Maize Downy Mildew and Effects of Varieties and Metalaxyl

Identification of Maize Downy Mildew Pathogen in Lampung and the Effects of Varieties and Metalaxyl on Disease Incidence

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ABSTRACT

Maize downy mildew (MDM) is concidered as a major problem in all maize growing areas in Indonesia including the Province of Lampung. The objectives of this study were (i) to identify the species of *Peronosclerospora* causing maize downy mildew (MDM) in Lampung, (ii) to determine the influence of varieties on the intensity of downy mildew and (iii) to determine the efficacy of metalaxyl to control MDM on some maize varieties. To identify *Peronosclerospora* causing MDM, the pathogens were observed under light microscope and scanning electrom microscope. Maize varieties response against pathogens and efficacy metalaxyl were researched in the field with the test plants exposed to plants showing MDM symptoms as the sources of inocula to mimic natural conditions. Three species of *Peronosclerospora* were found, i.e. *P. sorghi, P. maydis,* and *P. philippinensis*. *P. sorghi*, *P. maydis*, and *P. philippinensis*. However, the presence of the *Peronosclerospora* species reported here is tentative pending further studies with molecular techniques. On both varieties Pioneer 27 (P-27) and NK-22, AUDPC on F1 plants was greater than that on F2 plants. On P-27, the production of F2 was higher that that of F1 plants, but there is no significant difference in production between F1 and F2 of NK-22 variety. Seed treatment using metalaxyl was not effective to control downy mildew of maize.

***Keywords**:* maize downy mildew, metalaxyl, mildew, *Peronosclerospora sorghi, P. maydis, P. philippinensis*

INTRODUCTION

Downy mildews have been reported to cause crop diseases in many countries (Crandall et al., 2018; Rashid et al., 2013). Maize downy mildew (MDM) is a serious disease affecting maize production in many parts of Indonesia, including the Province of Lampung (Muis et al., 2016; Widiantini et al., 2015; Hikmahwati et al., 2011; Semangun, 2004). Infected maize plants show two types of symptoms, i.e. systemic symptom and local symptom. The systemic symptom occurs when the invading pathogen has reached its growing point (that is located at the top of the stalk tissue of the plant). In infected young plants, newly formed leaves show symptoms of small chlorotic spots. These patches develop into parallel with the leaf bone. The affected plants become dwarf. If the pathogen does not reach the growing point, local symptoms will occur in the form of chlorotic lines on the leaves. In the morning, there is a layer of white velvet, consisting of conidia and conidiophores of the fungus, on the underside of the affected leaf (Semangun, 2004).

MDM in Indonesia is caused by three *Peronosclerospora* species, i.e. *P. maydis, P. sorghi,* and *P. philippinensis* (Hikmahwati et al., 2011; Muis et al., 2016). Previously, *P. maydis* was reported to be restricted to Indonesia and Australia, while *P. sorghi* distributed worldwide including America, Asia, Africa, Europe and Australia. Janruang & Unartngan (2018) reported that *P. maydis* was found in several locations in Thailand. *P. philippinensis* is widely distributed in Asia (International Maize and Wheat Improvement Center [CIMMYT], 2012). In Lampung, the causal agent of MDM was reported to be *P. sorghi* (Muis et al., 2016) and *P. maydis* (Muis et al., 2013; Semangun, 2014). In Central Lampung, the causal agent of MDM was reported to be *P. sorghi* (Muis et al., 2016), *P. maydis* (Muis et al., 2013). It was probably both spesies of *Peronosclerospora* existed in Central Lampung. More extensive survey should be conducted to determine what spesies of *Peronosclerospora* causing MDM in Lampung.

MDM is often controlled by planting resistant varieties and/or preplanting seed treatment using the synthetic fungicide metalaxyl (Semangun, 2004). The use of fungicide metalaxyl has caused the emergence of resistant *Peronosclerospora* population (Widiantini et al., 2015; Surtikanti, 2013; Isakeit & Jaster, 2005). The occurrence of metalaxyl resistance was also reported in other pathogens. For example, out of 116 isolates of *Phytophthora infestans* causing potato late blight tested for metalaxyl resistance, 25.9% were found to be resistant, 19.8% was intermediate and 54.3% was sensitive to metalaxyl (Aav et al., 2015).

Thus, maize plants are often damaged due to MDM although hybrid improved maize seeds are planted. In addition, farmers often use F2 seeds that are harvested from original F1 hybrid seed that may be considered expensive. Thus, it should be ascertained whether the response of various varieties and their progenies (F1 and F2) are different against pathogens that exist in the field. In addition, it is necessary to assess whether a specific variety or the progenies showed different responses to metalaxyl.

The objectives of this study were to identify the species of *Peronosclerospora* causing MDM in the Province of Lampung, to determine the influence of maize variety and its progeny on the intensity of MDM, and to determine the efficacy of metalaxyl to control MDM on some maize varieties.

METHODS

The first part of this research was identification of the pathogen causing MDM in all major maize production areas in the Province of Lampung. In addition, three experiments were conducted from April 2016 to May 2017. They were (1) the effect of maize variety and its progeny on MDM, (2) the efficacy of metalaxyl on NK-22 F1 and F2, (3) the efficacy of metalaxyl on P-27 F1 and F2.

