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Research Paper

Leaf diseases on *Eucalyptus pellita* in plantation forest at Sebulu, East Kalimantan

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Abstract. *Arsensi I, Mardji D. 2016. Leaf diseases on* Eucalyptus pellita *in plantation forest at Sebulu, East Kalimantan*. *Eucalyptus pellita* is often grown in monoculture, can be susceptible to disease whether grew in the nursery or the field. Currently in the plantation of PT Surya Hutani Jaya, Sebulu is developing *E. pellita*, derived from seed and clonal. The results were later called family. To determine the benefits of trees, the company deliberately does not preserve this area so there will be generated trees (family) that excel in both productivity and resistance to pests and diseases. This study is aimed at determining the symptoms and signs of disease on the leaves, the microorganisms that cause disease on the leaves as well as the incidence and severity of pathogen that attacks the leaves of *E. pellita*. The research took place at PT Surya Hutani Jaya, Sebulu, Kutai Kartanegara Regency, East Kalimantan and continued with the identification of pathogens at the Laboratory of Forest Protection, Faculty of Forestry, University of Mulawarman. The object of this research was *E. pellita* of a 6 year old plantations, spacing of 3 × 2 m. The origin of *E. pellita* is a clone from Riau. Symptoms of the disease observed at the progeny test were leaf spot and leaf blight. The pathogens were *Cercospora* sp., *Pestalotia* sp., *Curvularia* sp., *Bipolaris* sp., *Marsonina* sp. and *Dactylaria* sp. The incidence of leaf spot pathogen was 83.3% and leaf blight was 80.6% of the severity of 9.7% and 12.5%, respectively.

Keywords: *Eucalyptus pellita*, symptom, sign, incidence, severity, progeny test.

INTRODUCTION

The need for wood as raw material for pulp / paper will remain high, and even tends to increase steadily. The emphasis of the wood supply is directed to the development of industrial forest plantations in large scale in the form of wooden gardens (trees farming). The need for wood raw material is so high that it causes the planting of monocultures and a short cutting rotation to be the primary choice (Kemenperin, 2012).

Eucalyptus pellita is often grown in monoculture, both in the nursery and in the field which is susceptible to disease (Semangun 2008). Given widespread planting of *E. pellita* in Kalimantan in general and in East Kalimantan in particular, it is necessary to do more intensive research. Because *E. pellita* wood is very valuable timber and in a very wide area, even a small decrease in the increment should be prevented. A decrease of the increment can be caused by disease-causing (pathogenic), both biotic and abiotic factors (SHJ 2012).

Currently on the plantation of PT Surya Hutani Jaya at Sebulu, *E. pellita* is being developed from seed and clonal selection with the results then called family. Selection is designed to generate the families that excel in order to increase productivity and meet the shortage of wood raw material pulp for the paper industry. In an effort to get the superior families, PT Surya Hutani Jaya has established a progeny test plantation of *E. pellita* known as Full Sib Progeny Test, which is the seeds harvested from 14.4 ha plantation areas in Sumatera. To know the benefits of trees, the company deliberately does not preserve this area, so that it will be generated tree (family) who excels both in productivity and resistance to pests and diseases (SHJ 2012).

This research was conducted to determine symptoms and signs of disease on the leaves of *E. pellita*; microorganisms that cause disease on the leaves; along with the incidence and severity of disease.

MATERIALS AND METHODS

Plantation forest of PT Suryani Hutani Jaya is located approximately 30 km northwest of Samarinda city and approximately 20 km north of Tenggarong city. The plantation forest is geographically situated between the

coordinates 0º31' N and 0º45' S, 116º45' E and 117º22' E, the width is 183,300 ha, altitude 50-500 m. Based on the classification of rainfall Schmidt and Ferguson, PT Surya Hutani Jaya is including climate A, average annual rainfall is 1,840 mm with the average number of rainy days are 82 days per year. Wet months occur almost throughout the year, while the dry months are in rare cases, because of the driest months still have rainfall above 60 mm per month. The intensity of average rainfall in the surrounding area is 22.08 mm per day. At the time of this study, the average of the daily temperature was 26,33ºC with a relative humidity of 86.17% (SHJ 2012).

The research was conducted at the progeny test plantation of PT Surya Hutani Jaya at Sebulu Regency, East Kalimantan and continued with the identification of pathogens in the Laboratory of Forest Protection, Faculty of Forestry, University of Mulawarman.

