Research paper

Correlations of Anatomical and Chemical Leaf Characteristics of Eucalyptus Clones with Spontaneous Leaf Spot Disease Severity Associated with *Phaeophleospora* Fungi

Sri Rahayu,[1,2] Ifert Ehrlick Tudon,[1] Eko Bhakti Hardiyanto[1]

【Summary】

Recently, leaf spot disease caused by *Phaeophleospora* spp. fungi has become a severe problem in eucalyptus clonal plantations. These pathogens can kill *Eucalyptus* trees, and cases can be found in different stages of tree development, ranging from seedlings in nurseries to trees in the field. Before deploying a large-scale *Eucalyptus* clonal plantation, selecting clones resistance to disease is important in addition to selecting for optimal growth and wood properties. In this research, we explored anatomical and chemical leaf characteristics associated with potential leaf spot disease resistance. The study was conducted on a 6-mo-old eucalypt clonal plantation at a forestry company in South Sumatra, Indonesia. Three selected clones, i.e., clones 79 and 80 (*E. pellita* × *E. brassiana*), and clone 47 (pure *E. pellita*), were assessed for their growth, severity of spontaneously occurring disease, and leaf characteristics (the stomatal density, stomatal size, thickness of adaxial and abaxial palisade mesophyll, and phenol contents). Clone 79 was susceptible, while clones 47 and 80 were more resistant to the disease. The stomatal size and density and leaf phenol contents assessed from healthy clones were not good indicators for determining resistant clones. The thickness of the abaxial palisade parenchyma, however, was negatively correlated with disease severity. Comparing palisade mesophyll thickness is suggested to be a quick, simple, and cheap approach for a preliminary assessment of potential resistance against leaf spot disease among different *Eucalyptus* clones.

Key words: Clonal forestry, leaf disease, palisade mesophyll, *Eucalyptus pellita, Eucalyptus brassiana.*

Rahayu S, Tudon IE, Hardiyanto EB. 2020. Correlations of anatomical and chemical leaf characteristics of *Eucalyptus clones* with spontaneous leaf spot disease severity associated with *Phaeophleospora* fungi. Taiwan J For Sci 35(3):239-49.

[1] Faculty of Forestry, Universitas Gadjah Mada, Jalan Agro No. 1, Bulaksumur, Yogyakarta 55281, Indonesia.

[2] Corresponding author, e-mail:sri.rahayu2013@ugm.ac.id 通訊作者。

Received July 2020, Accepted December 2020. 2020年7月送審 2020年12月通過。

研究報告

桉樹營養系的解剖及化學特徵與*Phaeophleospora* spp.真菌所引起的自發性葉斑病嚴重程度的關係

Sri Rahayu[1,2] **Ifert Ehrlick Tudon**[1] **Eko Bhakti Hardiyanto**[1]

摘　要

近年來，*Phaeophleospora* spp.真菌所引起的葉斑病已對桉樹(*Eucalyptus*)營養系人工林地造成嚴重影響。從苗圃內的苗木到野外的樹木，均可見到病原菌造成不同生長時期桉樹死亡的案例。在進行大規模的桉樹造林前，除了考量生長和木材性質良好的性狀外，篩選具抗病性的營養系也顯得相當重要。本研究中，我們探討了與潛在葉斑病抗性相關的葉片解剖和化學特徵。該研究是在印度尼西亞南蘇門答臘的一家林業公司之6個月大桉樹營養系人工林中進行。針對挑選的3個營養系，即營養系79、80 (*E. pellita* × *E. brassiana*)、和47 (*E. pellita*)，分析它們的生長，發病嚴重程度和葉片特徵(氣孔密度、氣孔大小、近軸和遠軸端柵狀葉肉厚度以及酚含量)。營養系79呈現感病，而營養系47和80則呈現較高抗病性。健康營養系的氣孔大小，密度和葉酚含量，並不是評估營養系抗病性的良好指標。然而，遠軸端柵狀葉肉的厚度與染病的嚴重程度呈負相關。比較柵狀葉肉厚度可做為初步評估不同桉樹營養系對葉斑病潛在抗性一種快速、簡單且便宜的方法。

關鍵詞：營養系林業，葉病，葉柵葉肉，粗皮桉，吉桉。

短期標題：桉樹無性繁殖系的解剖與葉斑病的關係。

Sri Rahayu、Ifert Ehrlick Tudon、Eko Bhakti Hardiyanto。2020。 桉樹營養系的解剖及化學特徵與 *Phaeophleospora* spp.真菌所引起的自發性葉斑病嚴重程度的關係。台灣林業科學35(3):239-49。

