Epidemiology of bacterial wilt disease on Eucalyptus pellita F. Muell. in Indonesia

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Abstract. Productivity of forest plantation such as Eucalyptus pellita in Indonesia is strongly decreased by bacterial wilt disease (Ralstonia solanacearum Smith). However, studies of bacterial wilt disease on E. pellita in Indonesia is still inadequate, so several comprehensive research are required. This research was aimed to analyse the dominant silviculture factors and soil properties which can contribute to disease epidemiology. Observation on disease incidence was conducted by the transect method on 40 observation plots selected by the purposive random sampling in 4 provinces in Indonesia. Nine silviculture factors were collected by tracking the identity and the history of plot maintenance, while soil properties were analysed from soil samples. The correlation among disease incidence to silviculture factors and soil properties was carried out by chi-square and multiple regression analysis, respectively. Planting age, Eucalyptus clones, and root malformation were the top 3 of silviculture factors which has shown significant correlation to disease incidence. Early growth of Eucalyptus sp. plant was a critical period of the disease epidemic, especially in the susceptible clone. On the other hand, soil texture (percentage of sand, clay, and silt) were the dominant variables of soil properties which strongly affected the disease incidence.

1. Introduction
Bacterial wilt disease has become an important threat in wide and intensive Eucalyptus planting. It becomes even more serious when the plants are propagated from cuttings [1, 2]. This disease was firstly reported by Machmud [3] on Eucalyptus in Indonesia, then described on lowland Eucalyptus plantations since 2006 in Jambi. This disease has been widely spread in Riau, Palembang and East Kalimantan. Outbreak of this disease that occurred in Indonesia on 2010-2011 caused damage in nurseries and plantations with estimated losses up to IDR 16 billion [4].

The pathogen, namely R. solanacearum (RS) can colonize the host plants with no symptoms (latent infection) [5]. Vascular tissue of the host contains large bacterial populations but are asymptomatic [6]. Latent infection in propagative materials on another plant such as potato, banana, and geranium is believed to be the pathogen’s long-distance distribution pathway [7]. In the case of the Eucalyptus tree, planting using latently infected seedlings into the field has caused disease outbreak [8]. Stressed due to silviculture in the field can trigger symptom expression later on.
Different eucalyptus species, including *E. pellita* and *E. urophylla* shows variations in disease resistance. Variations in this resistance have been identified on various *Eucalyptus* species in Brazil [9]. The variations in clones within *E. pellita* species showed different levels. This fact indicates that strong interaction between the clones resistance and environmental (biotic and abiotic) factors is applied [10].

Bacterial wilt research on *Eucalyptus* in the most tropical and subtropical regions showed that the disease incidence was closely related to stress conditions [11]. Various studies on the influence of environmental factors on bacterial wilt disease have been carried out, including those related to environmental temperature [12], the biological and chemical properties of the soil [13, 14], and poor silviculture practices [11]. In Indonesia, *E. pellita* is a dominant *Eucalyptus* species planted, but we have limited information about bacterial wilt on *E. pellita*. So, the purpose of this research was to determine the trigger factors (silviculture practices and soil properties) for bacterial wilt disease incidence on *Eucalyptus* sp.

2. Methods

2.1. Assessment of disease incidence
Observation was conducted on eucalyptus plantation in Riau, Jambi, South Sumatra and East Kalimantan Province based on the distribution data of Indonesian eucalyptus wood production in 2017 [15]. A total of 40 plots was selected by purposive random sampling method. Assessment of disease incidence was carried out using the transect (5-2) method [16]. The starting point of observation was determined from the middle or corner of the plot and the 5-10th tree from the edge of the plot as 1st tree to be observed. A total of 5 trees in the 1st row was observed, then passed the next 2 trees before the next 5 trees until it reached 1% of the plants number in the plot (Fig. 1). Disease incidence (DI) was calculated by the formula below:

\[
DI = \left( \frac{n}{N} \right) \times 100\%
\]

With, \( n \): number of infected trees; \( N \): Number of total trees.

2.2. Silviculture data collection
Silviculture data were collected by tracing the identity and history of plot maintenance. The data consisted the information of land slope, plant age, seedling type, weed coverage, planting rotation, herbicide toxicity, site class, eucalyptus clones and root malformation (Table 1).
Table 1. Silviculture practice variables and class categories

| Variables                  | Categories                                      |
|---------------------------|-------------------------------------------------|
| Land slope                | Flat (0% ≤ x < 8%)                              |
|                           | Sloping (8% ≤ x < 15%)                          |
|                           | Medium (15% ≤ x < 25%)                          |
|                           | Steep (25% ≤ x < 40%)                           |
| Plant age                 | 0-4 mo                                          |
|                           | 5-8 mo                                          |
|                           | 9-12 mo                                         |
|                           | > 12 mo                                         |
| Seedling                  | Tissue Culture                                  |
|                           | Mini Cutting                                    |
| Weed coverage             | Low (≤ 30%)                                      |
|                           | Medium (30% < x < 70%)                          |
|                           | High (> 70%)                                    |
| Planting rotation         | 2                                               |
|                           | 3                                               |
|                           | 4                                               |
|                           | 5                                               |
|                           | 6                                               |
| Herbicide toxicity        | Yes                                             |
|                           | No                                              |
| Site class                | I                                               |
|                           | II                                              |
|                           | III                                             |
|                           | IV                                              |
|                           | V                                               |
|                           | VIII                                            |
| Eucalyptus clones         | EP0077AA                                        |
|                           | EP0221AA                                        |
|                           | EP0286AA                                        |
|                           | EP0361WK                                        |
|                           | EP0364WK                                        |
|                           | EP0497AA                                        |
|                           | EP5147AA                                        |
|                           | EP0335WK                                        |
| Root malformation         | Yes                                             |
|                           | No                                              |