Identification of *Peronosclerospora* Species Causing MDM in Lampung

First, maize plants in vegetative phase showing systemic or non-systematic symptoms were observed in eight regencies or city in Lampung, namely Central Lampung, South Lampung, East Lampung, North Lampung, West Tulang Bawang, Bandar Lampung, Pasawaran, and Pringsewu. Efforth was made to take plant samples from two fields from each regency/city and to take two plants from each field.

Plant samples were uprooted with the soil around the base of the stem and then was placed into polybags individually. Each plant was covered with transparent plastic bag to reduce stress during transportation to the laboratory and to avoid the pathogen spread among the sample plants. During the identification process in the laboratory, the plants were placed individually in a 1-m high plastic enclosure also to prevent pathogens from spreading among the sample plants.

Pathogen sporulation – in which timing is crucial - was stimulated by modified procedure from Rustiani et al. (2015). A maize plant in a polybag was transferred to the laboratory. In the afternoon in the laboratory, the third leaf from the shoot with the symptoms was washed by rubbing the leaves with two fingers while rinsed with running

water and then dried with tissue paper. The washing was aimed to keep moisture and ensure clean stomata from dirts and fungus propagules. Then, the infected maize plant was covered with transparent plastic and placed in the room at 17 ^{o}C and incubated for ± 7 hours. At 04.00 am, conidia were harvested by scraping the whitish layer on the underside of the leaf and placed on an object glass. The observations were performed under a light microscope to measure the length and width of the conidia and the length and number of branching levels of conidiophores.

Observation was also conducted with scanning electron microscope (SEM) at the Integrated Laboratory and Technology Innovation Center (LTSIT) at University of Lampung with the following procedure. The procedure was started by taking leaf sample showing MDM symptoms at around 01.30. Prior to analysis, the sample was dried and free from moisture. The leaf sample was placed on a double-glazed cell holder called carbon type, then coated with gold metal in vacuum condition with a sputter coater that sample coating to strengthen the conduction properties. Coating was done for 60 seconds. After that, the coated sample was mounted in the stage holder and inserted into the SEM chamber. Then, SEM ZEISS EVO MA 10 was used to take pictures.

Oospore observation was done by scraping leaves showing advanced symptoms using scalpel and placing the the results of the scraping on a drop of water on a glass object. The observation was made under light microscope. Identification was based on the description outlined by CIMMYT (2012), Hikmahwati et al. (2011) and Ahmad et al. (1994).

Effect of Maize Variety and its Progeny on MDM

Treatments were arranged in a randomized block design with four treatments and four blocks (replications). The varieties used were NK-22 (F1 and F2) and P-27 (F1 and F2). NK-22 and P-27 were popular among maize growers in Lampung.

For the preparation of this study, the land was cleared in advance of the remnants of weeds and other plants, then the soil was dug as deep as ± 20 cm to destroy chunks of soil and leveling of land that has been dug. Further process was tilling so that the soil became loose and to make plots of 2 x 2 m with a distance of 0.75 m between plots. Crop rows were 25 cm appart and three maize seeds were planted at each planting hole in anticipation of not all seeds grew well. After the plants were 5 days old, thinning was done to leave one plant per hole.

Fertilization was done twice. The first fertilization was done with half-dose of urea and all dose of TSP and KCl 2 weeks after planting. The second fertilization was

done with the remaining half dose of urea 6 weeks after planting. The dose of the fertilizer was that urea fertilizer at 300 kg/ha, SP-36 200 kg/ha, and KCl 50 kg/ha. Thus, the fertilizer needs of plants per plot with a size of 2 m x 2 m namely urea of 120 g/plot, SP-36 of 80 g/plot, KCl of 20 g/plot.

Variables to be measured were disease incidence and disease severity, length and width of stomata, the number of stomata, and production. Data was analyzed by analysis of variance and followed by least significant difference test (LSD) at 5% level.

Inoculation was done with a procedure to mimic natural field conditions (Semangun, 2004). For that, two plants showing MDM symptoms were planted in consistent pattern among experimental plots 7 days after planting. The plants with symptoms used as the source of inoculum were positioned so that all plants had similar chance of being inoculated naturally. Caring for the plants included watering of plants after planting maize seeds and weeding that was done manually by removing the weeds growing around the planting area.

To measure the disease incidence and severity, observations were made every week after planting for 5 weeks. Disease incidence was calculated with the formula of I = n/N x 100% with I = disease incidence (%), n = number of plants showing symptoms, and N = total number of plants observed. Disease severity was calculated using modified procedure of Pataky et al. (1998), namely: $S = \Sigma (n_i x v_i)/(N \times V) \times 100\%$ with S = severity of disease, n_i = number of plants from each category of attack, v_i = value score for each category of attack, N = the total number of plants observed, and V = the value of the highest score of the disease. The scale values of each category were as follows: 0 = no symptoms observed, 1 = when <10% of leaf surface showed the symptoms, 2 = when 10-25% showed the symptoms, 3 = when 25-50% showed the symptoms, and 4 = when more than 50% of leaf surface showed the symptoms.