INTRODUCTION

Eucalyptus species are globally important planted trees due to their fast growth, valuable wood properties, and wide adaptability (Orwa *et al*. 2009). Several *Eucalyptus* species have long been grown in many tropical regions, including Indonesia. However, in recent years, only *E. pellita* has been planted on a large scale across Indonesia due to its resistance or tolerance to pests and diseases (Nambiar et al. 2018). So far, *E. pellita* is the best alternative species to replace *Acacia mangium* which is very susceptible to *Ceratocystis* disease and monkey and squirrel attacks after 3 or 4 successive rotations (Tarigan et al. 2011). Relying only on a single species of *Eucalyptus* is certainly a risky management policy; thus other alternatives, i.e., different *Eucalyptus* species and hybrids, need to be tested for their productivity and resistance to pests and disease.

Eucalyptus hybrids are essentially a cross-breed between 2 or more eucalypt species which possess a combination of desirable characteristics. *Eucalyptus* species with potential to be developed as hybrids in Indonesia are limited, as the majority of sites for plantation development are located in lowland areas with high rainfall and humidity, where many eucalypts are unsuitable to be grown, as they are susceptible to leaf diseases or poor growth. However, there are a number of species with high potential for hybrid de-

velopment, namely *E. pellita*, *E. grandis*, *E. brassiana*, *E. camaldulensis*, and *E. urophylla*. Normally, *E. pellita* serves as the female parent in interspecific hybridizations, combining its best traits, particularly its tolerance to disease, with desirable traits such as growth and wood properties of other species (Prasetyo et al. 2018).

In forestry companies that have adopted clonal forestry techniques, vegetative propagation of the best selected *Eucalyptus* clones is carried out to a greater or lesser degree of sophistication to capture the full genetic potential of the clones. The yield of *Eucalyptus* plantations will continue to increase as greater numbers of improved clones are developed and best silvicultural practices are applied (Zobel 1993).

One of the virulent diseases in *Eucalyptus* clonal plantations recently observed in South Sumatra is leaf spot disease caused by *Phaeophleospora* spp. Recently, leaf spot disease has emerged as a significant threat to eucalypt plantations in a number of South East Asian countries including Indonesia, Thailand, and Vietnam, and consequently the disease has been considered worthy of a proper management response.

Phaeophleospora spp. can cause the death of *Eucalyptus* trees, and they have been detected in different tree development stages, ranging from seedlings in nurseries to trees in the field with symptoms of reddish leaf patches and black spores on the leaf surface (Old et al. 2003, Videira et al. 2017). Before deploying a large-scale plantation of *Eucalyptus* and its hybrids, selection of clones resistant to disease is of paramount importance in addition to selecting for optimal growth and wood properties. To date, all *Phaeophleospora* species are known to be associated with leaf spot diseases of plants (Taylor and Crous 1999). The association between leaf anatomy and resistance to leaf pathogens was investigated in a number of studies, including *Mycosphaerella fijiensis* M. Morelet on bananas (Craenen et al. 1997), frog-eye leaf spot (*Cercospora sojina* Hara) on soya beans (Yang, 2000), and *Mycosphaerella berkeleyi* W.A. Jenkins on groundnuts (Jyosthna et al. 2004), as well as *Teratosphaeria* leaf disease on *Eucalyptus globulus* (Smith et al. 2017). Stomata are located at the frontline between internal plant tissues and the surrounding environment, making them convenient gates for phytopathogens including *Phaeophleospora* spp. However, the infective process of *Phaeophleospora* spp. has not been studied in detail.

Stomata play an important role as a natural entry point for pathogens; thus leaves with higher numbers of stomata per unit area will have greater chances that fungal mycelia can penetrate (Smith 2006). On the other hand, it was proposed that the first stage of the defense mechanism of plants involves a rapid accumulation of phenols at the infection site, which inhibits the growth of pathogens that penetrate into plant cells. Several phenols are regarded as a pre-infection inhibitors, providing plants with a certain degree of basic resistance against pathogenic microorganisms (Satisha et al. 2008). A simple indicator of leaf properties can possibly be used to identify susceptible clones to *Phaeophleospora* spp. before selected clones are released into the field as operational plantations. The aim of this study was to identify leaf properties (including leaf stomatal properties, the proportion of palisade mesophyll, and phenol leaf contents) potentially associated with leaf spot disease resistance of eucalypt clones of *E. pellita* and its hybrids.