2.3. Soil sampling and analysis

One composite soil sample consisting 5 individual samples that represented each observation plot was observed. Soil samples were taken from 0-20 cm depth at each sampling point. Analysis of soil properties included soil pH, organic carbon (C), total nitrogen (N), phosphate (P) and sulphur (S), potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na) and soil texture (percentage of sand, clay, and slit). Soil chemical analysis was carried out using the method described by BPT [17]. Soil chemical content was measured on dried samples of heated soil at 105 °C; while pH measurement was carried out by watering the soil (water:soil = 1:5). Organic-C content was measured by the Walkey and
Black method, N-total content by the Kjeldahl method, P-total content by extracting 25% HCl. Exchangeable cation (Ca\(^2+\), Mg\(^2+\), K\(^+\), and Na\(^+\)) was measured by atomic absorption spectrophotometry (AAS).

2.4. Correlation analysis of silviculture and soil properties factor with disease incidence

The disease incidence (DI) was grouped into 4 level, namely free (DI = 0%), low (0 < DI ≤ 5%), moderate (5 < DI ≤ 10%) and high (DI ≥ 10%). Firstly, the value of silviculture factors was grouped into classes, then the data were arranged in a contingency table with the disease incidence as columns and silviculture factors as rows. The correlation status was determined by using chi-square test (P ≤ 0.05). For soil properties, multiple regression analysis was carried out on the soil variable data obtained from 40 locations. Regression analysis was performed using the IBM SPSS Statistics 20 Program.

3. Results and discussion

3.1. Incidence and bacterial wilt symptoms

The bacterial wilt disease incidence from various locations was varied, except in East Kalimantan where the disease was never found. The soil types in all plots are mineral soil; although the plots have different height and elevation, it classified as lowlands, which are optimum sites for E. pellita plantations [18]. The initial disease symptoms are yellowing of the leaves followed by branches wilting. The wilting invaded to entire branches and caused plant death. Color changes, turning blackish-brown, occurred in the inner cortex of the stem associated with bacterial ooze (Fig. 2). This bacterial exudates from the plant stem can be used to distinguish plants infected by bacterial wilt disease compared other wilting diseases caused by Ganoderma philipii (root rot disease) or Ceratocystis sp. (stem and cancer fungus). Recent studied has shown that Ralstonia was not solely occurred in the exudate [11].

![Figure 2](image)

**Figure 2.** Bacterial wilt sign and symptoms in E. pellita. (a) wilting and drying branches symptom on 2-mo-old eucalyptus; (b) further symptom: the dry up and die branches on 9-mo-old eucalyptus; (c) bacterial exudates (ooze) on 2-mo-old eucalyptus; (d) colour changes turning blackish-brown on eucalyptus 23-mo-old stem.

3.2. The relationship between silviculture factors and disease incidence

There were 3 out of 9 variables on silviculture factors that showed significant correlation with disease incidence. These factors were plant age, clones type, and root malformation (Table 2). Young eucalyptus plants obtain more critical condition to disease epidemic, especially in the susceptible clones. These findings have contrary result with those reported by Wardlaw [19] that trees between 2 and 4-yr-old are most susceptible to infection by Ralstonia species. This might be suspected because of latently infected plants are planting to the field. Our laboratory analyses have shown that Ralstonia are often detected asymptomatically in seedling in susceptible clones (Fahrizawati, unpublished).

Fig. 3 showed the corresponding analysis of these 3 significant variables. Free-diseases plots are corresponding into clones EP0077AA, EP0361WK and EP0286AA; while the high level of disease
incidence plots is corresponding into clones EP5147AA and EP0221AA. The screening of bacterial wilt genetic materials in growth chamber has shown that clones EP0077AA and EP0361WK are resistance while the clones EP5147AA and EP0221AA are susceptible [20]. The character of clones become dominant variable compared to plant age and root malformation in influencing the disease incidence. Variations in resistance have been identified for various eucalyptus clones [12, 8, 21]. Root malformation conditions such as J-root and root knotting can trigger plant stress. The malformed root system can cause the creation of wounds as the entry point of bacterial infection, weaken the host’s defense systems, and reduce water absorption by root [11, 22]. Susceptible clones combined with root malformation at immature plantation can exacerbate the disease incidence in the field.

| Table 2. Correlation between silviculture practices and bacterial wilt incidence* |
|---------------------------------|----------|---------|
| Silviculture practices          | Sig.     | χ²      |
| Land slope                      | 0.5493   | 8.399   |
| Plant age                       | 0.0292*  | 18.593  |
| Seedling                        | 0.1490   | 5.482   |
| Weed coverage                   | 0.4601   | 6.139   |
| Planting rotation               | 0.0573   | 20.613  |
| Herbicide toxicity              | 0.4949   | 2.888   |
| Site class                      | 0.1894   | 19.751  |
| Eucalyptus clone                | 0.0004*  | 49.748  |
| Root malformation               | 0.0003*  | 18.506  |

* Indicates parameters correlated with the disease incidence on P <0.05.