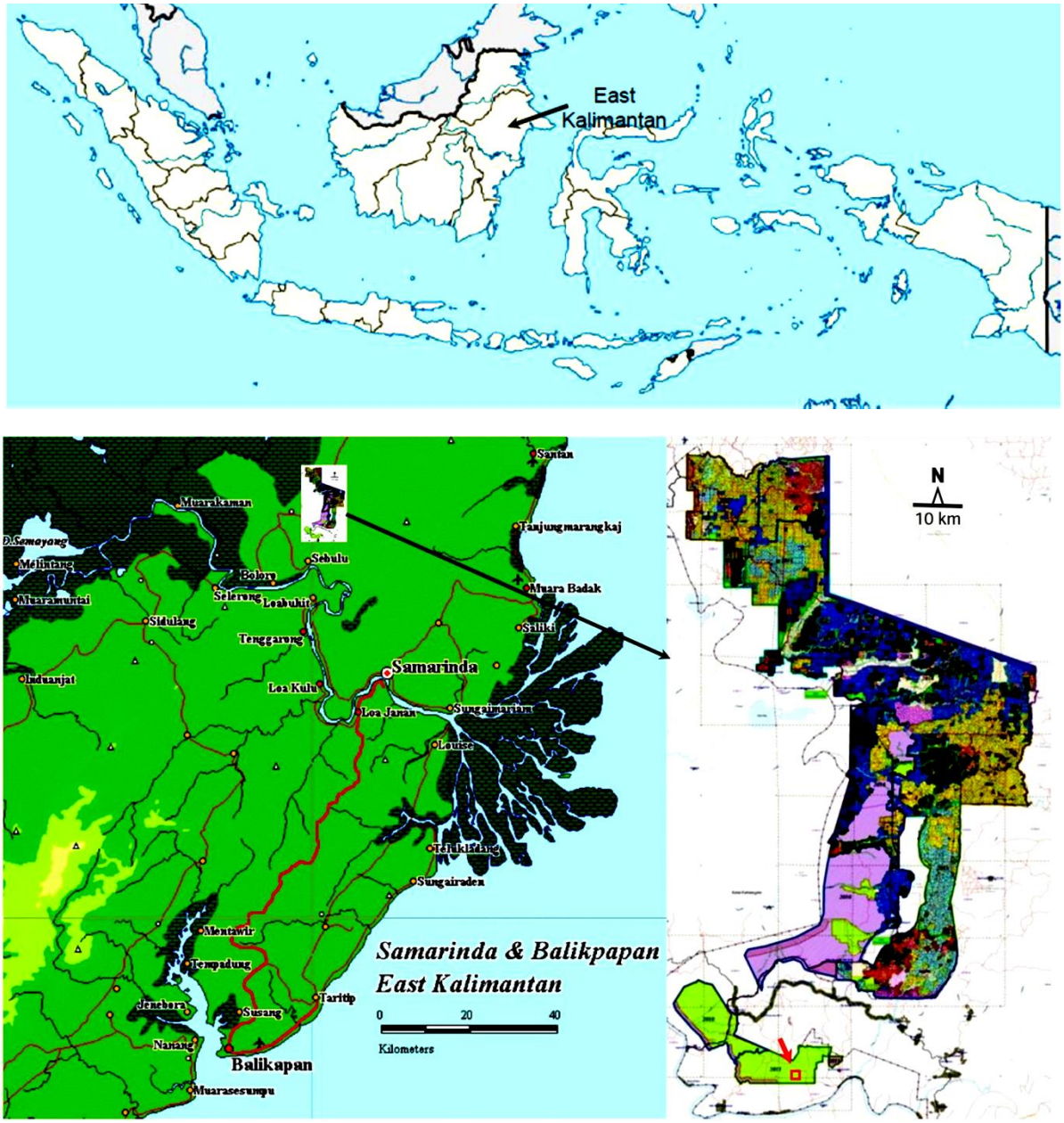

Figure 1. Study site in full sib progeny test (red arrow) of PT Surya Hutani Jaya at Sebulu Regency, East Kalimantan

The research was done as follows:

a. Establishment of research plots. Research plots were placed in Full Sib Progeny Test plots A34 zone 34. The width of research plot area was 2 ha, plant spacing of 3 × 2 m, the number of plants in one subplot was 12 trees in 72 m^2 subplots.

b. Determining observed trees. Number of trees observed were 30 families, consisted of 12 trees for each family that repeated 6 times, so that the total number of trees observed were 30 families × 6 replicates × 12 trees = 2160 trees.