MATERIALS AND METHODS

Based on a previous preliminary study, specific leaf spot symptoms caused by *Pha-*

eophleospora spp. were clearly observable on 6-mo-old *Eucalyptus* trees in the field. Subsequently the study was conducted at a 6-mo-old eucalypt plantation belonging to PT Musi Hutan Persada, a forestry company growing eucalyptus in South Sumatra, Indonesia. Three clones, viz., clones 47, 79, and 80, were used in this research. A preliminary study on clones 79 and 80, both *E. pellita* × *E. brassiana* hybrids, indicated that they were respectively susceptible and resistant to *Phaeophleospora* spp., while clone 47, a pure *E. pellita*, was resistant.

The following characteristics were assessed: 1) leaf spot disease severity caused by *Phaeophleospora* spp.; 2) leaf stomatal attributes; 3) thickness of the palisade mesophyll; and 4) constitutive total leaf phenols.

Disease severity of leaf spot caused by *Phaeophleospora* spp.

In order to assess the severity of spontaneously occurring leaf spot disease caused by *Phaeophleospora* spp., an investigation was conducted on 6-mo-old trees in the field. Trees were planted at a 3 × 2-m spacing. Observed traits were disease severity (DS) based on index scores as described in Table 1. Each clone was observed in 3 blocks, and in each block 40 trees were systematically selected, with 4 trees in every row. Leaf samples were systematically taken from 30% of the branches of every sample tree (from lower, middle, and upper stems), with 10 leaves from every branch. Tree height was also measured in order to assess the effect of the disease on the growth of young eucalypt trees.

Disease severity was calculated as follows (modified from Chester 1959):

$$\textit{Disease severity (DS)} = \frac{(z1 \times n)+(z2 \times n)+(z3 \times n)+(z4 \times n)+(z5 \times n5)}{z \times N} \times 100\%$$

where *z1*, *z2*, … *z5* are index scores of leaf spot, n is the number leaves with scores of 1 to 5, *N* is the total number of leaves, and *Z* is the highest index score (5).

With regard to DS, the level of tree resistance was categorized as follows: resistant (R; DS = 0~20%), somewhat resistant (SR; DS 21~40%), tolerant (T; DS = 41~60%), somewhat susceptible (SS; DS = 61~80%), and susceptible (S; DS = 81~100%).

Leaf stomata

Leaf stomatal density, stomatal size, and stomatal proportion were assessed for clones 47, 80, and 79 at the age of 6 mo. Stomata were observed on 5 parts of each leaf lamina: the tip, left, right, middle, and base. A thin layer of clear nail polish was spread on the bottom side of the leaf surface and then allowed to dry. Clear sticky tape was applied to the top of the nail polish and pressed down to make a good connection with the nail polish and slowly released. The sticky tape was placed on a microscope slide. Slides were ob-

Table 1. Index scores of leaf spot symptoms caused by *Phaeophleospora* spp. on 6-mo-old *Eucalyptus* clones in the field

No.	Stage of symptomatic leaf	Index score
1	Necrosis spots	1
2	Necrosis spots with chlorosis	2
3	Necrosis spots with chlorosis and spores emerging	3
4	Necrosis spots with chlorosis, blight, and spores emerging	4
5	Necrosis spots with chlorosis, blight, and spores emerging, followed by leaf curling	5

served under an Olympus CX33 light microscope (Olympus, Tokyo, Japan) at 1000x and 400x magnifications. Images were taken with an OptiLab camera (Miconos Inc) to calculate and measure the number, size, and density of stomata in each clone.

Measurement of palisade mesophyll

Leaves were sampled from the 4th leaf pair from the shoot tip to assure that all leaf samples were of the same age. Five healthy leaves were sampled from each healthy 6-mo-old seedling. Leaf samples were prepared by standard free-hand sectioning. Sections were cut with smooth strokes and placed on a Petri plate with water to keep the sample fresh. The sample was then mounted on a microscope slide for observation. Slides were viewed under an Olympus CX33 light microscope. Images were taken with an OptiLab camera to calculate the adaxial and abaxial palisade mesophyll lengths.

