



## Ecosystem Monitoring on Leaves of Leaf Rust Disease of Maize (*Zea mays* L.)

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### Abstract

Endemic leaf rust disease always occurs in almost all maize plantations in Indonesia. Furthermore, the development of this disease differs concurrently and is greatly influenced by the ecological conditions of maize cultivation. Therefore, this study fills the epidemiological gap of diseases that has not been conducted against the epidemiology of maize rust. This identifies the causes of leaf rust that attacked the maize plants in two locations, namely Bayur and Muang Dalam, Lempake, Samarinda, Indonesia. This study also analyzed the relationship or model between ecological factors of temperature, humidity, and soil fertility on the intensity of leaf rust and the infection rate of maize leaf rust. Measurement of disease intensity, the rate at which it developed, soil fertility and temperature and humidity of the area are conducted in this study. Meanwhile, soil fertility also influenced disease progression and the nutrient-poor soils in two sites cause leaf rust disease to develop well. The identification results showed that the cause of maize leaf rust was *Puccinia sorghi* Schw. Therefore, the temperature accompanied by the increased humidity is directly proportional to the development of the leaf rust.

**Keywords:** maize leaf disease identification; plant disease epidemiology; *Puccinia sorghi* Schw; relative humidity; temperature

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### INTRODUCTION

Maize leaf rust is an airborne disease because its transmission mode is through the air and grows profusely when the pathogens encounter their host, such as maize. The pathogen penetrates the leaf, infects the plant and multiplies on the leaves (Surtikanti, 2011). The development of this disease is strongly influenced by its ecological conditions, including the temperature and humidity factors, as well as a plant supporting factor, namely soil fertility (Dhami et al., 2015). It is observed that 100% relative humidity and a temperature of 25 to 30°C sporulation of Gray Leaf Spot (GLS) on maize are high, but the number and expansion

of lesions are not significantly different at temperature > 25°C. GLS develops slowly when the average daily temperature falls below 20°C. In summer maize, the disease incidence is relatively high in the mountains and the valleys, while it is very low in the terai. However, the disease has attacked the winter and spring maize in the Terai of Nepal (Subedi, 2015).

Agricultural ecosystems are simpler and less stable, making them prone to disease development (Tilman et al., 2002). The stability of an ecosystem is determined by the diversity of structures and the characteristics of its components. Furthermore, this is achieved when an understandable and controllable interaction

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exists between components (Robertson et al., 2014).

Agroecosystems are dynamic, constantly changing in time and place, and are highly sensitive to influences within and outside the ecosystem. Therefore, to achieve ecosystem management goals, information about its state and dynamics obtained through monitoring activities is required. Furthermore, these activities are conducted to gain information about the state of the ecosystem, including weather, water, soil, pest and disease populations, natural enemies, crop damage and plant growth (Médiène et al., 2011).

According to statistical data, maize production in East Kalimantan increased in 2015, reaching 112,522 tons from the previous year (BPS-Statistics of Kalimantan Timur Province, 2016). Meanwhile, this has occurred due to an increase in productivity of 39.35%. Also, according to the field data, one of the constraints of maize production is the attack of leaf rust disease (Kusyanto and Hasmara, 2017).

Leaf rust is a disease that attacks maize plants caused by the fungus *Puccinia sorghi* Schw (Soenartingsih et al., 2013). The factors that influence leaf rust disease are abiotic and biotic environmental factors such as climate as well as pests, respectively. Asynchronous climate change increases the development rate of fungus, inhibiting the growth of the maize plant itself. Therefore, it is necessary to control the leaf rust disease (Burhanuddin, 2015).

The agroecological approach strives to improve crop yields simultaneously while also understanding the processes that permit their maintenance (Wezel et al., 2014). However, the primary goal is to determine the long-term sustainability of agricultural systems. The primary foundation of agroecology is the ecosystem concept, defined as a functional system of complementary relationships between living organisms and their environment, delimited by arbitrarily chosen boundaries in space and time and appears to maintain a steady yet dynamic equilibrium (Gliessman, 1995; Ponisio and Ehrlich, 2016). In addition, Sopialena (2018) stated that the environment is one of the dominant factors in disease and its progression. Therefore, the research on ecosystems contributes to the development of sustainable agricultural systems (Perfecto and Vandermeer, 2015). The emergence of a disease requires at least

three factors, such as the host plant, pathogen and environmental factors (Wakman and Burhanuddin, 2010).

