalypt clone 847. Our findings corroborate with several other previous studies that have reported genetic and physiological variability in populations of *A. psidii* (Aparecido et al., 2003; Castro et al., 1983; Coelho et al., 2001; Ferreira, 1981; Graça et al., 2013, 2011; Joffily, 1944; MacLachlan, 1938; Stewart et al., 2018). Furthermore, as in previous studies (Graça et al., 2011; Xavier, 2002), the set of eucalypt clones tested was shown to be effective for differentiation of *A. psidii* races. As a result, these eucalypt clones, as well as the *A. psidii* isolates (representing races 1, 4, and 5), tested in this study continue to show great potential for use in screening trials to select MR-resistant genotypes of eucalypts.

In this current study, *A. psidii* EUMG-5 isolate (race 5) infected a larger number of eucalypt clones tested. For all infected genotypes, EUMG-5 (race 5) was more virulent/aggressive than races 1 and 4. This is consistent with results reported by Graça et al. (2011) for *A. psidii* race 4, which was virulent to a larger number of eucalypt clones compared to race 1. However, the impact of *A. psidii* race 5 on MR transmission to eucalypts in the field remains unknown. To date, *A. psidii* race 4 has apparently not spread widely since it was first discovered on eucalypt clone BA6021 in 2008 (Graça et al., 2011), because it has not yet been detected in eucalypt plantations across Brazil. Provided that evolution of new *A. psidii* races continues to occur only over relatively long time periods (e.g., 15 years), and that new *A. psidii* races are not introduced, eucalypt breeding/resistance programs of forest industries of Brazil should have sufficient time to develop and plant new MR-resistant eucalypt genotypes. Alternatively, if sporadic outbreaks of *A. psidii* on eucalypts and other Myrtaceae species were to occur, MR may be controlled by fungicide applications (Pathan et al., 2020; Zauza et al., 2008). In this context, our findings are especially important to eucalypt breeding programs by providing increased understanding of *A. psidii* population variation and access to representative *A. psidii* isolates for continued development of new MR-resistant eucalypt genotypes.

5 | CONCLUSIONS

Overall, our results confirmed that the eucalypt clone GG100 is still resistant to *A. psidii* race 1 (UFV-2 isolate). Therefore, the resistance of this clone was not misclassified in previous studies, and the first hypothesis was refuted. Although the GG100 clone was infected by race 4 (EUBA-1 isolate) of *A. psidii*, showing some compliance with our second hypothesis, it was confirmed that the EUMG-5 isolate collected in the field corresponds to a new race of *A. psidii* because it successfully infects eucalypt clones 847, allowing us to accept the third hypothesis of this study.

The new race of *A. psidii* – represented by the isolate EUMG-5 and denominated to as *A. psidii* race 5 – is highly aggressive and capable of infecting a larger variety of eucalypt genotypes compared to *A. psidii* races 1 and 4. Furthermore, eucalypt clone 847 can be used to differentiate *A. psidii* races 4 and 5. Like *A. psidii* race 4, race 5 also overcame MR resistance conferred by the *Ppr1* gene in eucalypt clone G21 (heterozygous); however, only race 5 successfully infected the eucalypt clone G26, which is homozygous for *Ppr1* in this study.

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REFERENCES

Alfenas, A.C., Zauza, E.A.. V., Assis, T.F., 2003. First record of *Puccinia psidii* on *Eucalyptus globulus* and *E. viminalis* in Brazil. Australas. Plant Pathol. 32, 325–326. https://doi.org/10.1071/AP03021

Alfenas, A.C., Zauza, E.A.V., Mafia, R.G., Assis, T.F., 2009. Clonagem e doenças do eucalipo, 1st ed. Editora UFV, Viçosa, MG state, Brazil.

Alves, A.A., Rosado, C.C.G., Faria, D.A., Guimarães, L.M. da S., Lau, D., Brommonschenkel, S.H., Grattapaglia, D., Alfenas, A.C., 2012. Genetic mapping provides evidence for the role of additive and non-additive QTLs in the response of inter-specific hybrids of *Eucalyptus* to *Puccinia psidii* rust infection. Euphytica 183, 27–38. https://doi.org/10.1007/s10681-011-0455-5

Amorim, L., Rezende, J.A.M., Bergamin Filho, A., 2018. Manual de Fitopatologia: princípios e conceitos, 5st ed. Editora Agronômica Ceres, São Paulo, MG state, Brazil.