Constitutive total leaf phenols

One pair of healthy *Eucalyptus* leaves from the 1st 3 nodes was sampled per tree, with 3 trees from each clone. Leaf samples were prepared and extracted following the method outlined by Hagerman (1995). Freeze-dried, ground leaf material (0.5 g) was placed in a glass test tube with 5 mL of 70% acetone. Samples were sonicated for 30 min at 4℃, and centrifuged at 2800 g min^{-1} for 10 min. The supernatant was decanted and stored at 4℃. The process was repeated a further 3 times with supernatants combined for each sample. Leaf phenolic compounds were determined using the modified Prussian blue assay for the total phenolic content (Graham 1992). The concentration of phenols (per unit of dry weight) was determined in relation to a gallic acid standard (G-7384 Sigma, St. Louis, MO, USA).

Statistical analysis

An analysis of variance was used to test the effect of different clones on leaf spot disease severity, tree height, stomatal density, size of stomata, thicknesses of the abaxial and adaxial palisade mesophyll, and total leaf phenolic contents. A step-wise multiple linear regression analysis was used to determine which variables were significantly related to disease severity. All analyses were performed using the statistical package of R (vers. 3.6.1).

RESULTS

DS of leaf spot disease

The DS indicates the level of severity of leaf spot disease symptoms. Results from field observations showed that in the 6-mo-old plantation, clone 79 (DS = 76.4%) was somewhat susceptible to *Phaeophleospora* spp., while clones 80 (DS = 26.6%) and 47 (DS = 26.6%) showed some resistance. The growth performances of clone 79 and 80 at age 6 mo were almost the same, but that of clone 47 was significantly lower than the other clones. Mean heights of clones 79, 80, and 47 were 2.6, 2.1, and 1.8 m, respectively. The correlation between tree height and leaf spot disease severity was moderate and positive (R^2 = 0.47). This indicates that the leaf spot DS tended to increase with increasing tree height. The disease did not seem to disturb the growth of the tree at younger than an age of 6 mo.

Leaf stomatal attributes

Based on the stomatal density and size investigation (Fig. 1, Table 2), the stomata density and size of clones 79 and 80 did not significantly differ, but they significantly differed from those of clone 47. The size of stomata in clone 47 was smaller than and significantly different from those of clone 79

and 80. These findings indicate that stomatal attributes are not good indicators for identifying susceptibility of *E. pellita* and its hybrid clones to leaf spot disease.

Thickness of the palisade mesophyll

Results revealed that the thicknesses of the palisade mesophyll on both the adaxial and abaxial sides of clone 79 were thinner compared to those of clones 80 and 47 (Fig. 2). In addition, the DS and thickness of palisade mesophyll of these 3 clones were strongly negatively correlated, with correlation coefficients (r) of 0.88 and 0.94 for the adaxial and abaxial palisade mesophyll thicknesses, respectively. Leaves with a thicker palisade mesophyll tended to have lower DS and thus may be more resistant to *Phaeophleospora* spp. infection. The step-wise multiple regression analyses estimated that leaf spot DS on these 3 clones of *Eucalyptus* was only significantly related to the thickness of the abaxial palisade mesophyll through the following equation:

$LS = 48 - 0.14\ PA$, with $R^2 = 0.92$;

where LS is leaf spot DS and PA is the abaxial palisade mesophyll thickness.

Constitutive total leaf phenols

Results of the phenolic compound content analysis expressed as gallic acid equivalents showed that clone 80 had a significantly higher phenolic content than did clone 79 (Fig. 4). In addition, the 2 clones *(E. pellita* x *E. bassiana*) had significantly higher phenolic contents than did clone 47 (pure *E. pellita*). It is possible that clones 79 and 80 acquired a high content of phenol from *E. brassiana*; however an analysis of phenolic compounds in leaves of *E. brassiana* was not carried out in this study. The correlation between phenolic contents and leaf spot DS on 6-mo-old trees was low and negative (r = -0.198). This finding suggests that the phenolic content is not a good indicator for selecting resistant clones to leaf blight spot disease, in spite of the fact that polyphenols are a part of the