Research on temperature and light on maize conducted by Negeri et al. (2013) showed that the maize phenotype was strongly influenced by temperature. Plants have hypersensitivity symptoms as a result of low temperature. Hence, there is an interaction between temperature and maize genetics. The hypersensitive reaction of maize controlled by the resistance genes is suppressed when maize is grown at a temperature greater  $> 30^{\circ}\text{C}$ . Therefore, the phenotype of maize is influenced by an increase or decrease in temperature.

To date, no studies have been conducted on maize's epidemiological leaf rust disease. Therefore, the importance of this study is to fill the gap in knowledge of diseases epidemiology on maize to identify the causes of leaf rust that attack in two research locations, namely Bayur and Muang Dalam, Lempake, Samarinda, Indonesia. Also, to examine the relationship or model between ecological factors (temperature, humidity and soil fertility) and the intensity of leaf rust disease as well as its infection rate. Furthermore, the calculation of the ecosystem monitoring model and damage level was performed as a measurement reference used as an early warning system in the initial control steps.

## MATERIALS AND METHOD

### Location of the research

This study was conducted on dry land maize plants in Bayur (latitude of  $-0.4736368$  and longitude of  $117.1645419$ ) and Muang Dalam Village (latitude of  $-0.408272$  and longitude of  $117.1645419$ ), Lempake, Samarinda, East Kalimantan, Indonesia. Also, at Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Mulawarman. In addition, soil fertility was analyzed at Laboratory of Soil Science, Faculty of Agriculture, Universitas Mulawarman.

### Research design

#### Survey

A survey was conducted from January to April 2019 to determine the sampling sites. The first location was a maize field in Bayur Village, Lempake, which had previously been planted with long beans and had 144 maize

plants (Bonanza F1 varieties). Meanwhile, the fertilizers and pesticides used for maize crops are NPK as well as Basmilang, respectively. The second sampling location was a maize field in Muang Dalam, Lempake, with a population of 100 maize plants (Bonanza F1 varieties)



a.

and had previously been planted with paddy. The fertilizers used included NPK, and the pesticides are Toxafine and Gramoxone. The sites' conditions are shown in Figure 1, and the distance between Bayur and Muang Dalam Village is 6 km.



b.

Figure 1. Maize plantation in Bayur (a), Muang Dalam (b)

This disease was identified in the laboratory, where pustules from infected leaves were scrapped using a needle sprayed with alcohol. After which, there were placed on an object glass and given a drop of methylene blue and safranin. The morphology of the pathogen was observed using an optical microscope.

#### *Observation parameters*

Using a wet-dry ball thermometer, the temperature and humidity were measured daily at the two locations starting from 1st week after planting. The data were collected three times a day, including morning 6 AM, afternoon 12 AM and evening 6 PM. Furthermore, the pathogen was identified in the Laboratory of Plant Protection, Faculty of Agriculture, Universitas Mulawarman by scraping the pustule on the surface of the maize leaf using an ooze needle. Afterward, there were placed on a glass object and observed under the microscope. The soil nutrient and pH analysis were conducted in the Laboratory of Soil Science, Faculty of Agriculture, Universitas Mulawarman. The method used is total N (Khejdhal Method), potential-P (Extraction of HCl 25% using Bray or Olsen methods), available-K (1 N NH<sub>4</sub>OAc pH7) and soil pH (Electrometric Method using pH meter Hanna type HI 8424, by comparison, the liquid 1 : 2.5).