Data collected in the field were symptoms of disease on leaves of each family and sign of attack, namely microorganisms (pathogens) that attacked the leaves were identified by attaching masking tape to the upper and lower sections of the attacked leaves, embedded in an object glass and then examined under a microscope and compared with the literatures.

The severity of the pathogen attack was determined based on tree conditions modified from Mardji (1996) (Table 1).

Table 1. Determining the score of attack of pathogens on each tree of *Eucalyptus pellita*

Condition of tree	Score
Healthy i.e no symptoms of attack or present but the number of affected leaves are very few compared to the total number of leaves, the leaves are very lush until thick.	0
Low attack i.e the number of leaves and number of attacks on each of the affected leaves are few or loss or chlorotic leaves are few or plant looks healthy, but there are other symptoms such as stem canker, the leaves are lush until rare.	1
Moderately attack i.e the number of leaves and number of attacks on each of the affected leaves was a lot or fallen of chlorotic leaves was a lot or is accompanied by other symptoms such as stems canker, the leaves are rather sparse.	2
Severe attack i.e the number of leaves and number of attacks on each of the affected leaves was many or many leaves had fallen or frequent chlorosis or is accompanied by other symptoms such as stem canker stems, leaves are rare.	3
Dead i.e. entire leaf wilting or loss or no signs of life.	4

Identification of disease-causing microorganisms was done by pressing adhesive tape to the upper and lower sections of the affected leaves, embedded in an object glass and then examined under a microscope (Kumar 2013). This works had been done at least five times. Literatures used for the identification of pathogens were "Illustrated genera of imperfect fungi" by Barnett and Hunter (1998), "A manual of diseases of eucalypts in South-east Asia" by Old et al. (2003) and informations from the internet.

Symptoms and signs of attack were clarified qualitatively. Disease incidence (I) was calculated by comparing the number of trees attacked by the total number of observed trees in terms of percent according to James (1974) as follows:

$I = (n/N) \times 100\%$. Where n = number of diseased and dead trees. N = number of observed trees.

Disease severity (S) was calculated using a modified formula of de Guzman (1985), Singh and Mishra (1992) as follows: $S = \{(X1Y1 + X2Y2 + X3Y3 + X4Y4) / XY4\} \times 100\%$

Where X = number of observed trees. X1 = number of trees having score 1. X2 = number of trees having score 2. X3 = number of trees having score 3. X4 = number of trees having score 4. Y1 to Y4 = score of 1 to 4.

To determine the condition of stand and level of resistance of each family of *E. pellita* against disease is shown in Table 2.

Table 2. Determining of stand condition and resistance level of each family of *Eucalyptus pellita* with disease

Severity of disease (%)	Stand condition	Resistance level
0 – 1	Healthy (H)	Highly resistant (HR)
>1 – 25	Low damaged (LD)	Resistance (R)
>25 – 50	Moderately damaged (MD)	Moderately resistant (MR)
>50 – 75	Heavy damaged (HD)	Susceptible (S)
>75 – 100	Very heavy damaged (VHD)	Highly susceptible (HS)

RESULTS AND DISCUSSION

Symptom and Sign

a). Cercospora leaf spot

Symptoms appearing on the leaves were patchy necrosis, pale brown, oval to irregular, measuring 5 × 2 cm, lined with a darker brown color, two or more necrosis could be fused, old necrosis was decayed so that the leaves become perforated. The cause was a fungus *Cercospora* sp. (Figure 2a) The spore of this fungus was

hyaline colored (pale). Long cylindrical with one end rounded and the other end was tapered and consisted of several septa.

Most of *Cercospora* species are plant pathogens and showed a high host specificity. Genera *Cercospora* has been divided into several genera on the basis of new criteria such as conidiomatal structural and morphological features of the mycelium, conidiophores, conidiogenous cells and spores of pigmentation (Crous and Braun, 2003). Nair (2000) stated that *Cercospora* sp. attacked the leaves of *Acacia mangium* and saplings of dipterocarp in Indonesia that resulted in the loss of leaves.

b). Pestalotia leaf spot

Symptoms were patches of brown necrotic irregular shapes measuring 6.5 × 3 cm, with specks of brown to black in the middle, concentric circles, bounded brown young and old, and the veins of leaves were infected. Uninfected parts of the leaf remained green. The cause was a fungus *Pestalotia* sp. (Figure 2b). *Pestalotia* sp. produces spores with three to five cells, three cells in the middle were thick-walled. Two cells bases and tip were hyaline and thin-walled. One end had two to 3 three tails.