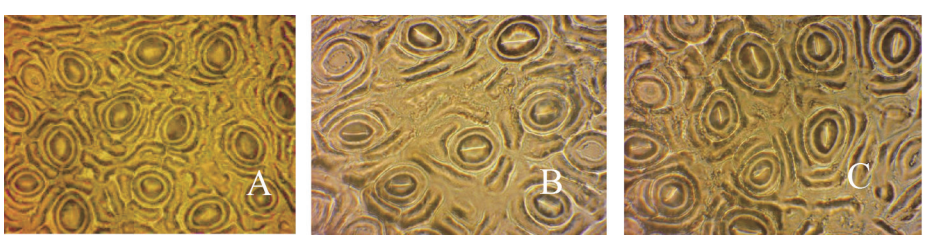

Fig. 1. Leaf stomata of 6-mo-old clones: (A) 47 (pure *Eucalyptus pellita*), (B) Eucalyptus hybrid clone 79, and (C) clone 80 (all pictures were captured at 1000×).

Table 2. Stomatal density and size of stomata of 6-mo-old *Eucalyptus* clones

	Clone		
Characteristic	47	79	80
	E. pellita	E. pellita × E. brassiana	*E. pellita* × *E. brassiana*
Mean stomata density (no. of stomata mm^{-2})	542.3a	355.9c	335.8c
Size of stomata (μm^2)	119.1c	212a	204.0a

Notes: Numbers within the same row with the same letter do not significantly differ by the least significant difference test at $p = 0.05$.

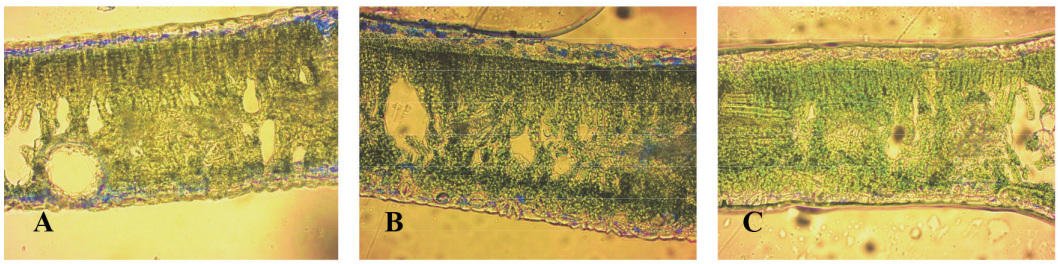

Fig. 2. Palisade mesophyll of 6-mo-old healthy leaves of *Eucalyptus* clones. (A) Clone 47, (B) clone 80, and (C) clone 79 (all pictures were captured at 400×).

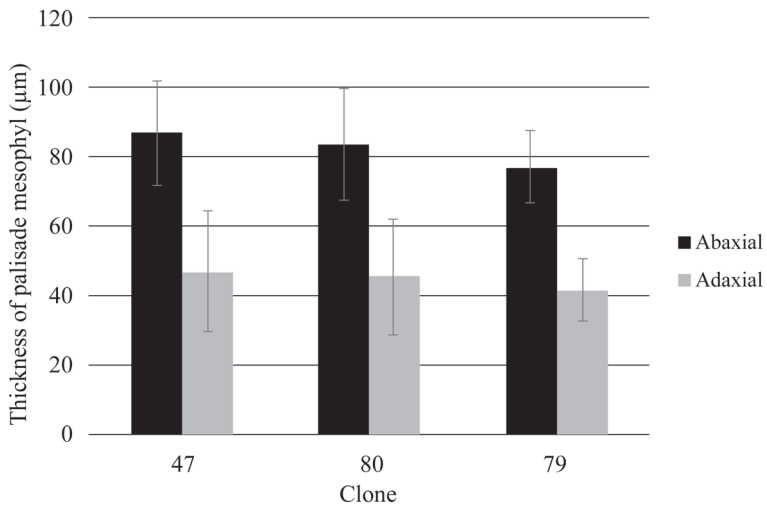

Fig. 3. Average thickness of the palisade mesophyll layer (µm^2) on 3 *Eucalyptus* leaf clones. Bars indicate 1 standard error.