#### **Data analysis**

##### *Disease intensity*

The following formula is used to calculate the disease intensity (Equation 1).

$$I = \frac{\sum (ni \times vi)}{Z \times N} \times 100\% \quad (1)$$

Where; I represent the disease intensity (%), ni represent the numbers of plant leaves attacked, vi represent the scale of the attack, N represent the total number of leaves observed and Z represent the highest scale of attack categories

##### *Infection rate*

According to Jeffers (1965), the following formula is used to calculate the disease infection rate (Equation 2).

$$r = \frac{2.3}{t_2 - t_1} \left( \log_{10} \frac{x_2}{1 - x_2} - \log_{10} \frac{x_1}{1 - x_1} \right) \quad (2)$$

Where; r represent the infection rate, 2.3 represent the number of natural logarithmic conversion results to ordinary logarithm (Ln x = 2.3 Log x), t represent observation time interval, x<sub>2</sub> represent the proportion of diseased leaves at t<sub>2</sub>, x<sub>1</sub> represent the proportion of diseased leaves at baseline.

Table 1. Scale category of leaf rust disease (Jeffers, 1965)

Scale	Attack of the leaf area
1	1 – 5%
3	5 – 11%
5	> 11 – ≤ 25%
7	> 25 – ≤ 75%
9	> 75 – 100%

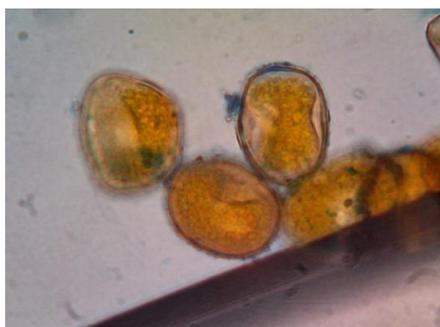
Table 2. Infection rate criteria adopted from (Jeffers, 1965)

Infection rate (unit of the week)	Criteria
< 0.11	Mild
> 0.11 – < 0.50	Moderate
> 0.50	Severe

## RESULTS AND DISCUSSION

### Fungal identification

*P. sorghi* Schw often appeared after silking in maize, having an early symptom of chlorotic specks on the leaf. Meanwhile, the obvious sign of this pathogen was golden-brown pustules or bumps on the above-ground surface of the plant tissue. These bumps were uredospores that could spread to other plants and cause further infection.



a.



b.

Figure 2. Microscopic *P. sorghi* Schw methylene blue staining (a), safranin staining (400x) (b)

Fungal spores that spread through the air landed on the surface of healthy leaves. Although they were observed 11 times, leaf rust occurred at 55 days or 9th week after inoculation, as shown in the Table 3.

The early pustules grew gradually and only existed in the margins of maize leaves, as shown in Figure 3. Pustules were circular to round and orange to brown, and they rarely grow under the surface of maize leaves. Spores spread throughout the surface of maize leaves during their harvested period. RPK (1972) and Dey et al. (2015) stated that the leaf rust disease on maize

Also, they were circular and powdery due to spores breaking through the leaf surface. The bumps are only about 1 to 2 mm each and are numerous with equal frequencies on upper and lower leaf surfaces (Burhanuddin, 2015; Dey et al., 2015).

Figure 2 shows details of *P. sorghi* Schw based on the laboratory analysis of maize rust on leaves from two areas, Bayur and Muang Dalam plantations preferring humidity > 75% and temperature > 20°C. The spores were unable to live without their host, hence, these are termed obligate parasites. First, *P. sorghi* scrubbed the pustules on the surface of maize leaves with a needle placed them on the object-glass, after which methylene blue and safranin staining was added and lastly viewed directly under a microscope.

Observations under a microscope with a 400x enlargement showed oval and round shapes. This is in accordance with Jeffers (1965), stating that the *P. sorghi* Schw uredospore was yellowish to golden colour. The walls of the spores thickened it at both ends to golden. Also, its walls are thick with 4 to 5 holes-equator. Teliospore is brown, smooth, elliptical and round at both ends.

caused by the fungus *P. sorghi* Schw occurred every growing season. Rust pustules usually occur in high relative humidity and high-temperature conditions, with characteristic chlorotic specks on the leaf surface. These soon developed into powdery, brick-red pustules as the spores broke through the leaf surface. The pustules were oval or elongated, about 1/8 inch long and scattered sparsely or clustered together. The leaf tissue around it was likely to be yellow or die, leaving lesions of dead tissue forming a band across the leaf, resulting in their death when severely infected. According to the age of the pustules,

the red spores turned black and continued to erupt through the leaf surface. Some parts which are also prone to infection are the husks, leaf sheaths and stalks (Adegbite, 2011; Suriani et al., 2019).

