The presence of *Austropuccinia psidii* and the threat to Myrtaceae plantations in Indonesia

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**Abstract.** *Austropuccinia psidii* is an invasive pathogenic rust that infects the Myrtaceae family. This rust is a threat to Myrtaceae plantations around the world due to its widespread distribution. In this study, we observed the presence of *A. psidii* in three species of Myrtaceae, i.e. *Melaleuca cajuputi*, *Syzygium myrtifolium*, and *Syzygium polyanthum* planted in Yogyakarta and Sukabumi. The symptoms of infection were yellow-reddish spot in young leaves, presence of urediniospores in infected spot, foliage, and branch dieback. To confirm the presence of *A. psidii* on those trees, a molecular detection was performed using specific primer for *A. psidii* (Ppsi1/Ppsi6) on DNA samples extracted from diseased leaves. The presence of *A. psidii* was proved by the presence of DNA amplicon sized around 500bp in all samples collected from three different hosts. In this study, *S. myrtifolium* was firstly reported to be infected by this rust in Indonesia. Further study about the presence and the economic impact of this pathogen in Indonesia should be conducted. Indonesia has many species numbers of Myrtaceae and some species are important for medicines, herbs, foods, and as industrial plants. A strategy to control this pathogen should be established to avoid large economic losses in Myrtaceae plantations in Indonesia.

1. Introduction

*Austropuccinia psidii* is a pathogenic fungus that has a broad host range of the Myrtaceae family. This rust was firstly reported in Brazil infects *Psidium guajava* in 1884 [1]. *Austropuccinia psidii* has a wide distribution around the world with a wide range of hosts. It has been detected to infects *Pimenta dioicain* in Florida [2], *Eucalyptus amplifolia* and *E. rudis* in Japan [3], *Metrosideros polymorpha* in Hawaii [4], *Syzygium jambos* in Hainan island of China [5], *Myrtus communis* in Africa [6], *Agonis flexuosa*, *Callistemon viminalis* and *Syncarpia glomulifera* in New South Wales, Australia [7].

Indonesia has a large number of Myrtaceae plants, with an estimation to have around 30 genera with an unknown exact number [8]. Myrtle plant is widely used as medicine, herbs, ornamental plants, foods, and industrial plants. This family has many economic benefits, such as *Syzygium*, this genus has important roles in medicines, herbs, fruit production, and furniture. *Syzygium polyanthum* is one of the commonly known species in the *Syzygium* genus, it is widely used and cultivated by the local community of Indonesia [9]. It’s usually used as spices and traditional medicines [10]. Infection of *A. psidii* has been recorded in *S. polyanthum* in Yogyakarta Indonesia [11]. The presence of *A. psidii* should be...
considered as a threat to Myrtaceae species, as it shows the easy transmissions from one Myrtaceae plant to another. The local spread of *A. psidii* occur via wind and the long-distance transmission occur via living plant tissue transportation [12].

*A. psidii* and *A. myrtifolium* are also considered economically important genera in Myrtaceae. *Eucalyptus* is a multipurpose tree. Many species of *Eucalyptus* are harvested for pulps and paper industry, medical plants, aromatic plants, timber, fuelwoods, etc [13]. These benefits make eucalypts planted as industrial trees in many countries around the world including Indonesia [14], in addition, these plants are also able to grow under a wide range of climatic conditions and are well-known for their fast-growing which makes them suitable for a short period time of plantation [13]. However, these plants are susceptible to several diseases that can affect their growth. One of these diseases is myrtle rust caused by *A. psidii* infection. This pathogen attacks young organs such as leaves, and impaired the photosynthesis process in an infected leaf of eucalypts [15]. The first report about *A. psidii* infections in Indonesia was recorded on *E. pellita* in North Sumatra in 2015 [12]. It was reported again in the next five years on clone *E. grandi* x *E. pellita* at PT. Toba Pulp Lestari, located in Toba Samosir regency, North Sumatra [16]. This disease can become a threat for *Eucalyptus* plantations in Indonesia in the future. Meanwhile, infection of *A. psidii* on *Melaleuca* genera was first reported on *M. leucadendra* in Riau, Sumatra island in 2015 [12], and reported again on *M. cajuputi* in Yogyakarta, Java island in 2020 [11].