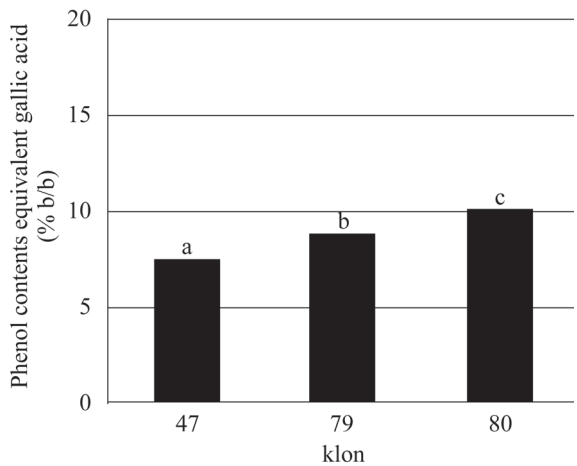

Fig. 4. Phenolic contents as gallic acid equivalents of healthy leaves of clones of *Eucalyptus pellita* (47) and its hybrids (79 and 80).

complex immune system which can be activated in tissues under stress.

DISCUSSION

As there might be a mixture of different *Phaeophleospora* spp. (Videira et al. 2017) associated with *Eucalyptus* leaf spot, isolation to obtain pure and specific *Phaeophleospora* spp. for artificial inoculation experiments will require sophisticated, expensive, and time-consuming assessments, including molecular analyses. Therefore, the assessment of leaf spot severity caused by *Phaeophleospora* spp. in the present study was conducted using spontaneous infection in the field. In addition, our preliminary study found that infection by *Phaeophleospora* spp. resulted in specific leaf spot symptoms only in trees aged 6~12 mo. The most obvious symptom was observed in 6-mo-old trees (Fig. 5C). Subsequently, an evaluation of leaf spot DS based on spontaneous infection in the field was conducted on 6-mo-old trees. It is suggested that evaluation of leaf spot DS using artificial inoculation with certain species *Phaeophleospora* can be carried out in the future.

Based on anatomical assessments, it is clear that most conidia of *Phaeophleospora* spp. entered leaf cells through the stomata, while most pycnidia were formed on the lower side of the leaf epidermis (Fig. 5A). However, up to now, there is little information about the infection mode of *Phaeophleospora* spp. in leaves. Smith et al. (2017) reported that stomata abundance of *E. globulus* had no significant role in determining resistance to *Teratosphaeria* leaf disease, which was previously referred to as *Mycosphaerella* leaf disease.

Research on the response of *Eucalyptus* species to foliar pathogens is usually conducted at the cellular level of young leaves. For example, in *E. globulus*, there is some evidence that leaf density (influenced by the internal leaf structure) may contribute to its resistance to *Mycosphaerella* species (Smith et al. 2006). A higher parenchyma cell density might enhance a plant's resistance by preventing infection of certain *Mycosphaerella* species due to their inability to penetrate closely packed palisade cells (Park et al. 2000). This theory is supported by studies associating the density of palisade parenchyma cells with resistance to several foliar diseases (Basra et al. 1985, Mayee and Suryawanshi 1995, Yang 2000). Although resistance to *Mycosphaerella* infection is likely due to a combination of several traits, there appears to be a relationship between resistance and the thickness of the palisade mesophyll (Smith et al. 2006).

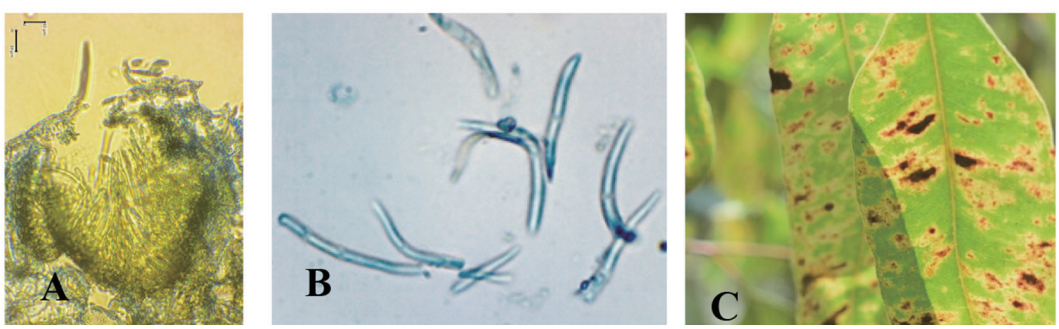

Fig. 5. Conidia of Phaeophleospora spp. in vivo. (A) Pycnidium on the lower epidermis surface (400x), (B) conidia (1000×) collected from (C) specific leaf spot symptom of 6-mo-old young Eucalyptus hybrids in the field.