AUDPC and infection rate (r) were calculated by using data on disease severity. The area under the disease progress curve (AUDPC) was calculated with following function (Shaner & Finney, 1977):

$$AUDPC = \sum_{i=1}^{n-1} \left[(X_{i+1} + X_i)/2 \right] \times \left[t_{i+1} - t_i \right]$$

with Xi = disease severity on the i^{th} date; ti = time (days) at the i^{th} observation; and n = total number observation. The infection rate was calculated with following function (van der Plank, 1963):

$$r = \frac{1}{t_2 - t_1} \left(-\ln\left(-\ln\left(X_2\right)\right) + \ln\left(-\ln\left(X_1\right)\right) \right)$$

with X_1 and X_2 = disease severity at dates of measurement, t_1 and t_2.

Observation of the stomata was carried out to determine whether the number and size of stomata affected the MDM intensity. The observation was done when the plants were 2 weeks old by taking the third leaves of two plants per plot. To make stomata preparations, leaf cuts were taken at two points of the third leaf of each plant. Then the lower surface of the leaf was spread with clear nail polish and left to dry. Polish exfoliated with insulation and affixed to glass slide and then observed to determine the number of stomata in 1 mm2, and the length (µm) and width (µm) of the stomata.

To measure production variable, maize plants were harvested 3 months after planting when the maize husk turned brown. Harvesting was done by opening the husk and then picking maize cobs. Maize cobs then separated per plot. After harvesting, the maize cobs were dried and weighed.

The Efficacy of Metalaxyl to Control MDM

To test the efficacy of metalaxyl to control the disease, two separate experiments were conducted. In the first experiment, F1 and F2 of NK-22 were tested, while in the second one, F1 and F2 of P-27 were used. Soil tillage was done as mentioned above. The experimental design used was randomized block design with four replications. The treatments were arranged factorially with two factors, i.e. NK-22 at two levels (F1 and F2) and metalaxyl in the Saromyl 35 SD trademark formulation in at three levels (0; 1.25; and 2.5 g/kg of seed). According to the recommendations on Saromyl 35 SD label, the dose for maize seed treatment is 1.25 g / kg of seed. One experimental unit consisted of one plot with 18 maize plants.

Before planting, maize seeds were treated in accordance with the predetermined metalaxyl dose. The maize seeds were planted to the depth of 3-4 cm with spacing of 25 x 75 cm. Three maize seeds were planted at each planting hole in anticipation of not all seeds grew well. After the maize seeds grew, thinning was done to leave one plant per planting hole so that there were 18 plants per experimental plot.

After the maize seed was planted, when the soil was dry, the plants were watered using tap water that had been provided. Fertilization was done as described above. Similarly, the observed variables and observation and data analysis were similar to previous experiment described above.

In the second experiment, maize variety of P-27 (F1 and F2) was used. The experimental method was the same as that in the first experiment except that P-27 was used.

RESULTS AND DISCUSSION

Peronosclerospora Species Causing MDM in Lampung

The morphological characteristics and morphometry of *Peronosclerospora* are presented in Table 1. The results of this research showed that there were three species of *Peronosclerospora* found attacking maize plants di the Provine of Lampung, i.e. *P. sorghi, P. maydis,* and *P. philippinensis*. *P. sorghi, P. maydis,* and *P. philippinensis* was found in 6, 5, and 2 regency/city of the Province of Lampung. From each district/field, one to three species could be found (Table 2). The morphological illustrations that were observed under light microscope and SEM could be seen in Figure 1. This was in agreement with the results of observations made by Muis et al. (2016). *P. australiensis* infecting maize in Australia (Shivas et al., 2012) has not been reported in Indonesia.

All *P. sorghi* and *P. philippinensis* isolates produced oospores that were spherical or subspherical. The oospore wall of *P. philippinensis* was slightly smoother than that of *P. sorghi* (Figure 2). No *P. maydis* isolate was observed to produce any oospore. This was in agreement with the morphological characteristics reported by CIMMYT (2012) suggesting that *P. maydis* was observed not to produce any oospore.

There were few problems in identification of what species of *Peronosclerospora* infecting maize in Lampung. Some characteristics were overlapping in that some isolates produced oval and ovoid to cylindrical (Table 1; Figure 1 g). Similar results were found by Bock et al. (2000) who found significant different isolate morphology of *P. sorghi* isolated from different geographic locations and hosts in Africa. In addition, the size of spherical conidia found varie d. For example, the average diameter of spherical conidia found in plant 1 grown in Hajimena Bandar Lampung City was 12.9 µm, while that of spherical conidia from plant 3 grown in Trimurjo Central Lampung was 21.9 µm. This range was different from that of conidial size proposed in CIMMYT (2012). This identification was not in total agreement with Widiantini et al. (2015) who proposed that conidial with oval shape to be *P. maydis.*

Therefore, the presence of the *Peronosclerospora* species reported here is tentative pending further studies with molecular techniques. We suggest that further studies are to be conducted to identify the species based on the results of morphological and DNA sequence observations (Janruang and Unartngam, 2018). In addition, in further studies more attention should be given to sexual structures such as oogonia and oospora and/or molecular methods as suggested by Shivas et al. (2012).