Stevens (1966) stated that genera *Pestalotia* comprises more than 200 species. Most of the species are economically very detrimental. According to Barnett and Hunter (1998), this fungus is parasitic or saprophytic. Simorangkir (2014) found that this fungus also attacks the hybrid clones of *E. grandis* × *E. derivative pellita* in the nursery of PT Toba Pulp Lestari Tbk. Toba Samosir, North Sumatera; *E. grandis* × *E. pellita* in the same location was more resistant compared to *E. grandis* × *E. urophylla* against *P. theae* due to the lower severity of their attacks (Sembiring 2015). Nair (2000) reported that *Pestalotia* sp. attacked sapling leaves of dipterocarp in Indonesia that led to leafing spots and fall off, while at the seedling and sapling of *Eucalyptus* spp. causing leaf spot. Suharti and Kurniaty (2013) noted that *Pestalotia* sp. attacked seedlings of nyamplung (*Callophyllum inophyllum*), meranti (*Shorea* sp.), kesambi (*Schleichera oleosa*) and gandaria (*Bouea macrophylla*) in the nursery of Nagrak research station at Bogor.

c). *Curvularia* leaf spot

Symptoms were patches of brown necrotic, roundish shape to irregular, measuring 0.5 × 0.5 cm, occurred between the vein of leaves, close distance to each other, two or more patches may be fused into a larger size, and the border between infected and healthy was reddish brown and chlorotic. The cause was a fungus *Curvularia* sp. Brown spores consisting of three to five cells, one cell curve (Figure 2c). Barnett and Hunter (1998) reported that *Curvularia* sp. is generally parasitic or saprophytic. This fungus is cosmopolitan, meaning that the spread is very wide, living in tropical and subtropical regions, can be found in plant litter or soil, as pathogens in tropical and subtropical plants; this fungus is also the cause of allergic diseases in humans such as asthma and sinusitis (Micro-Solutions Inc. 2015). Research by Nadarajah (2015) resulted that the fungus causes a significant lot of diseases such as black spot on grain, black spots on rice, rotten stem of rice and rice sheath blight worldwide. According to Suharti and Kurniaty (2013), the fungus *Curvularia* sp. also attacked seedlings of nyamplung (*C. inophyllum*), suren (*Toona sureni*), gandaria (*B. macrophylla*) in the nursery and rasamala (*Altingia excelsa*) in the pruning garden at the Nagrak research station at Bogor.

d). Bipolaris leaf spot

Symptoms were reddish brown necrotic spots surrounded by round shape chlorotic, size between 0.5 × 0.5 cm to 1 × 1 cm, close distance from one spot to another, two or more patches can be fused so become a larger patch. The cause is a fungus *Bipolaris* sp. The spores were brown, consisting of several bulkhead (phragmosporous), elliptical slightly curved (Figure 2d). Ellis (2015) stated, that the stage of sexual/teleomorph called *Cochliobolus* sp. *Bipolaris* species is similar to *Dreschlera* and *Exserohilum*. The only difference between the morphology of *Bipolaris* and *Dreschlera* is that the spores germinate from each cell of *Dreschlera* whereas *Bipolaris* spores germinate only from the end of the cell. *Exserohilum* spores have a cup-like structure that is visible at the base of the cell (EMLab 2015). Genera of *Bipolaris* consists of many species which are responsible for significant diseases with worldwide distribution. The species are involved in leaf spot, blight, root rot and other diseases, especially in high value crops in the family Poaceae, including rice, corn, wheat and other hosts (Ellis 1971). The fungus is harmful to staple crops of rice and wheat resulting in famine in a large number of human population in some countries in the world, such as the famine in India in 1943-1944 as a result of the attack of *B. oryzae* on rice plants (Ou 1985). This fungus that attacked *Eucalyptus* sp. in Taiwan was *B. cynodontis* (Farr and Rossman 2013).