Aparecido, C.C., Figueiredo, M.B., Furtado, E.L., 2003. Influência da temperatura sobre a infecção, formação de teliosporos e produção de basidiosporos de *Puccinia psidii* (Uredinales) - Pesquisa Google. Summa Phytopathol. 29, 239–243.

Arnaud-Haond, S., Duarte, C.M., Alberto, F., Serrão, E.A., 2007. Standardizing methods to address clonality in population studies. Mol. Ecol. https://doi.org/10.1111/j.1365-294X.2007.03535.x

Assis, T.F., Mafia, R.G., 2007. Hibridação e clonagem, in: Borém, A. (Ed.), Biotecnologia Florestal. UFV, Viçosa, MG, Brazil, pp. 93–121.

Beresford, R.M., Turner, R., Tait, A., Paul, V., Macara, G., Yu, Z.D., Lima, L., Martin, R., 2018. Predicting the climatic risk of myrtle rust during its first year in New Zealand. New Zeal. Plant Prot. 71, 332–347. https://doi.org/10.30843/nzpp.2018.71.176

Butler, J.B., Freeman, J.S., Vaillancourt, R.E., Potts, B.M., Glen, M., Lee, D.J., Pegg, G.S., 2016. Evidence for different QTL underlying the immune and hypersensitive responses of *Eucalyptus globulus* to the rust pathogen *Puccinia psidii*. Tree Genet. Genomes 12, 39. https://doi.org/10.1007/s11295-016-0987-x

Butler, J.B., Potts, B.M., Vaillancourt, R.E., Lee, D.J., Pegg, G.S., Freeman, J.S., 2019. Independent QTL underlie resistance to the native pathogen *Quambalaria pitereka* and the exotic pathogen *Austropuccinia psidii* in *Corymbia*. Tree Genet. Genomes 15, 72. https://doi.org/10.1007/s11295-019-1378-x

Carnegie, A.J., Giblin, F.R., 2020. *Austropuccinia psidii* (myrtle rust). Invasive Species Compendium. Wallingford, UK CABI. URL https://www.cabi.org/isc/datasheet/45846 (accessed 10.28.20).

Carnegie, A.J., Kathuria, A., Pegg, G.S., Entwistle, P., Nagel, M., Giblin, F.R., 2016. Impact of the invasive rust *Puccinia psidii* (myrtle rust) on native Myrtaceae in natural ecosystems in Australia. Biol. Invasions 18, 127–144. https://doi.org/10.1007/s10530-015-0996-y

Carnegie, A.J., Lidbetter, J.R., Walker, J., Horwood, M.A., Tesoriero, L., Glen, M., Priest, M.J., 2010. *Uredo rangelii*, a taxon in the guava rust complex, newly recorded on Myrtaceae in Australia. Australas. Plant Pathol. 39, 463–466. https://doi.org/10.1071/AP10102

Carvalho, A.D., Alfenas, A.C., Maffia, L.A., Carmo, M.G.F., 1998. Resistência de espécies, progênies e procedências de *Eucalyptus* à ferrugem, causada por *Puccinia psidii* Winter, Pesquisa Agropecuária Brasileira.

Castro, H.A., Krugner, T.L., Iderilha, C.H.F., Capello, M.S., Marchi, A.B., 1983. Inoculação cruzada de *Eucalyptus*, goiaba (*Psidium guajava*) e jambeiro (*Syzygium jambos*) com *Puccinia psidii* - Pesquisa Google. Fitopatol. Bras. 8, 491–497.

Coelho, L., Alfenas, A.C., Ferreira, F.A., 2001. Physiologic variability of *Puccinia psidii* - the rust of *Eucalyptus*. Summa Phytopathol. 27, 295–300.

Coutinho, T.A., Wingfield, M.J., Alfenas, A.C., Crous, P.W., 1998. Eucalyptus rust: A disease with the potential for serious international implications. Plant Dis. 82, 819–825. https://doi.org/10.1094/PDIS.1998.82.7.819

du Plessis, E., McTaggart, A.R., Granados, G.M., Wingfield, M.J., Roux, J., Ali, M.I.M., Pegg, G.S., Makinson, J., Purcell, M., 2017. First report of myrtle rust caused by *Austropuccinia psidii* on *Rhodomyrtus tomentosa* (Myrtaceae) from Singapore. Plant Dis. 101, 1676–1676. https://doi.org/10.1094/PDIS-04-17-0530-PDN

Fernandez Winzer, L., Berthon, K.A., Carnegie, A.J. et al., 2019. *Austropuccinia psidii* on the move: survey based insights to its geographical distribution, host species, impacts and management in Australia. Biol Invasions 21, 1215–1225. https://doi.org/10.1007/s10530-018-1891-0

Ferreira, D.F., 2011. Sisvar: Um sistema computacional de análise estatística. Cienc. e Agrotecnologia 35, 1039–1042. https://doi.org/10.1590/S1413-70542011000600001

Ferreira, F.A., 1983. Ferrugem do eucalipto. Rev. Árvore 7, 91–109.