Table 3. The observation results on leaf rust distribution in Bayur and Muang Dalam fields

Observation week to	Number of pustules	
	Bayur	Muang Dalam
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	149	276
10	2,413	3,655
11	11,151	9,361

#### Disease intensity

Disease occurrence is the proportion of individual hosts or organs affected by the disease

regardless of how severe it might be, while its severity is the proportion of infected host surfaces to the total observed host surface, having symptoms expressed as a wide percentage to leaf surface area. Furthermore, this also refers to disease intensity (Agrios, 2005).

It has been observed that the average disease intensity ranged from 27 to 81% (Table 3), from moderate to severe criteria. The disease attacked the lower leaves until they went up, leaving the younger ones on the top. This was due to the disease's preference for humid and shady environments. Plant diseases were scored (assessed) by comparing plant symptoms to score tables from the Directorate of Protection. (Table 1).

#### The infection rate of leaf rust disease

The infection rate was obtained based on the proportion of diseased plants (percentage of disease), calculated every week according to the progression. The calculation of disease infection rates in the two research sites is shown in Table 4. Since the average rate of rust infection between the two places was not different, it implies that rust has been endemic in the area.



a.



b.

Figure 3. Early pustules appear (a), pustules spread throughout the leaf surface (b)

Table 4. Results for calculation of Bayur infection rates and Muang Dalam infection rate

Place	Infection rate (unit week)		Mean average	Mean average temperature (°C)	Mean average RH (%)
	r1	r2			
Bayur	2.887	1.953	0.739	29.12	70
Muang Dalam	2.160	1.424	1.792	29.39	70
Mean average	2.523	1.688	1.081		

Note: r1 = infection rate 1; r2 = infection rate 2; RH = relatif humidity

The observations are taken from 24 and 20 samples of plant leave in Bayur and Muang Dalam fields, respectively, which

appeared to be increasing due to environmental conditions, especially temperature and humidity. The disease's development is also aided by

significant rainfall, which causes infection to increase over time (Kinyua et al., 2011; Sopialena and Palupi, 2017).

The average infection rate in both places was more than 0.50 units week<sup>-1</sup> due to the rapid spread of spores. The attacks by the fungus of *P. sorghi* were too aggressive. Host varieties that are not resistant to disease and environmental factors support the development of pathogens. According to Jeffers (1965), the infection rate is used to identify the vulnerability of aggressive organisms, as well as the conducive status of the environment to disease development. A value of *r* greater than 0.5 units day<sup>-1</sup> (Table 2) implies that an aggressive pathogen and the host variety are susceptible to disease as well as weather support and vice versa.

The infection rate in the Bayur field at the beginning and end of the observation was 2.887 units week<sup>-1</sup> and 1.953 units week<sup>-1</sup>, respectively. Meanwhile, the level of infection in Muang Dalam at the beginning and end of the observation was 2.16 units week<sup>-1</sup> and 1.424 units week<sup>-1</sup> 4, respectively. The results above are similar to the research conducted by Thorson and Martinson (1993); Pap et al. (2013), which revealed that relative humidity of 95% was optimal for germ tube elongation and appressoria formation. It was then supported by de Nazareno and Madden (1992), stating that sporulation was high at 100% relative humidity and 25 to 30°C temperature. However, the number and

expansion of lesions were not significantly different with > 25°C temperature. Disease intensity occurred more in warm and humid conditions (Rahayu et al., 2018; 2020; Rochi et al., 2018). For example, rust disease was slow to develop when the mean daily temperature dropped below 20°C (Sucher et al., 2017).

#### Observation of these spread area

Based on the study results, pustules in leaf rust appeared in the 9th week, increasing weekly. Firstly, the pustule was visible on the edge of the leaf, then it spread to the center of the leaf. Finally, in the 13th week, its number increased to 80% of the leaves' surface covered by the rust pustule.

The symptom of *P. sorghi* Shcw pathogen attack was the absence of a golden brown pustule on the leaf's surface. Furthermore, this lump was a collection of uredospore that penetrated the leaf's surface, turning the yellow patches next to the spots to brownish. Meanwhile, the brown lump could turn blackish due to many pustules on the leaf. In stricken crops, symptoms increases covering the entire maize leaf (Menkir et al., 2006; Puspawati and Sudarma, 2016).