e). Marssonina leaf blight

The symptoms were elongated reddish brown necrosis on the edge of the leaf that extends to midrib of the leaves, measuring between 2 × 1 × 1 cm to 7 cm. Irregular shape with darker outer edges than in the middle

brighter and chlorosis. The cause was a fungus *Marssonina* sp. (Figure 3a) The spores of *Marsonina* sp. were ovoid elongated, pale brown, consisting of two cells. Barnett and Hunter (1998) stated, that this fungus is parasitic mainly on leaves. *Marssonina* sp. is a fungus that can survive in the fallen leaves or twigs infected in the past year. Spores develop on leaves on the ground infect and grow on new leaves in the spring of the following year (Guyon 2005). The disease is most virulent in humid weather and a common disease in the entire northwest Pacific on leaves of *Populus* spp. (Micro-Solutions Inc. 2015). The fungal hyphae infect the leaves directly on the surface of the epidermis without having to go through stomata (Spiers and Hopcroft 1984).

f). Dactylaria leaf blight

The symptoms were patches of yellow necrotic and dark brown with brown spots in the middle bound by chlorosis, necrotic spots bound by a darker color measuring 7 × 3 cm. The cause was a fungus *Dactylaria* sp. The spores were pale brown oval-shaped, consisting of two cells (Figure 3b).

g). Cylindrocladium leaf blight

Symptoms were brown necrosis which occurred from the end to 3/4 the width of the leaves, measuring between 6 × 5 cm to 9 × 6 cm, the portion of borders on the unhealthy was dark brown. The midrib and vein of the leaves were attacked. The cause was a fungus *Cylindrocladium* sp. (Figure 3c). *Cylindrocladium* is asexual (anamorphic) stage, whereas in the sexual stage (teleomorphic) belongs to genera *Calonectria*. The results showed that the spores of *Cylindrocladium* sp. were pale (hyaline), slender and elongated shape like a rod. *Cylindrocladium* is a fungus that has widely spread and is a pathogen which has many hosts including *Eucalyptus* spp. in the nursery and on the plantation (Old et al. 2003). Crous (2002) suggested that there are 39 species of *Cylindrocladium* in the world, among which there are 24 species, including pathogens in *Eucalyptus* spp., in which 15 species are discovered in Southeast Asia. Cordell et al. (2004) reported that the disease is caused by several species of *Cylindrocladium* (especially in America was *C. scoparium, C. crotalariae* and *C. floridanum*) that can attack plant needles and broad leaves of many species. In the middle of north and northeast America as well as in the Province of Ontario, the most species commonly affected are *Pinus resinosa*, *P. strobus*, *Picea mariana* and *P. glauca*. In South America, seedlings of *Juglans nigra*, *Liriodendron tulipifera*, *Liquidambar styraciflua*, *Eucalyptus* and *Pinus strobus* are most frequently affected. Additionally, *Prunus serotina*, *Cornus florida*, *Quercus rubra*, *Cercis canadensis* and several kinds of shrubs are also susceptible to *Cylindrocladium*. This leaf blight is also one of the most destructive diseases of *E. grandis* forest in Colombia. Leaf blight disease can cause weight loss. Thus reducing the productivity of *E. grandis* wood in the forest zone with high humidity. The results of evaluation of experimental clones *Eucalyptus* it turns out there is a big difference in resistance to infection by *C. spathulatum* (Rodas et al. 2005). Blum et al. (1992) reported, that *C. clavatum* can cause damping off and root rot on *Eucalyptus* spp., while *C. scoparium* causes spot, blight and root rot. Provenances *E. torelliana*, *E. citridodora*, *E. deglupta* and *E. maculata* are resistant to both *Cylindrocladium* spp. Six provenances of five species (*E. robusta, E. resinifera, E. urophylla, E. grandis* and *E. citriodora*) are resistant only against *C. scoparium*. Ten other provenances including eight species (*E. grandis, E. pellita, E. urophylla, E. pilularis, E. camaldulensis, E. resinifera, E. grandis* and *E. maculata*) are quite resistant to *C. clavatum*, but only eight provenances including six species (*E. deanei, E. grandis, E. pellita, E. saligna, E. urophylla* and *E. pilularis*) are quite resistant to *C. scoparium*. Species of *C. clavatum* is more aggressive than *C. scoparium* against many *Eucalyptus* spp. and it also has a wider host range than *C. scoparium*.

Incidence and Severity of Disease

Leaf spot

This disease ranks second most commonly prevalent at the site. The average incidence of attack was the highest on family Ep12 (89.5%) and the lowest on family EP30 (16.7%). The average severity was the highest on family EP30 (29.2%) and the lowest on family Ep10 (9.7%) (Table 3).