Ferreira, F.A., 1981. Ferrugem do eucalipto — ocorrência, temperatura para geminação de uredosporos, produção de teliosporos, hospedeiro alternativo e resistência. Fitopatol. Bras. 6, 603–604.

Fonseca, S.M., Resende, M.D. V., Alfenas, A.C., Guimaraes, L.M.S., Assis, T.F., Grattapaglia, D., 2010. Manual Prático de Melhoramento Genético do Eucalipto, 1st ed. UFV, Viçosa, MG, Brazil.

Glen, M., Alfenas, A.C., Zauza, E.A.V., Wingfield, M.J., Mohammed, C., 2007. *Puccinia psidii*: A threat to the Australian environment and economy - A review. Australas. Plant Pathol. https://doi.org/10.1071/AP06088

Goyeau, H., Halkett, F., Zapater, M.F., Carlier, J., Lannou, C., 2007. Clonality and host selection in the wheat pathogenic fungus *Puccinia triticina*. Fungal Genet. Biol. 44, 474–483. https://doi.org/10.1016/j.fgb.2007.02.006

Graça, R.N., Aun, C.P., Guimarães, L.M.S., Rodrigues, B.V.A., Zauza, E.A.V., Alfenas, A.C., 2011. A new race of *Puccinia psidii* defeats rust resistance in eucalypt. Australasian Plant Pathology 40, 442–447. https://doi.org/10.1007/s13313-011-0056-8

Graça, R.N., Ross-Davis, A.L., Klopfenstein, N.B., Kim, M.-S., Peever, T.L., Cannon, P.G., Aun, C.P., Mizubuti, E.S.G., Alfenas, A.C., 2013. Rust disease of eucalypts, caused by *Puccinia psidii*, did not originate via host jump from guava in Brazil. Mol. Ecol. 22, 6033–6047. https://doi.org/10.1111/mec.12545

Gray, Y.H. 2000. It takes two transposons to tango: Transposable-element-mediated chromosomal rearrangements. Trends Genet. 16, 461–468. doi: 10.1016/S0168-9525(00)02104-1

Joffily, J., 1944. Ferrugem do eucalipto. Bragantia 4, 475–487. https://doi.org/10.1590/s0006-87051944000300001

Junghans, D. T., Alfenas, A.C., Brommonschenkel, S.H., Oda, S., Mello, E.J., Grattapaglia, D., 2003a. Resistance to rust (*Puccinia psidii* Winter) in *Eucalyptus*: mode of inheritance and mapping of a major gene with RAPD markers. Theor. Appl. Genet. 108, 175–180. https://doi.org/10.1007/s00122-003-1415-9

Junghans, Davi T., Alfenas, A.C., Maffia, L.A., 2003b. Rating scale to eucalypts rust severity evaluation. Fitopatol. Bras. 28, 184–188. https://doi.org/10.1590/S0100-41582003000200012

Kawanishi, T., Uematsu, S., Kakishima, M., Kagiwada, S., Hamamoto, H., Horie, H., Namba, S., 2009. First report of rust disease on ohia and the causal fungus, *Puccinia psidii*, in Japan. J. Gen. Plant Pathol. 75, 428–431. https://doi.org/10.1007/s10327-009-0202-0

Lima, B.M., Teixeira, J.E., Gazaffi, R., Garcia, A.A., Grattapaglia, D., Valle, R.K., Camargo, L.E., 2011. Identification of a novel QTL contributing to rust resistance in *Eucalyptus*. BMC Proc. 5, P32. https://doi.org/10.1186/1753-6561-5-S7-P32

MacLachlan, J.D., 1938. A rust of the pimento tree in Jamaica. Phytopathology 28, 157–170.

Marlatt, R.B., Kimbrough, J.W., 1979. *Puccinia psidii* on *Pimenta dioica* in south Florida. Plant Dis. 63, 510–512.