Inoculation carried out by using symptomatic plants as the source of inoculum resulted in that some inoculated plants showed symptoms of MDM since the plants

were 1 week old. As the plants were older, more plants showed symptoms. At first maize leaves showed symptoms of chlorosis on young leaves. Chlorotic symptoms expanded to all the leaves and form lines parallel to the veins. On the lower surface of the leaves, there was white layer which consisted of *Perenosclerospora* conidiophores and conidia. Some attacked plants did not produce any cob, while some diseased plants produced cobs with fewer kernels than that formed in healthy plants.

Effect of Maize Variety and its Progeny on MDM

In general, data of disease severity, AUDPC, and infection rate (r) were in line (Table 3). On both varieties P-27 and NK-22, disease severity, AUDPC, and r on F1 plants was greater that that on F2 plants, except that in NK-22 both disease severity and r were not significantly different. The smallest AUDPC occurred on P-27 F2 although it was not significantly different from that in NK-22 F2. The greatest AUDPC was on NK-22 F1 alhthough it was not significantly different from that in P-27 F1. The greatest r occurred on NK-22 F1 although it was not significantly different from that on NK-22 F2 and P-27 F1. The smallest r occurred on P-27 F2 although it was not significantly from those in P-27 F1 and NK-22 F2. The graphic of AUDPC of NK-22 and P-27 both F1 and F2 could be seen at Figure 3 (a, b).

The number of stotama was different among varieties, while the length and width of stomata hole were not significantly different between different types of plants tested in this study (Table 4). It seems that stomata did not affect the occurrence or severity of the disease. This conclusion was drown from the fact that, while the P-27 F1 plant with relatively high intensity of the disease had fewer than the number of stomata NK-22 F1 plants with relatively high intensity, P-27 F2 plants with low intensity of the disease had stomata at most (Table 4). However, Pudjiwati et al. (2013) found that, in conjunction with the maize breeding program, low density trichomes and stomatal density characters increase resistance to MDM. More observations are needed to confirm these data.

As of production variable, the variety significantly affected the production of maize. In general the influence of varieties on the production was in line with the influence of varieties on the intensity of the disease that was the lower the intensity of the disease in a variety of the higher production. On both P-27 and NK-22, the production of F2 was higher that that of F1 plants (Table 3). When released, P-27 was resistant to MDM, while NK-22 was susceptible. However, both varieties were heavily damaged by the disease. In addition, metalaxyl that was effectively control MDM beforehand becoming ineffective (Molinero-Ruiz et al., 2008; Rashid et al., 2013). In

the future, more breeding programs should be conducted to find more alternative lines or varieties in order to control the disease. Wiser et al. (2005) showed that the genetics of resistance of maize was very complex and suggested that certain breeding schemes may be more suitable for certain diseases. Rashid et al. (2013) implemented several shcemes to evaluate or to screen the resistance of maize cultivars or lines to MDM.

The Efficacy of Metalaxyl to Control MDM

The results of the second experimental showed that up to 2.5 g per kg seed, metalaxyl was not effective to control MDM on NK-22 both F1 and F2 (Table 5). Other researchers reported the same fenomena that metalaxyl was not effective to control MDM (Aav et al., 2015; Widiantini et al., 2015; Surtikanti, 2013; Isakeit & Jaster, 2005).

The problem of fungal pathogen resistance to fungicides is not uncommon in other cases and the use of molecular techniques developed to detect the occurance of resistance of fungal pathogens to fungicides (Ishii, 2006) should also be developed to detect the incidence of *Peronosclerospora* resistance to metalaxyl. This could contribute to formulate integrated control of MDM.

Metalaxyl and other phenylamide fungicides are site-specific fungicides and inhibit the polymerization of r-RNA. The use of these fungicides frequently selected resistant population pathogens. There is high risk for emergence of resistance in all oomycetes as only one or two semi-dominant genes involve in probably monogenic mechanism (Gisi & Sierotzki, 2008).

In addition, NK-22 F1 was more susceptible than NK-22 F2 to the pathogen as shown by AUDPC on all NK-22 F1 and NK-22 F2 plants. Apparent infection rate was not defferent among treatments (Table 5). This was in line with the results of the first experiment in that F2 varieties were better that F1 in resistance to MDM.

The third experiment resulted that P-27 F1 plants was more susceptible than P-27 F2 plants as shown disease severity and AUDPC, although the r was not significantly different (Table 6). Metalaxyl generally was not effective to control downy mildew on Pioneer plants, except that P-27 F1 treated with 2 g metalaxyl per kg seed reduced AUDPC if compared by F1 plants untreated with the fungicide. Apparent infection rates on all F1 plots were greater than those on all F2 plots. The graphic of AUDPC of NK-22 and P-27 both F1 and F2 plants treated with metalaxyl could be seen at Figure 3 (c, d, e, f).

Metalaxyl resistance pathogens also occurred in some other diseases (Gisi & Sierotzki, 2008; Molinero-Ruiz et al., 2008). Molinero-Ruiz et al. (2008) reported that the use of metalaxyl to *Plasmopara halstedii* causing downey mildew in sunflower was stopped due to the emergence of resistant population and was substituted by metalaxyl-M (mefenoxam). After several years, resistant *P. halstedii* population occurred and should be considered in disease management.