Figure 3. Corresponding analysis result.

Silviculture practices can be used to manage plant diseases by creating favorable environment to plants, but not to pathogens [23]. This can affect the microclimate and plant fitness that reduce the disease development [10]. The land slope, weed coverage, planting rotation, seedling type, and site class did not show a significant correlation to the disease incidence. These factors are not enough to influence microclimate changes and plant fitness as well as can inhibit disease progression.
3.3. The relationship between soil properties and disease incidence
The soil properties variables showed varying results. Based on the United States Department of Agriculture's Natural Resources Conservation Service, soil pH range on almost all eucalyptus plantations plots is classified as extremely acidic (3.5-4.5). Soil acidification resulted on bacterial wilt disease outbreak [24]. However, eucalyptus pathosystem did not a strong correlation. The free-disease plots can also occur in very extreme acidic soils. The acidity of the soil is an essential factor in plant diseases because it can influence other soil factors [25]. Organic-C, N-total and P-available content showed medium to high levels, while exchangeable cations ranged from low to very low levels (Table 3).

Table 3. Soil properties analysis result correlated to disease incidence.

| Variables       | Minimum | Maximum | Mean  | Sig.  |
|-----------------|---------|---------|-------|-------|
| pH (H2O)        | 3.10    | 5.00    | 4.00  | 0.566 |
| C-org (%)       | 0.59    | 34.30   | 3.70  | 0.944 |
| N-total (%)     | 0.06    | 2.75    | 0.30  | 0.387 |
| P-avail (mg/kg) | 0.00    | 79.42   | 13.82 | 0.173 |
| S-total (mg/kg) | 40.00   | 6126    | 993.85| 0.725 |
| K+ (me/100 g)   | 0.03    | 0.40    | 0.14  | 0.248 |
| Mg+ (me/100 g)  | 0.01    | 3.23    | 0.26  | 0.958 |
| Ca2+ (me/100 g) | 0.10    | 4.81    | 0.49  | 0.711 |
| Na2+ (me/100 g) | 0.01    | 0.15    | 0.06  | 0.814 |
| Silt (%)        | 5.00    | 77.00   | 28.71 | 0.048*|
| Clay (%)        | 1.00    | 46.00   | 24.94 | 0.053 |
| Sand (%)        | 10.00   | 90.00   | 46.26 | 0.050 |

* Indicates parameters correlated with the disease incidence on P <0.05.

Multiple regression analysis was used to determine the relationship among the level of disease incidences (dependent variable) which is affected by more than 1 soil properties content (independent variable). Table 3 shows that soil textures (percentage of sand, clay, and silt) are the dominant variables of soil properties which strongly affecting the disease incidence. Silt content was correlated positively with the disease incidence in the field. Medium to high-disease incidence mostly occurred in soils with 20%-50% silt content (clay loam to silty clay soil) while the free or low-disease incidence occurs in 5%-17% silt content (loamy sand soil) (Fig. 4). This phenomenon is possibly related to the bacterial population that decline faster in sandy soils (low silt) than in clay soils [26].

This study showed that silviculture practices and soil properties may become the trigger factors for bacterial wilt disease. Age planting, eucalyptus clones, root malformations and silt content can contribute to the disease epidemic. Integrated pest management (IPM) using resistant genetic materials is proven to be efficient and applicable to forestry plantations. Screening for resistant planting material [21] is an alternative solution for the problem, but this cannot be used as single strategy, once the bacterial concentration may influence plant tolerance. Deployment of clone resistance combined with the
elimination of bacterial entrance site in the nursery, root malformation in the field, maintenance the bacterial population in low concentration and adequate nutrition are the proper way to control bacterial wilt in a eucalyptus plantation.

Figure 4. Overlay of disease incidence level on soil particle size scale diagram. Disease incidence level was differentiated into Free: green; Low: yellow; Medium: orange; and High: red dot.

4. Conclusion
Bacterial wilt disease epidemic is influenced by silviculture practices and soil properties. Planting age, eucalyptus clones, and root malformation are silviculture factors showing significant correlation to disease incidence. Early growth of eucalyptus plant is a critical period of the disease epidemic, especially in the susceptible clones. Moreover, plant stress caused by root malformation contributes to disease incidence. Soil texture (percentage of sand, clay, and silt) are the dominant variables of soil properties which most strongly affect the disease incidence. Silt content was correlated positively with the disease incidence in the field.

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