McTaggart, A.R., Roux, J., Granados, G.M., Gafur, A., Tarrigan, M., Santhakumar, P., Wingfield, M.J., 2016. Rust (*Puccinia psidii*) recorded in Indonesia poses a threat to forests and forestry in South-East Asia. Australas. Plant Pathol. 45, 83–89. https://doi.org/10.1007/s13313-015-0386-z

McTaggart, A.R., Shuey, L.S., Granados, G.M., du Plessis, E., Fraser, S., Barnes, I., Naidoo, S., Wingfield, M.J., Roux, J., 2018. Evidence that *Austropuccinia psidii* may complete its sexual life cycle on Myrtaceae. Plant Pathol. 67, 729–734. https://doi.org/10.1111/ppa.12763

Miranda, A.C., Moraes, M.L.T., Tambarussi, E.V., Furtado, E.L., Mori, E.S., da Silva, P.H.M., Sebbenn, A.M., 2013. Heritability for resistance to *Puccinia psidii* Winter rust in *Eucalyptus* grandis Hill ex Maiden in Southwestern Brazil. Tree Genet. Genomes. https://doi.org/10.1007/s11295-012-0572-x

Morin, L., Talbot, M.J., Glen, M., 2014. Quest to elucidate the life cycle of *Puccinia psidii* sensu lato. Fungal Biol. 118, 253–263. https://doi.org/10.1016/j.funbio.2013.12.004

Pathan, A.K., Cuddy, W., Kimberly, M.O., Adusei-Fosu, K., Rolando, C.A., Park, R.F., 2020. Efficacy of fungicides applied for protectant and curative activity against myrtle rust. Plant Dis. 104, 2123–2129. https://doi.org/10.1094/PDIS-10-19-2106-RE

Pegg, G., Taylor, T., Entwistle, P., Guymer, G., Giblin, F., Carnegie, A., 2017. Impact of *Austropuccinia psidii* (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in south east Queensland. PLoS One 12, e0188058. https://doi.org/10.1371/journal.pone.0188058

Pegg, G.S., Giblin, F.R., McTaggart, A.R., Guymer, G.P., Taylor, H., Ireland, K.B., Shivas, R.G., Perry, S., 2014. *Puccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact. Plant Pathol. 63, 1005–1021. https://doi.org/10.1111/ppa.12173

Razali, N.M., Cheah, B.H., Nadarajah, K., 2019. Transposable elements adaptive role in genome plasticity, pathogenicity and evolution in fungal phytopathogens. Int. J. Mol. Sci. 20, 3597.

Rosado, T.B., Tomaz, R.S., Ribeiro Junior, M.F., Rosado, A.M., Guimarães, L.M. da S., Araújo, E.F. de, Alfenas, A.C., Cruz, C.D., 2010. Detection of QTL associated with rust resistance using IBD-based methodologies in exogamic *Eucalyptus* spp. populations. Crop Breed. Appl. Biotechnol. 10, 321–328. https://doi.org/10.1590/S1984-

70332010000400006

Roux, J., Greyling, I., Coutinho, T.A., Verleur, M., Wingfeld, M.J., 2013. The myrtle rust pathogen, *Puccinia psidii*, discovered in Africa. IMA Fungus 4, 155–159. https://doi.org/10.5598/imafungus.2013.04.01.14

Ruiz, R.A.R., Alfenas, A.C., Ferreira, F.A., Vale, F.X.R., 1989. Influência da temperatura, do tempo de molhamento foliar, fotoperíodo e da intensidade de luz sobre a infecção de *Puccinia psidii* em eucalipto. Fitopatol. Bras. 14, 55–61.

Santos, A.P., Gomes, R.L., Furtado, E.L., Passos, J.R. de S., 2020a. Quantifying losses in productivity by the rust in eucalypt plantations in Brazil. For. Ecol. Manage. 468, 118170. https://doi.org/10.1016/j.foreco.2020.118170

Santos, S.A., Vidigal, P.M.P., Guimarães, L.M.S., Mafia, R.G., Templeton, M.D., Alfenas, A.C., 2020b. Transcriptome analysis of *Eucalyptus grandis* genotypes reveals constitutive overexpression of genes related to rust (*Austropuccinia psidii*) resistance. Plant Mol. Biol. 104, 339–357. https://doi.org/10.1007/s11103-020-01030-x

Silva, P.H.M., Miranda, A.C., Moraes, M.L.T., Furtado, E.L., Stape, J.L., Alvares, C.A., Sentelhas, P.C., Mori, E.S., Sebbenn, A.M., 2013. Selecting for rust (*Puccinia psidii*) resistance in *Eucalyptus grandis* in São Paulo State, Brazil. For. Ecol. Manage. 303, 91–97. https://doi.org/10.1016/j.foreco.2013.04.002