CONCLUSIONS

Based on the results of this research, we conclude that MDM in the Province of Lampung was caused by *P. sorghi, P. maydis,* and *P. philippinensis*. *P. sorghi* and *P. maydis* were found more frequently and had wider distribution than *P. philippinensis.* However, the results of this identification is tentative pending further studies with molecular techniques. In both P-27 and NK-22, F1 plants were more susceptible to MDM compared to F2 plants. Metalaxyl was not effective to control MDM.

ACKNOWLEDGEMENTS

We thank to Reasearch Insitute of the University of Lampung for providing grant for this research. We also thank to Ir. Dad Resiworo J. Sembodo, M.S. whose farm was used to do the field experiment.

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Table 1

Morphology and morphometry (µm) of conidiophores and conidia of Peronosclerospora *from diseased maize plants*

Field Location	Plant	Conidiophore Length Average (Range) (µm)	Bran-Ching Level	Conidia Form	Width Average (Range) (µm)	Length Average (Range) (µm)
Bandar Lampung City						
Hajimena	1	244.2 (217.0-299.0)	3	Spherical	12.9 (10.9-14.9)	14.2 (11.5-15.9)
	2	276.2 (224.0-340.0)	2 & 3	Oval	16.3 (11.7-21.5)	20.4 (12.4-33.0)
Rajabasa	1	288.3 (250.0-396.0)	3 & 4	Sperical, Subspherical	16.5 (13.8-18.4)	17.4 (14.6-19.4)
South Lampung Regency						
Sidomulyo	1	242.4 (197.0-281.0)	2 & 3	Oval	14.6 (10.8-17.9)	17.9 (13.7-22.7)
	2	224.6 (144.0-265.0)	2 & 3	Oval	16.5 (12.3-19.3)	19.9 (14.7-24.4)
T. Bintang	1	211.0 (184.0-244.0)	2 & 3	Oval	12.9 (10.9-15.2)	17.2 (12.4-19.6)
Central Lampung Regency						
B. Jaya 1	1	269.3 (204.0-307.0)	3 & 4	Spherical Subspherical	14.9 (10.9-14.9)	16.4 (12.2-20.8)
	2	272.5 (238.0-318.0)	3 & 4	Spherical	16.0 (11.0-19.5)	16.6 (11.9-20.3)
B. Jaya 2	1	295.2 (222.0-367.0)	3 & 4	Spherical Subspherical	16.0 (14.0-18.0)	17.1 (15.3-19.5)
Trimurjo	1	211.8 (174.0-250.0)	3	Oval	17.6 (14.3-20.2)	22.8 (20.6-26.6)
	2	215.2 (185.0-252.0)	3	Oval to cylindrical	17.2 (14.8-19.2)	22.7 (21.1-24.4)
East Lampung Regency						
Pekalongan	1	204.6 (160.0-266.0)	2 & 3	Oval	16.4 (15.7-17.3)	19.7 (17.7-21.2)
S. Nuban	1	226.2 (198.0-260.0)	2	Oval	20.7 (17.8-23.0)	23.2 (20.2-24.9)
North Lampung Regency						
Abung Jaya	1	318.2 (246.0-359.0)	3 & 4	Spherical	19.4 (17.7-21.4)	20.1 (18.9-22.3)
	2	270.6 (211.0-346.0)	3	Spherical	15.0 (11.3-17.6)	16.4 (11.7-19.9)
B. Umpu 1	1	279.8 (225.0-325.0)	2, 3, & 4	Ovoid to cylindrical	16.0 (12.7-18.1)	23.0 (18.4-28.2)
B. Umpu 2	1	253.0 (203.0-281.0)	3	Spherical	16.4 (13.1-18.8)	17.4 (13.7-20.3)
Pesawaran Regency						
Tegineneng	1	242.2 (181.0-341.0)	2 & 3	Oval	16.0 (12.9-19.0)	18.4 (13.1-21.0)
Trimulyo	1	452.4 (286.0-578.0)	3 & 4	Spherical	21.9 (18.8-25.1)	22.6 (19.8-26.0)
Tulang Bawang Barat Regency						
G. Timbul	1	384.2 (224.0-473.0)	3	Spherical	17.5 (15.5-19.2)	18.3 (15.7-19.2)
Pringsewu Regency						
Srikaton	1	279.4 (180.0-422.0)	3	Oval	18.3 (15.0-19.9)	21.4 (15.9-25.5)
	2	271.9 (189.0-387.0)	3 & 4	Spherical Subspherical	20.3 (19.1-21.6)	21.4 (20.3-23.5)

Table 2

Spesies of Peronosclerospora *identified from specimen of diseased maize plants in the Province of Lampung*