Higher palisade densities (or volume fractions) were associated with resistance to several leaf spot pathogens, including *Cercospora* species (Basra et al. 1985), and a tighter packing of palisade cells was also observed in resistant families of *E. globulus* compared to susceptible families. Evidence from past studies and the present study suggests that compact palisade layers may slow down or prevent hyphal development due to the inability of some *Mycosphaerella* species to penetrate and colonize tightly packed palisade cells (Park et al. 2000, Smith et al. 2006). Although deviations in the thickness of palisade mesophyll among resistant (clone 47), tolerant (clone 80), and susceptible (clone 79) clones appeared very small and might be significant only in direct comparison, most *Phaeophleospora* spp. pycnidia formed on the lower side of the leaves; thus the role of the abaxial palisade mesophyll appeared to be of paramount importance in preventing hyphal development in leaf cells. In this regard, it is suggested that the thickness of the palisade mesophyll of selected candidate clones be investigated and quantified. Further testing of resistant or susceptible clones to leaf spot pathogens will determine the suitability of using the palisade density and internal leaf structure as indicators for *Eucalyptus* clone selection.

Compounds such as phenols, salicylic acid, chloroisonicotinic acid, and benzolthiadiazole-7-carbothioic acid S-methyl ester are able to induce systemic and acquired resistance against a wide range of microbial pathogens in a variety of plants (Sticher et al. 1997). Polyphenols are a part of the complex immune system, which can be activated in tissues under stress (Feucht 1994). The involvement of phenols in plant disease resistance is based to a large extent on their cytotoxicity, which is associated with their oxidation products (Aver'yonav and Lapikova 1994). Results of our current study suggested that the phenolic content is not a good indicator for selecting resistant clones to leaf spot disease. In our study, leaf samples were taken from healthy trees. Since the accumulation of phenols normally occurs in infected leaves, the analysis of phenol contents in the future study should be conducted on infected leaves from the field or under artificial inoculation.

CONCLUSIONS

This pioneer study on correlations of anatomical and chemical leaf characteristics of *Eucalyptus* clones with spontaneous leaf spot disease severity associated with *Phaeophleospora* fungi reports important findings that can be summarized as follows. 1) Clones 79 and 80 (*E. pellita* × *E. brassiana* hybrids) and clone 47 (*E. pellita*) showed different responses to leaf spot disease caused by *Phaeophleospora* spp. Clone 79 was more susceptible then clone 80, while clone 47 was strongly resistant to leaf spot disease. 2) Leaf stomatal size and density of different clones were poorly correlated with leaf spot disease severity. 3) The leaf phenolic content was not a good indicator for determining resistant clones. 4) The abaxial palisade mesophyll was the only good indicator of resistant clones. Assessing the thickness and number of palisade layers on greater numbers of different *E pellita* clones and their hybrids is recommended to confirm the findings of the current study.

ACKNOWLEDGMENTS

The authors are thankful to the Management of PT. Musi Hutan Persada for the permission to conduct the study and its R&D for providing helpful assistance both in the field and in the laboratory. Universitas Gadjah Mada provided research grant support through RTA (funding and support for gradu-

ate research student) in 2019.

LITERATURE CITED

Aver'yanov AA, Lapikova VP. 1994. Participation of active forms of oxygen in the mechanism of ferulic acid toxicity. Biol Bull 4:352-7.

Basra RK, Kaur S, Dhillon M. 1985. Anatomical and biochemical studies of the resistance and susceptibility of the groundnut varieties to *Cercospora* leaf spot. Ann Biol 1:7-12.

Castro-Díez P, Puyravaud J, Cornelissen J. 2000. Leaf structure and anatomy as related to leaf mass per area variation in seedlings of a wide range of woody plant species and types. Oecologia 124:476-86. Doi: 10.1007/PL00008873

Chester KS. 1959. How sick is the plant? In: Horsfall JGH, Diamond A editors. Plant pathology, Vol. 1. New York: Academic Press. ISBN: 9780123956774.

Craenen K, Coosemans J, Oritz R. 1997. The role of stomatal traits and epicuticular wax in resistance to *Mycosphaerella fijiensis* in banana and plantain (*Musa* spp.). Tropicultura 15:136-40.

Crous PW, Wingfield MJ. 1997. New species of *Mycosphaerella* occurring on *Eucalyptus* leaves in Indonesia and Africa. Can J Bot 75:781-90.

Feucht W. 1994. The localization of phenols at the cellular and tissue level. Acta Horticult 381:803-15.