In this study, we reported a new host of *A. psidii* in Indonesia, i.e. *S. myrtifolium*. We also reported the new location of this rust infection in Java i.e. Sukabumi, West Java, and Sleman, Yogyakarta. Molecular detection using a specific primer [17] was performed to confirm the infection of *A. psidii* in those plants. The presence of *A. psidii* in several place and host in Indonesia can threaten Myrtaceae plantation in other genera and species in another location that has not been reported to be infected yet. The awareness about this disease and infection symptoms should be raised to prevent further losses in the economy and biodiversity of the Myrtaceae plant in Indonesia.

2. Materials and methods

2.1. Sample collection

*Austropuccinia psidii* sample were collected from three Myrtaceae species i.e *Syzygium polyanthum*, *Melaleuca cajuputi*, and *Syzygium myrtifolium*. Young leaves and branches that showing the symptoms of *A. psidii* infection (the presence of urediniospore) were collected and documented. Infected leaves of *S. polyanthum* were collected from three different areas i.e. the arboretum of Center for Forest Biotechnology and Tree Improvement Yogyakarta (CFBTI), a private garden in Sleman, Yogyakarta, and a privat garden in Sukabumi, West Java. Meanwhile, diseased *M. cajuputi* and *S. myrtifolium* were only collected from CFBTI’s arboretum.

2.2. Score the susceptibility of the host to *Austropuccinia psidii*

We used a disease rating system developed by Pegg [18] to record the susceptibility of Myrtaceae species to *Austropuccinia psidii* with the following scale: 1. The presence of sori is on <10% of expanding leaves and shoots with limited sori is rated as relatively tolerant (RT), 2. The presence of sori on 10-50% of expanding leaves and shoots with limited to multiple sori in the infected area is rated as moderately susceptible (MS), 3. The presence of sori is on 50-80% of expanding leaves and shoots with the rust infection expand to the older leaves, and juvenile stems, multiple sori that causing blight and leaf or stem distortion is rated as highly susceptible (HS), and 4. The presence of sori on all expanding leaves, juvenile stems, and shoots, with foliage, stem, and shoot dieback is rated as extremely susceptible (ES).

2.3. DNA extraction

DNA extraction was performed using a procedure developed by Glen [19]. Leave tissue with pustules was excised and placed in a 2 mL microtube and 250 μL of extraction buffer [20] was added to the tubes.
Two metal bead were placed in the tubes and shaken with a mini-bead-beater for 5 mins. Then, the tubes were incubated at 65°C for 1 hour. After incubation the tubes were centrifuged at 14,000 rpm for 15 mins, the supernatant was removed and placed in a new tube. A total of 10 µL silica glass milk and 600 µL of NaI 100% were added to the supernatant for DNA purification. The tubes were vortexed and incubated in an ice box for 15 mins, shaken occasionally. Tubes were centrifuged again at 14,000 rpm for 20 secs and the supernatants were discharged. A total of 750 µL of wash solution were added to the pellet and were centrifuged at 14,000 rpm for 20 secs. The supernatant was discharged and 750 µL of ethanol 100% was added to the pellet. Tubes were centrifugated at 14,000 for 20 secs and the supernatant was discharged again. The pellets were dried in a laminar air flow for 1-1.5 hours. After dried, 25 µL of TE buffer were added to the pellet and vortexed. Tubes were incubated at 45°C for 10 mins then centrifuged again at 14,000 rpm, for 2 mins. The DNA pellets were stored at -20°C before the DNA analysis.

2.4 Austropuccinia psidii detection with specific primer

Molecular detection to confirm the presence of Austropuccinia psidii was performed by amplification of DNA template with a pair of specific primer Ppsi1 (5’- TTC TAC CTT ATT ACA GGT TAG AAG C-3’) and Ppsi6 (5’- GTC ATA TTG ACA GGT TAG AAG C-3’) [17]. PCR mix reaction contained 6.25 µL of ddH2O, 12.5 µL of PCR buffer My Taq Red Mix (Bioline), 0.625 µL of Ppsi1 as a forward primer, and 0.625 µL of Ppsi6 as a reverse primer. The mixture was vortexed and centrifuged after the addition of 5 µL of DNA template. PCR was performed using a GeneAmp PCR System 9700 ver 3.08 machine with one cycle of predenaturation at 94°C for 3 mins, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1 min, followed by one cycle of final extension at 72°C for 10 mins [17]. DNA amplicons were visualized in 1% electrophoresis agarose gel run with 100V for 30 minutes.