Table 3. Incidence (I) and severity (S) of leaf spot disease (%), condition of *Eucalyptus pellita* stands and resistance levels in progeny test

No	Family	Leaf spot		SC	LR	No	Family	Leaf spot		SC	LR
		I	S					I	S		
1	Ep1	50.0	20.8	LD	R	16	Ep16	33.3	19.4	LD	R
2	Ep2	47.9	13.8	LD	R	17	Ep17	18.8	22.2	LD	R
3	Ep3	45.8	20.8	LD	R	18	Ep18	37.5	16.7	LD	R
4	Ep4	83.3	16.7	LD	R	19	Ep19	81.3	26.4	MD	MR
5	Ep5	58.3	26.4	MD	MR	20	Ep20	20.8	18.1	LD	R
6	Ep6	66.7	25.0	LD	R	21	Ep21	72.9	23.4	LD	R

7	Ep7	68.8	22.2	LD	R	22	Ep22	64.6	13.9	LD	R
8	Ep8	45.8	22.2	LD	R	23	Ep23	60.4	16.7	LD	R
9	Ep9	54.2	26.4	MD	MR	24	Ep24	70.8	19.4	LD	R
10	Ep10	41.6	**9.7**	LD	R	25	Ep25	64.6	18.1	LD	R
11	Ep11	39.6	11.1	LD	R	26	Ep26	39.6	19.4	LD	R
12	Ep12	**89.5**	16.7	LD	R	27	Ep27	70.8	11.1	LD	R
13	Ep13	54.2	19.4	LD	R	28	Ep28	56.3	12.5	LD	R
14	Ep14	79.2	16.7	LD	R	29	Ep29	70.8	23.6	LD	R
15	Ep15	77.1	12.5	LD	R	30	Ep30	**16.7**	**29.2**	MD	MR

Each figure is the average of 12 trees. SC = stand condition. LR = level of resistance. LD = low damaged. MD = moderately damaged. R = resistance. MR = moderately resistant. Figures in bold are the highest and lowest.

There were 18 families with disease incidence over 50%, while 12 families was below 50%. The disease severity on all families were below 25% and thus the conditions of *Eucalyptus* stand in the study site can be categorized as minor damage and resistant against leaf spot pathogen. Except EP5, Ep9, Ep 30 and Ep19 were moderately resistant against leaf spot pathogen because the severity was below 50% with the condition of moderately damaged. Diseases on the leaves caused by pathogenic fungi did not cause any economic harm because the old leaves would always be replaced by fresh leaves. Leaf spot pathogen has a potential to cause harm in the future because the leaf is a vital organ for plants. The process of photosynthesis occurs in the leaves containing chlorophyll with the help of sunlight producing starch (carbohydrates) used for plant growth. Photosynthesis is a biochemical process of food substances such as carbohydrate formation performed by the plants, especially plants that contain chlorophyll or using nutrients, carbon dioxide, water and sunlight energy assistance (Salisbury and Ross 1999).

Leaf blight

Leaf blight disease was also noted in the study site. The average incidence of attacks was the highest on family Ep19 (89.6%) and the lowest was Ep2 (25.0%) (Table 4). The average severity of leaf blight was the highest on family Ep24 (12.5%) and the lowest on Ep1, Ep2, Ep15, EP20, Ep21 and Ep28 (1.4%).

Table 4. Incidence (I) and severity of the attack (S) (%) of leaf blight, condition of *Eucalyptus pellita* stands and resistance levels in progeny test