Regency/City	District/Field	Plant	Species
Bandar Lampung	Hajimena	1	*P. maydis*
		2	*P. sorghi*
	Rajabasa	1	*P. maydis*
South Lampung	Sidomulyo	1	*P. sorghi*
		2	*P. sorghi*
	T. Bintang	1	*P. sorghi*
Central Lampung	B. Jaya 1	1	*P. maydis*
		2	*P. maydis*
	B. Jaya 2	1	*P. maydis*
	Trimurjo	1	*P. sorghi*
		2	*P. philippinensis*
		3	*P. maydis*
East Lampung	Pekalongan	1	*P. sorghi*
	S. Nuban	1	*P. sorghi*
North Lampung	Abung Jaya	1	*P. maydis*
		2	*P. maydis*
	B. Umpu 1	1	*P. philippinensis*
	B. Umpu 2	1	*P. maydis*
Pesawaran	Tegineneng	1	*P. sorghi*
Tulang Bawang Barat	G. Timbul	1	*P. maydis*
Pringsewu	Srikaton	1	*P. sorghi*
		2	*P. maydis*

Table 3

Disease severity, AUDPC, infection rate (r), and production of maize F1 and F2 of NK-22 and P-27

Veriety	Disease severity (%)	AUDPC	r	Production (g)
P-27 F2	11 a	0.04 a	0.02 a	6.17 b
NK-22 F2	63 b	0.19 ab	0.06 ab	3.96 ab
P-27 F1	59 b	0.25 bc	0.06 ab	1.33 a
NK-22 F1	75 b	0.30 c	0.07 b	3.29 a

Note.Numbers at one collum followed by the same letter are not different according to LSD (≤5%).

Table 4

The number of stomata and their size formed by NK-22 dan P-27 F1 dan F2 maize

Treatment	Number of Stomata per mm^2	Lengt of Stomata hole (µm)	Width of Stomata hole (µm)
NK-22 F1	71.50 b	32.89 a	13.10 a
NK-22 F2	67.75 ab	34.36 a	13.96 a
P-27 F1	62.75 a	34.39 a	12.61 a
P-27 F2	81.75 c	32.21 a	12.99 a

Note. Numbers at one collum followed by the same letter are not different according to LSD ($\leq$5%).

Table 5

Disease severity, AUDPC and infection rate (r) of downy mildew of F1 and F2 NK-22 maize variety treated with metalaxyl at three concentration levels

Variety and Metalaxyl Concentration	Disease severity (%)	AUDPC	r (unit/day)
NK-22 F2 2.5 g	49 a	0.14 a	0.05
NK-22 F2 1.25 g	55 ab	0.18 a	0.06
NK-22 F2 0 g	57 ab	0.19 a	0.06
NK-22 F1 1.25 g	77 c	0.30 b	0.09
NK-22 F1 2.5 g	69 bc	0.28 bc	0.07
NK-22 F1 0 g	80 c	0.34 c	0.09

Note. Numbers at one collum followed by the same letter are not different according to LSD (≤5%).

Table 6

Disease severity, AUDPC and infection rate (r) of downy mildew of F1 and F2 P-27 maize variety treated with metalaxyl at three concentration levels

Variety and Metalaxyl Concentration	Disease severity (%)	AUDPC	r (unit/day)
P-27 F2 2.5 g	4 a	0.01 a	0.01 a
P-27 F2 1.25 g	4 a	0.01 a	0.01 a
P-27 F2 0 g	7 a	0.03 a	0.01 a
P-27 F1 1.25 g	77 b	0.35 b	0.08 b
P-27 F1 2.5 g	79 bc	0.36 bc	0.08 b
P-27 F1 0 g	88 c	0.41 c	0.08 b

Note. Numbers at one collum followed by the same letter are not different according to LSD ($\leq$5%).