3. Result and discussion

Three Myrtaceae plants observed in this study were Syzygium polyanthum, Melaleuca cajuputi, and Syzygium myrtifolium. The symptoms are shown in figure 1, including the presence of yellow urediniospores in infected spots of the young tissue. The urediniospores are often found on the lower surface of the leaves. Once urediniospore is established on the leaf surface it will form a pustule structure called uredinia [21]. Young organs such as young leaves are more susceptible of myrtle rust infection because it has thinner wax layer than the old leaves [22]. During observation, we found a range of disease severity. The uredinia are covering around 50-80% of S. polyanthum young leaves, as shown in figure 1. Sori were also found in older leaves. The disease scoring was performed using a disease rating system developed by Pegg [18], therefore we rated S. polyanthum as highly susceptible. The uredinia are covering around 50-80% of the young leaves of M. cajuputi. We also found that the older leaves were infected, therefore this host is rated as highly susceptible. A different response was found in S. myrtifolium. The uredinia or sori are covering less than 10% of leaves (figure 1), and no symptoms were found on the older leaves, therefore we categorized this host as relatively tolerant. Syzygium myrtifolium is known for its resistance to diseases and pests [23]. The specific study about the host-pathogen interaction between S. myrtifolium and A. psidii has not been reported yet, therefore a further study to observe the response to this rust infection is needed.

Necrotic lesions were found in young leaves of S. polyanthum with bright yellow urediniospores, while in the old leaves, the necrotic lesions were brown with dried sori which pale in color (figure 1). A study stated that the lesions have two types of spores i.e. urediniospore and teliospore or the mix of it [24]. During the observation, we only found the urediniospore because the color is bright yellow, meanwhile the teliospore has a dark brown color in it [24]. In addition, teliospores are rare in nature but often found in some species of Myrtaceae such as S. jambos [25]. Similar symptoms occur in M. cajuputi, we observed the yellow sori not only on the young leaves but also in shoot tips and the branches. In older leaves we found many dried sori on brown lesions (figure 1). Symptoms on resistant hosts were shown in S. myrtifolium. The rust infections were only shown as reddish flecks/lesions with
limited yellow urediniospores, no infection found in old leaves of *S. myrtillosum*. This symptom is formed by hypersensitive reaction (HR) in the host plant. The hypersensitive reaction usually occurs without sporulation but it can be varied depends on the resistance level [26].

**Figure 1.** *Austropuccinia psidii* infecting young leaf of *Syzygium polyanthum* (a), old leaves of *S. polyanthum* (b), young leaves of *Melaleuca cajuputi* (c), old leaves of *M. cajuputi* (d), and young leaves of *Syzygium myrtillosum* (e,f).

*Austropuccinia psidii* has become a threat to Myrtaceae plantation in many countries, the evidence of its presence in Indonesia can threaten Myrtaceae plantation in Indonesia. The first report of *A. psidii* infection in Indonesia was in 2015 [12], this rust infected two hosts i.e. *Eucalyptus pellita* and *Melaleuca leucadendra* in North and South Sumatra. Five years later in 2020 this rust was reported to infect *Eucalyptus grandis x Eucalyptus pellita* clone from North Sumatra [16]. The industrial crops *Eucalyptus spp.* are important for pulp production in PT. Toba Pulp Lestari, Tbk from Toba Samosir. This rust pathogen mostly attack the young plants in nurseries of industrial forest plantations. During several infection tests on *Eucalyptus* clones, the hybrid of *E. grandis x E. pellita* was found to be more resistant to *A. psidii*. These hybrid clones are still infected by the rust with mild symptoms such as the limited presence of sori on leaves and some yellow spots in the infected areas [16]. *Eucalyptus grandis* is widely used in the paper production industry in Brazil [24]. This becomes a real concern as *E. grandis* is one of the most susceptible host to *A. psidii* infections [27]. The economic losses caused by this rust are mainly due to infections on young trees and seedlings. This rust is now considered as one of the serious threats to *Eucalyptus* industrial plantation in the world [1].

Rust infection in *M. leucadendra* was not only reported to be present in Riau, Sumatra Island [12], but it was also present in Yogyakarta according to an observation in the arboretum of CFBTI [11]. Cajuput is an important plant that produces essential oils (cajuput oils), that has been planted widely as industrial plants in Sumatra and other areas in Indonesia such as Java. Perum Perhutani (a state forest
enterprise of Indonesia) was estimated to manage about 24,000 hectares of cajuput plantations in Java and has 10 processing units for cajuput oil production with a total capacity of up to 53,760 tons/year of cajuput leaves. Commercial production of cajuput oil in Java reaches 300 tons oil per year. The increasing presence of this pathogenic rust in Java will threaten cajuput plantations on this island because this rust can spread quickly with wind-borne spores [12]. The local transmission that occurs in the plantation areas or in the nursery can cause significant economic losses. The example of a significant impact of *A. psidii* on Myrtaceae plantation had been recorded in Rio de Janeiro state, this rust infected guava fruit and the economic loss of this disease was estimated. Fruit production is reduce up to 90% because of *A. psidii* infections. This rust caused lesion on mature guava fruits thus decreased the fruit quality. The lesion was also causing secondary infections that made opportunistic insects eating maturing fruits [28].