No	Family	Leaf blight		SC	LR	No	Family	Leaf blight		SC	LR
		I	S					I	S		
1	Ep1	72.9	**1.4**	LD	R	16	Ep16	56.3	9.7	LD	R
2	Ep2	**25.0**	**1.4**	LD	R	17	Ep17	77.1	4.2	LD	R
3	Ep3	27.1	11.1	LD	R	18	Ep18	81.3	2.7	LD	R
4	Ep4	50.0	9.7	LD	R	19	Ep19	**89.6**	5.6	LD	R
5	Ep5	54.2	6.9	LD	R	20	Ep20	64.6	**1.4**	LD	R
6	Ep6	58.3	6.9	LD	R	21	Ep21	45.8	**1.4**	LD	R
7	Ep7	52.1	4.2	LD	R	22	Ep22	58.3	2.7	LD	R
8	Ep8	37.5	4.2	LD	R	23	Ep23	85.4	4.2	LD	R
9	Ep9	64.6	5.6	LD	R	24	Ep24	70.8	**12.5**	LD	R
10	Ep10	64.6	6.9	LD	R	25	Ep25	68.8	6.9	LD	R
11	Ep11	33.3	2.8	LD	R	26	Ep26	41.7	4.2	LD	R
12	Ep12	64.6	5.6	LD	R	27	Ep27	54.2	5.6	LD	R
13	Ep13	77.8	11.1	LD	R	28	Ep28	58.3	**1.4**	LD	R
14	Ep14	68.8	8.3	LD	R	29	Ep29	77.1	5.6	LD	R
15	Ep15	50.0	**1.4**	LD	R	30	Ep30	62.5	2.8	LD	R

Each figure is the average of 12 trees. SC = stand condition. LR = level of resistance. LD = low damaged. R = resistance. Figures in bold are the highest and lowest.

There were 24 families that the incidence of attack was greater than 50% and only 6 families with the incidence below 50%. The severity of the attack on all families was below 25%. Hence the conditions of *E. pelita* stand in the study site can be categorized as minor damage with the level of resistance against the leaf blight pathogen.

Symptoms of leaf diseases on *E. pellita* found in progeny test were leaf spot and leaf blight. The causal agents were the fungi *Cercospora* sp., *Pestalotia* sp., *Curvularia* sp., *Bipolaris* sp., *Marsonina* sp. and

Dactylaria sp. The incidence of leaf spot was 83.3% and leaf blight was 80.6%, while the severity of leaf spot was 9.7% and leaf blight 12.5%.

This study revealed that the pathogen on leaves of *E. pellita* were many. Since the leaf is an important organ for photosynthesis process, then the disease can directly disturb plant physiological process. It is needed to protect the plant against leaf disease by means of planting resistant families, where this study were identified as family Ep1, Ep2, Ep7, Ep16, Ep20, Ep21, Ep22, Ep24 and Ep30. These families can be used as mother trees to find resistant seedlings against leaf disease, just in line with the program of full sib progeny test plantation by the company of PT Surya Hutani Jaya.

CONCLUSION

Symptoms of the disease observed at the progeny test were leaf spot and leaf blight. The pathogens were *Cercospora* sp., *Pestalotia* sp., *Curvularia* sp., *Bipolaris* sp., *Marsonina* sp. and *Dactylaria* sp. The incidence of leaf spot pathogen was 83.3% and leaf blight was 80.6% of the severity of 9.7% and 12.5%, respectively.