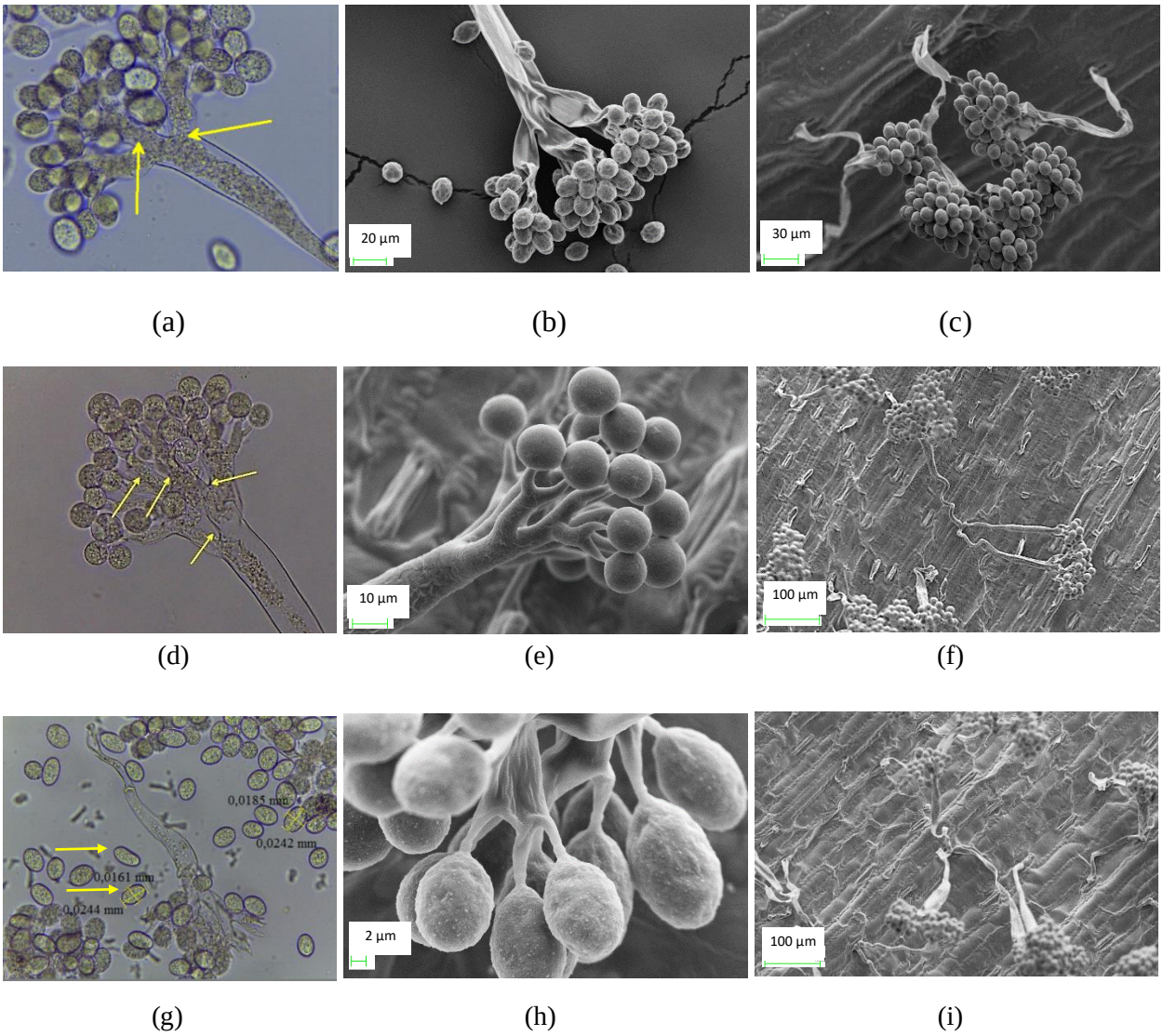
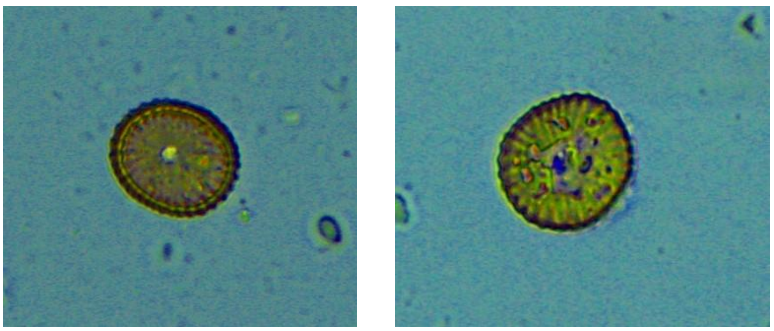
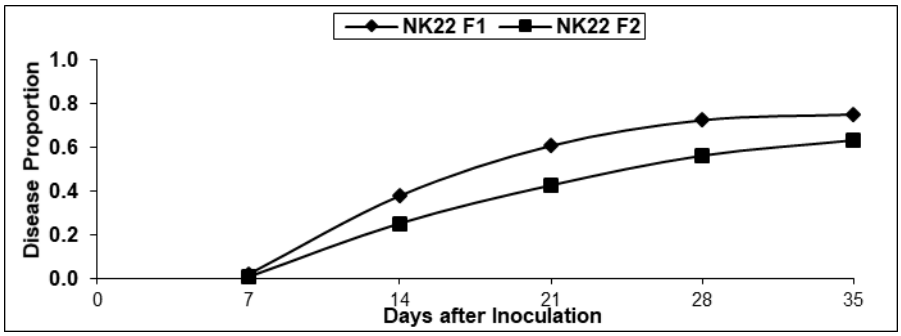


Figure 1. Conidia and conidiophores of *Peronosclerospora*: *P. sorghi* (a, b, c), *P. maydis* (d, e, f), and *P. philippinensis* (g, h, i) observed under light microscope (a, d, g) or scanning electrom microscope (b, c, e, f, h, i). Arrows in (a) and (d) show type of branching and arrows in (g) show cylindrical conidia. Conidiophores emerge singly (c) or in group (f, i) from stomata

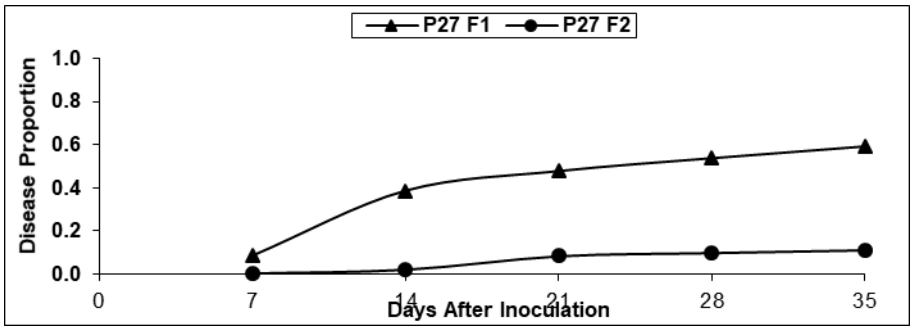


(a) (b)