Silva, R., Silva, A., Rodella, R., Serrão, J., Zanuncio, J., Furtado, E., 2017. Pre-infection stages of *Austropuccinia psidii* in the epidermis of *Eucalyptus* hybrid leaves with different resistance levels. Forests 8, 362. https://doi.org/10.3390/f8100362

Stewart, J.E., Ross-Davis, A.L., Graça, R.N., Alfenas, A.C., Peever, T.L., Hanna, J.W., Uchida, J.Y., Hauff, R.D., Kadooka, C.Y., Kim, M.-S., Cannon, P.G., Namba, S., Simeto, S., Pérez, C.A., Rayamajhi, M.B., Lodge, D.J., Arguedas, M., Medel-Ortiz, R., López-Ramirez, M.A., Tennant, P., Glen, M., Machado, P.S., McTaggart, A.R., Carnegie, A.J., Klopfenstein, N.B., 2018. Genetic diversity of the myrtle rust pathogen (*Austropuccinia psidii*) in the Americas and Hawaii: Global implications for invasive threat assessments. For. Pathol. 48, e12378. https://doi.org/10.1111/efp.12378

Tobias, P.A., Schwessinger, B., Deng, C.H., Wu, C., Dong, C.M., Sperschneider, J., Jones, A., Smith, G.R., Tibbits, J., Chagné, D., Park, R.F., 2020. Long read assembly of the pandemic strain of *Austropuccinia psidii* (myrtle rust) reveals an unusually large (gigabase sized) and repetitive fungal genome. *BioRxiv*.

Uchida, J., Zhong, S., Killgore, E., 2006. First report of a rust disease on ohia caused by *Puccinia psidii* in Hawaii . Plant Dis. 90, 524–524. https://doi.org/10.1094/pd-90-0524c

Vale, F.X.R., Fernandes-Filho, E.I., Liberato, J.R., 2003. Quant – A software for plant disease severity assessment, in: 8th International Congress of Plant Pathology. Plant Pathology Society, Christchurch, New Zealand, p. 105.

Vanderplank, J.E., 1968. Disease Resistance in Plants. New York: Academic Press

Winter, G., 1884. Repertorium. Rabenhorstii fungi europaei et extraeuraopaei. Cent. XXXI et XXXII. Hedwigia 23, 164–172.

Winzer, L.F., Berthon, K.A., Carnegie, A.J., Pegg, G.S., Leishman, M.R., 2019. *Austropuccinia psidii* on the move: survey based insights to its geographical distribution, host species, impacts and management in Australia. Biol. Invasions 21, 1215–1225. https://doi.org/10.1007/s10530-018-1891-0

Xavier, A.A., 2002. Histopatologia da interação *Puccinia psidii* e virulência de isolados do patógeno em espécies de Myrtaceae. UFV.

Yong, W.T.L., Ades, P.K., Tibbits, J.F.G., Bossinger, G., Runa, F.A., Sandhu, K.S., Taylor, P.W.J., 2019. Disease cycle of *Austropuccinia psidii* on *Eucalyptus globulus* and *Eucalyptus obliqua* leaves of different rust response phenotypes. Plant Pathol. 68, 547–556. https://doi.org/10.1111/ppa.12959

Zauza, E.Â.V., Couto, M.M.F., Maffia, L.Â., Alfenas, A.C., 2008. Eficiência de fungicidas sistêmicos no controle da ferrugem do *Eucalyptus*. Rev. Arvore 32, 829–835. https://doi.org/10.1590/S0100-67622008000500007

Zauza, E.A.V., Couto, M.M.F., Lana, V.M., Maffia, L.A., Alfenas, A.C., 2010. Vertical spread of *Puccinia psidii* urediniospores and development of eucalyptus rust at different heights. Australas. Plant Pathol. 39, 141–145. https://doi.org/10.1071/AP09073

Zauza, E.A. V., Lana, V.M., Maffia, L.A., Araujo, M.M.F.C., Alfenas, R.F., Silva, F.F., Alfenas, A.C., 2015. Wind dispersal of *Puccinia psidii* urediniospores and progress of eucalypt rust. For. Pathol. 45, 102–110. https://doi.org/10.1111/efp.12133

Zhuang, J.Y., Wei, S.X., 2011. Additional materials for the rust flora of Hainan Province, China. Mycosystema 30, 853–860.