#### Soil analysis results

The results of soil analysis of total-N (Khejdhal Method), potential-P (Extraction of HCl 25%), available-K (1 N NH<sub>4</sub>OAc pH7) and soil pH (Electrometric Method) is shown in Table 5.

Table 5. Soil analysis of Bayur and Muang Dalam

Location	pH	Total-N (%)	Potential-P (ppm)	Available-K (ppm)		
Bayur	5.01	0.44	40.37	171.5		
Muang Dalam	5.44	0.33	35.19	261.09		
Base cation (pH 7)						
Location	Ca <sup>++</sup> me 100g <sup>-1</sup>	CEC	Base saturation (%)			
Bayur	5.4666	4.12	0.39	0.3411	15.06	66.17
Muang Dalam	6.8700	3.88	0.85	0.4731	26.40	45.75

Table 5 shows that Bayur with pH 5.01 was included in the acid category. Total-N of 0.44%, potential-P and available-K was 40.37 ppm included in the high category and 171.50 ppm included in the very high category. Ca<sup>++</sup> 5.4666 ppm was classified as moderate, Mg<sup>++</sup> 4.12 ppm was classified as high, K<sup>+</sup> 0.39 ppm was categorized as low, Na<sup>+</sup> 0.3411 was classified as low, CEC 15.60 was classified as moderate

and base saturation was 66.17% included in the high category.

In Muang Dalam, pH 5.44 was included in the acid category. Total-N of 0.33%, potential-P and available-K was 135.19 ppm included in the high category and 261.09 ppm included in the very high category. Ca<sup>++</sup> 6.87 ppm was classified as moderate, Mg<sup>++</sup> 3.88 ppm was classified as high, K<sup>+</sup> 0.85 ppm was categorized

as low,  $\text{Na}^+$  0.4731 was classified as moderate, CEC 26.40 was classified as high and base saturation was 45.75% included in the medium category.

Comparing the results of the analysis on Bayur and Muang Dalam concerning the same disease attack showed that most of the criteria of the soil sampling conditions in both lands had poor soil fertility based on criteria of chemistry and physics of soil by the Institution of Soil Research. Meanwhile, Bogor and were not included in the categories and criteria for growing conditions of maize. Therefore, poor soil fertility affects the health condition of maize plants.

The soil was managed effectively to maintain its fertility by having a good understanding of the environmental condition of the soil in terms of its physical, chemical and biological components (Rosfiansyah and Sopialena, 2018). The more fertile the soil, the healthier the plant, and its resistance to pests and diseases is reduced. Therefore, good soil fertility inhibits the spread of leaf rust disease. Also, in accordance with the criteria for growing conditions, it spurs plants to protect themselves against disease due to adequate nutrition from the soil (Frac et al., 2018).

Similar research on the application of chemical fertilizers significantly affected GLS progress (Graef et al., 2018). Also, they reported that the GLS epidemic was significantly higher in non-fertilized plots than in fertilized plots. They also observed that a single application

of nitrogen increased the predisposition of plants toward GLS. However, a combined application of nitrogen and phosphorus at a recommended level significantly reduced the predisposition effect of a high nitrogen level. The unbalanced use of nutrients results in its deficiency in the host and loss of resistance status predisposed the plants to GLS (Graef et al., 2018). Subsequently, Dubey et al. (2019) and Panth et al. (2020) states that soil fertility increases the plant's resistance to pathogens.

#### Relationship between temperature, humidity against disease development

The observations and calculations showed the relationship between temperature and humidity factors on disease development. According to the regression results, the humidity and temperature factors were dominant and the regression equation  $Y = 0.062x + 25.17$  demonstrated a significant link with disease development factors, indicating a relationship between temperature and disease intensity (Figure 4). Meanwhile, the equation  $Y = 0.809x + 35.09$  showed the relationship between humidity and the intensity of the disease (Figure 5). These results were used to predict the development of leaf rust disease in maize plants. However, the two regressions, with an  $R^2$  value of 0.76 that shows the relationship between temperature and disease intensity and an  $R^2$  of 0.87 that shows the relationship between humidity and disease intensity, were significantly different.