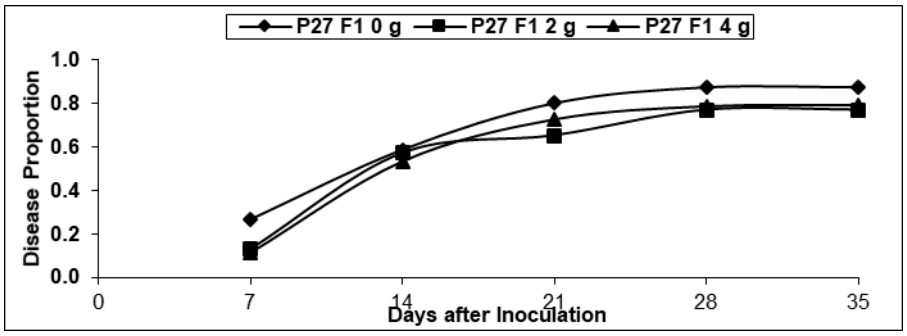
Figure 2. Oospores of *Peronosclerospora*: *P. sorghi* (a) and *P. philippinensis* (b)


NK22 F1
NK22 F2
Disease Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
Days after Inoculation

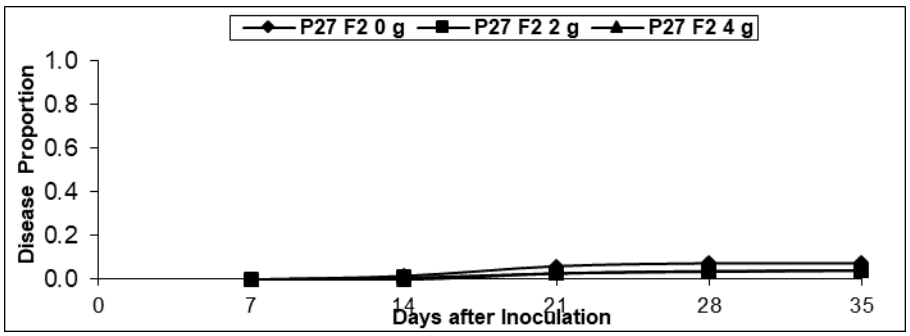
(a)


P27 F1
P27 F2
Disease Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
Days After Inoculation

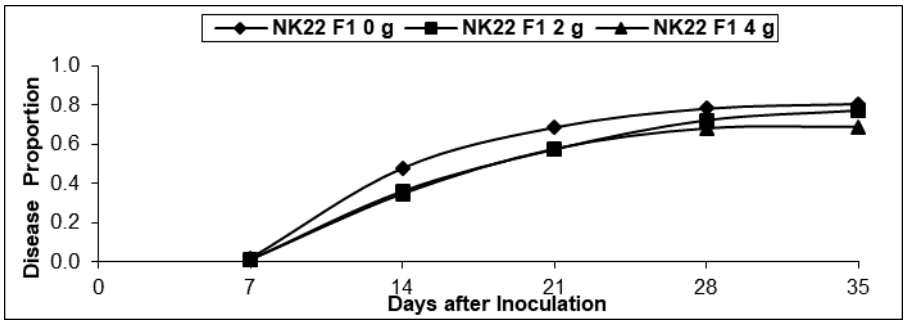
(b)


P27 F1 0 g
P27 F1 2 g
P27 F1 4 g
Disease Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
Days after Inoculation

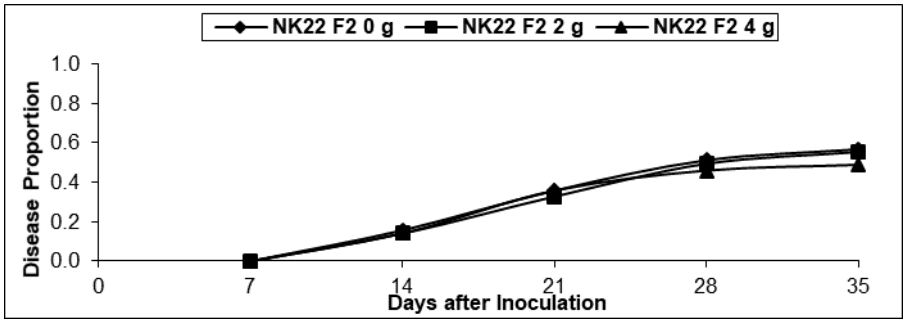
(c)


P27 F2 0 g
P27 F2 2 g
P27 F2 4 g
Disease Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
Days after Inoculation

(d)



(e)



(f)

Figure 3. Graphics of AUDPC from 7 to 35 days after inoculation: variety of NK-22 F1 and F2 (a); variety of P-27 F1 and F2 (b); F1 of P-27 treated with 0; 1.25 and 2.5 g metalaxyl (c); F2 of P-27 treated with 0; 1.25 and 2.5 g metalaxyl (d); F1 of NK-22 treated with 0; 1.25 and 2.5 g metalaxyl (e); F2 of NK-22 treated with 0; 1.25 and 2.5 g metalaxyl (f)