The information about *A. psidii* infections in Indonesia is still very limited, currently only two reports about this infection on Sumatra island [12,16] and one incident reported on Java island [11]. This study reported other incidents of *A. psidii* infection that were found in *S. polyanthum* in the new location in Java island i.e. Sukabumi, West Java, and Sleman Yogyakarta. These two new incidents were found in *S. polyanthum*. This paper also revealed the new infection of *A. psidii* on *S. myrtifolium*. We assumed that *A. psidii* infection has been widely present in Java but has not been reported yet. It is still unknown when *A. psidii* has been infecting Myrtaceae plants in Java and what species that provide as host of this pathogen. It is likely to be reported to infect more species of Myrtaceae in the future. *Syzygium polyanthum* is a useful species that is widely used as a spice, traditional medicine, and as a source of food for pet birds [9]. Its utilization as an ethno-medical plant has been studied for a long time. The leaf has been proved to have many therapeutic potentials such as antitumor, antidiabetic, antioxidant, anticancer, antihypertensive, antimicrobial, and antidiarrheal [29]. We reported that the infections of *A. psidii* in *S. polyanthum* were found in the local garden, as this species has not been planted in the wide-scale plantation. We should be aware of this infection especially if it occurred in the residential areas with diverse local Myrtaceae plants because urediniospores can be transmitted locally by the wind, or accidentally transmitted by human hands from one diseased tree to the healthy one.

Molecular detection using PCR was developed to detect and confirm the presence of *A. psidii* in Myrtaceae plants [17]. In this study, we used the specific primer Ppsi1/Ppsi6 to detect the presence of *A. psidii* on *S. polyanthum, S. myrtifolium*, and *M. cajuputi* leaves. The PCR amplification using these species resulted in a 500 bp DNA amplicon (figure 2). This primers pair will specifically amplify the DNA ITS of *A. psidii* and will not amplify other species of *Puccinia/Austropuccinia* such as *Puccinia oxalidis, P. emiliae, P. boroniae*, and *P. hordei* [17]. Not all of the 16 specimens of our plant species used in this study were produced *A. psidii* amplicon, seven specimens, i.e 2, 10, 11, 12, 14, 15, and 18 were failed to produce *A. psidii* amplicon. These specimens may be successfully amplify the *A. psidii* amplicon with further analysis using nested PCR method as described in the previous study [17], but we did not attempt this method in our study. Molecular detection is more fast, specific, and accurate to confirm the *A. psidii* infection than conventional diagnosis [30]. This specific primers pair can detect *A. psidii* in vegetative plant materials such as stem, leaf, fruit, pollen, old degraded lesion, seed, and contaminated plant tissue, even in symptomless organs [17].
Figure 2. Amplicon of *A. psidii* from *S. polyanthum* in CFBTI arboretum (1-6), *M. cajuputi* in the north arboretum (7-12), *M. cajuputi* in the south arboretum (13-18), *S. myrtifolium* (19-21), *S. polyanthum* in Sleman (22-23), *S. polyanthum* in Sukabumi (24-26), Marker 100 bp.

4. Conclusions

*Austropuccinia psidii* was found in the three Myrtaceae species i.e. *Syzygium polyanthum*, *Syzygium myrtifolium*, and *Melaleuca cajuputi* from Yogyakarta and Sukabumi, West Java. The symptoms of infection are the presence of urediniospores in infected young leaves and branches, tissue lesions and leaves dieback. The disease severity was varied between different host species. *Syzygium polyanthum* and *M. cajuputi* trees are highly susceptible while *S. myrtifolium* is relatively tolerant to this rust infection. Molecular detection by the application of specific primers (Ppsi1/Ppsi6) provides a fast and accurate way to confirm the rust infection. The 500 bp amplicon of *A. psidii* was produced from all three species. The infection of *A. psidii* is considered a serious threat to Myrtaceae plantation in Indonesia as its presence has been reported from native and non-native Myrtaceae plants in several areas in Indonesia.

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