Graham HD. 1992. Stabilization of the Prussian blue colour in the determination of polyphenols. J Agric Food Chem 40:801-5. Doi: 10.1021/jf00017a018.

Hagerman AE. 1995. Tannin analysis. 2nd ed. Oxford, OH: Miami Univ.

Jyosthna MK, Reddy NPE, Chalam TV, Reddy GLK. 2004. Morphological and biochemical characterization of *Phaeoisariopsis personata* resistant and susceptible cultivars of groundnut (*Arachis hypogaea*). Plant Path Bull 13:243-50.

Mayee CD, Suryawanshi AP. 1995. Structural defence mechanisms in groundnut to late leaf spot pathogen. Ind Phytopathol 48:160-5.

Nambiar EKS, Harwood CE, Mendham DS. 2018. Paths to sustainable wood supply to the pulp and paper industry in Indonesia after diseases have forced a change of species from acacia to eucalypts. Aust For 81:148-61.

Old KM, Wingfield MJ, Yuan ZQ. 2003. A manual of diseases of eucalypts in South-East Asia. Jakarta, Indonesia: CIFOR, Available at http://www.patologiaforestal.com/descargas/Enfermedades%20en%20Eucalipto.pdf. Accessed 17 October 2019.

Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. 2009. Agroforestree Database: a tree reference and selection guide version 4.0. Available at http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp. Accessed 20 December 2019.

Park RF, Keane PJ, Wingfield MJ, Crous PW. 2000. Fungal diseases of eucalypt foliage. In: Keane PJ, Kile GA, Podger FD, Brown BN, editors. Diseases and pathogens of eucalypts. 153-239. Collingwood, Victoria, Australia: CSIRO.

Prasetyo A, Aiso-Sanada H, Ishiguri F, Wahyudi I, Wijaya IPG, Ohshima J, Yokota S. 2018. Growth characteristics and wood properties of two interspecific *Eucalyptus* hybrids developed in Indonesia. For Prod J 68(4):436-44.

Satisha J, Doshi P, Adsule PG. 2008. Influence of rootstocks on changing the pattern of phenolic compounds in Thompson seedless grapes and its relationship to the incidence of powdery mildew. Turk J Agric For 32:1-9.

Smith A. 2006. The development of strategies for the management and research of foliar pathogens on eucalypt plantations: using *Mycosphaerella* as a case study. Hobart, Tasmania: School of Agricultural Science, Univ of

Tasmania.

Smith AH, Gill WM, Pinkard EA, Mohammed CL. 2006. Anatomical and histochemical defence responses induced in juvenile leaves of Eucalyptus globulus and Eucalyptus nitens by Mycosphaerella infection. For Pathol. 37: 361–373. Doi: https://doi.org/10.1111/j.1439-0329.2007.00502.x.

Smith AH, Potts BM, Ratkowsky DA, Pinkard EA, Mohammed CL. 2017. Association of *Eucalyptus globulus* leaf anatomy with susceptibility to *Teratosphaeria* leaf disease. For Pathol 48(2). https://doi.org/10.1111/efp.12395.

Stitcher L, Mauch Mani B, Metraux. 1997. Systemic acquired resistance. Ann Rev Phytopathol 35:235-70.

Tarigan M, Roux J, van Week M, Tjahjono B, Wingfield MJ. 2011. A new wilt and dieback disease of A. mangium associated with *Ceratocystis manginecans* and *C. acaciivora* sp. nov. in Indonesia. S Afr J Bot 77:292-304.

Taylor JE, Crous PW. 1999. *Phaeophleospora faureae* comb. nov associated with leaf spots on *Faurea safigna* (Proteaceae), with a key to the species of *Phaeophleospora.* Fungal Divers 3:153-8.

Videira SIR, Groenewald JZ, Nakashima C, U Braun, RW Barreto, PJGM De Wit, PW Crous. 2017. Mycosphaerellaceae – chaos or clarity? Stud Mycol 87. Doi: 10.1016/j.simyco.2017.09.003.

Yang Q. 2000. Germplasm evaluation, inheritance, molecular marker and mechanism of resistance to frog-eye leaf spot. J Northeast Agric Univ 7:1-6.

Zobel BJ. 1993. Clonal forestry in the eucalypts. In: Ahuja MR, Libby WJ, editors. Clonal forestry II. Berlin, Germany: Springer-Verlag. ISBN: 9783642848155.