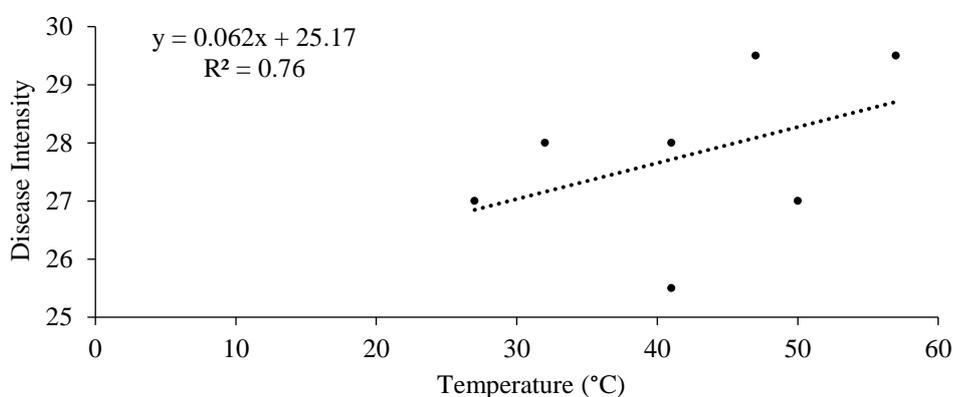


Figure 4. Regression relationship between temperature and intensity of disease

According to Figure 4, the average temperature during the three months observation was 29.120°C, while the emergence of leaf rust symptomatic plants was first characterized by pustules when the average

daily temperature reached 27°C with an average daily humidity of 64%, totaling 4 plants from 24 plant samples in Bayur. In Muang Dalam, at first, the emergence of leaf rust symptomatic plants was also characterized by the appearance

of pustules occurring when the average daily temperature reached 27°C with the average daily humidity of 78%, totaling 4 plants from

20 plant samples. Then, high average humidity leveled up to > 80%, which contributed to the appearance of leaf rust.

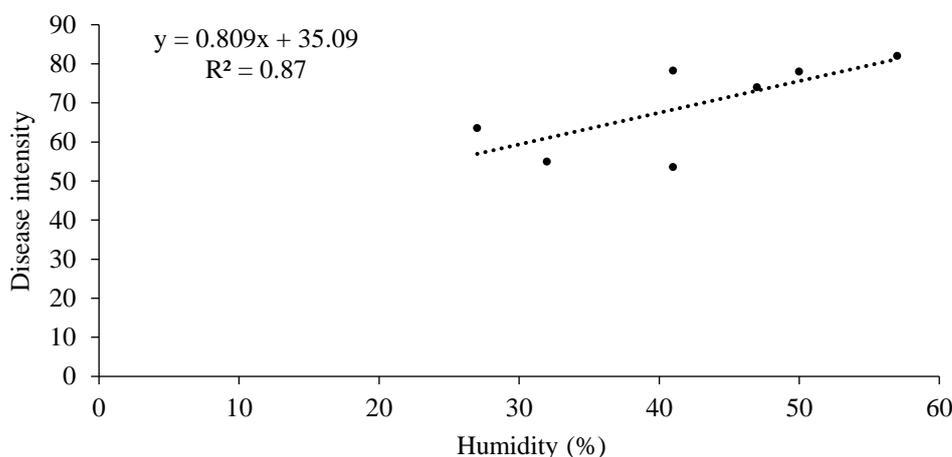


Figure 5. Regression relationship between humidity and intensity of disease

## CONCLUSIONS

It is concluded that the fungus causing leaf rust disease in maize is *P. sorghi* Schw. The average rate of rust infection between Bayur and Muang Dalam Village, Lempake, was not different. Temperature and humidity play a role in the development of leaf rust diseases. However, humidity plays a more important role. The higher the temperature accompanied by the increased humidity, the more leaf rust disease develops. Therefore, good soil fertility in accordance with the criteria for growing conditions spurs plants to protect themselves against disease by adequate soil nutrition.

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