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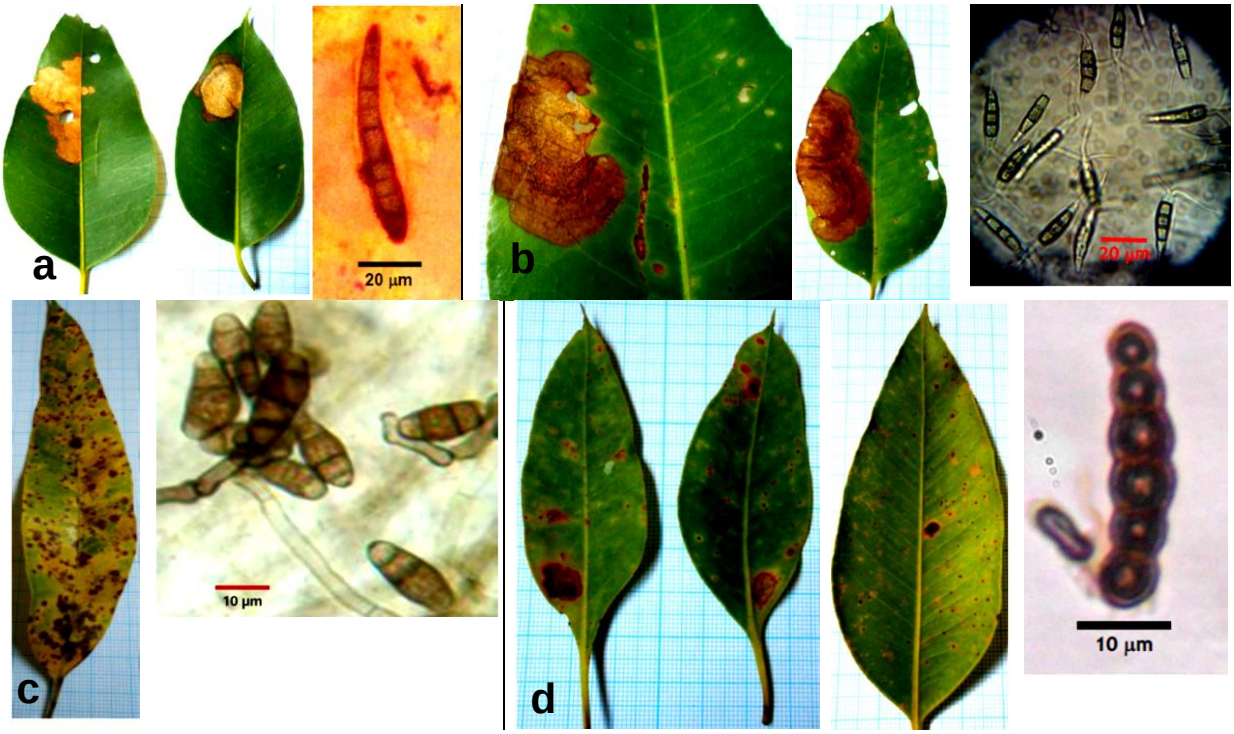
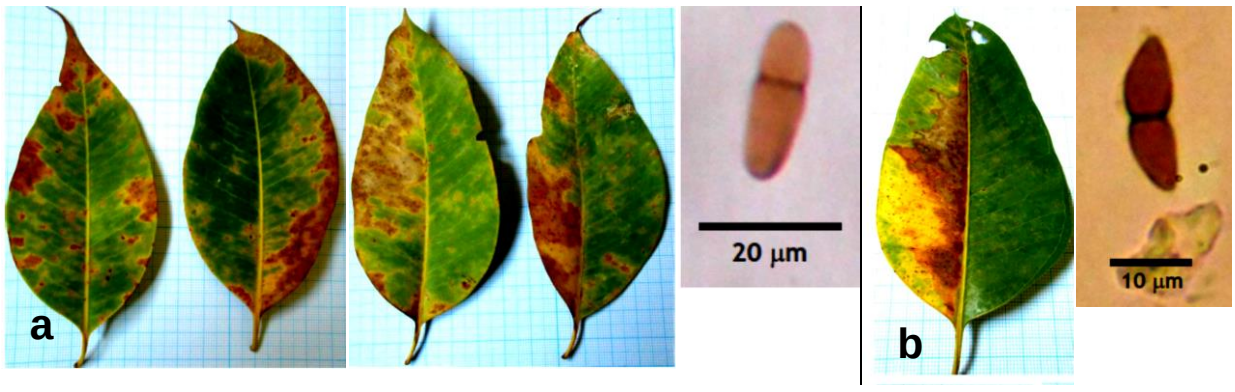


Figure 2. Leaf spot disease on *Eucalyptus pellita* caused by: a *Cercospora* sp. b *Pestalotia* sp. c *Curvularia* sp. d *Bipolaris* sp.



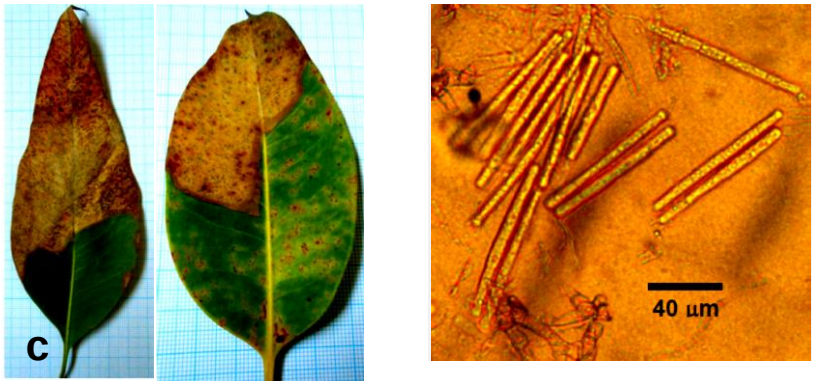


Figure 3. Leaf blight disease on *Eucalyptus pellita* caused by: a *Marssonina* sp. b *Dactylaria* sp. c *Cylindocladium* sp.