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

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Authors' contributions

This work, which included performing experiments and writing the manuscript, was done in collaboration among all 11 authors. Author CG initially had idea about this research and then together with authors JP and SRD designed the work. Authors DIRC, AA, ES and AHZP made contribution in the field work as well as collecting and tabulating data. Author PBT and Widyastuti managed observation with scanning electrom microscope SEM, while authors Ivayani, JP and SRD made contribution in other laboratory work. Authors Ivayani and TM performed statistical analysis. Author CG wrote first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Maize downy mildew (MDM) is considered as a major problem in all maize growing areas in Indonesia including the Province of Lampung.
Objectives: 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.
Methodology: To identify *Peronosclerospora* causing MDM, the pathogens were observed under light microscope and scanning electron microscope. Maize varieties response against pathogens and efficacy metalaxyl were studied in the field with the test plants exposed to plants showing MDM symptoms as the sources of inocula to mimic natural conditions.
Results: *Peronosclerospora sorghi, P. maydis,* and *P. philippinensis* were found to cause MDM in Lampung. 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 than that of F1 plants, but there was not 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.
Conclusion: Three species of *Peronosclerospora* were identified as disease-causing pathogen of MDM in Lampung, but these results were tentatively pending for further studies through molecular techniques. F1 plants were more susceptible than F2 plants to MDM in both P-27 and NK-22. Metalaxyl was not effective in controlling MDM.

Keywords: Maize downy mildew; metalaxyl; Peronosclerospora sorghi; P. maydis; P. philippinensis.

1. INTRODUCTION

Downy mildews have been reported to cause crop diseases in many countries [1,2,3]. Maize downy mildew (MDM) is a serious disease affecting maize production in many parts of Indonesia, including the Province of Lampung [4,5,6]. Infected maize plants show two types of symptoms, i.e. systemic symptoms, and local symptoms. The systemic symptoms occur 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 as small chlorotic spots. These patches further develop into parallel with the leaf bone. The affected plants become dwarf. If the pathogen does not reach the growing point, local symptoms 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 [4].

MDM in Indonesia is caused by three species of *Peronosclerospora*, i.e. *P. maydis, P. sorghi,* and *P. philippinensis* [4,6]. Earlier, *P. maydis* was reported to be restricted to Indonesia and Australia, while *P. sorghi* distributed worldwidely including America, Asia, Africa, Europe and Australia. Janruang and Unartngan [7] reported that *P. maydis* was found at several locations in Thailand. *P. philippinensis* is widely distributed in Asia as reported by International Maize and Wheat Improvement Center [CIMMYT] [8]. In Central Lampung, the causal agent of MDM was reported to be *P. sorghi* [4] and *P. maydis* [9]. It was probably both species of *Peronosclerospora* exist in Central Lampung. More extensive survey is needed to determine which species of *Peronosclerospora* is responsible for causing MDM in Lampung.

MDM is often controlled by planting resistant varieties and seed treatment with certain synthetic fungicide such as metalaxyl [5,6]. The use of fungicide metalaxyl has caused resistance in *Peronosclerospora* population [5,10,11]. The occurrence of metalaxyl resistance was also reported in other pathogens. Out of 116 isolates of *Phytophthora infestans* causing potato late blight tested for metalaxyl resistance and it was observed that 25.9% were resistant, 19.8% were intermediate and 54.3% were sensitive to metalaxyl [12].

Thus, maize crop is often affected by MDM although hybrid improved maize varieties are planted. In addition, farmers often use F2 seeds that are harvested from 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)

respond differently 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.

2. MATERIALS AND METHODS

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

2.1 Identification of *Peronosclerospora* Species

First, maize plants at vegetative stage shown systemic or local 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. Efforts were made to collect plant samples from two fields from each regency/city.

Selected plants were uprooted along with soil around the base of the stem and then were kept into polybags, individually. Each plant was covered with transparent plastic bag to avoid stress while transportation to laboratory and to avoid pathogens' spread among the sampled plants. During identification in laboratory, plants were kept individually in 1m high plastic enclosure to prevent pathogens spread to sampled plants.

Pathogen sporulation, in which timing is crucial, was stimulated by modified procedure of Rustiani et al. [13]. A maize plant in polybag was transferred to the laboratory. In the afternoon, the third leaf from the shoot with symptoms was washed by rubbing 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 dirt and fungal propagules. Then, the infected maize plant was covered with transparent plastic and placed in the room at 17°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 recorded under a light microscope to measure length and width of conidia, and length and number of branching levels of conidiophores.

Observations were also made with scanning electron microscope (SEM) at the Integrated Laboratory and Technology Innovation Center (LTSIT) at University of Lampung. It was started by taking leaf sample showing MDM symptoms at around 01.30. Prior to analysis, the sample was dried to avoid 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 shown advanced symptoms using scalpel and placing scraped biomass on a drop of water on a glass slide. The observations were made under light microscope. Identification was based on the description outlined by CIMMYT [8], Hikmahwati et al. [6] and Ahmad et al. [14].

2.2 Effect of Maize Varietyand Its Progeny on MDM

Experiment was conducted in a randomized block design (RBD) with four treatments and four replications. NK-22 (F1 and F2) and P-27 (F1 and F2) varieties were chosen because of popularity among maize growers in Lampung.

For this purpose, in advance weeding was done in field Then, land leveling, and ploughing was done. Plots of 2 x 2 m size at 0.75 distance were made. Crop rows were 25 cm apart and three maize seeds were sown/planting pit. After 5 days of sowing, thinning was done and one plant per pit was maintained.

Fertilizers were applied twice. In the first application, half dose of urea and entire dose of TSP and KCl was applied after 2 weeks of

planting. Remaining half of urea was applied after 6 weeks of planting. Urea 300 kg, SP-36 200 kg and KCl 50 kg/ha was applied in experimental plots.

Observations on disease incidence, disease severity, length and width of stomata, number of stomata, and production were recorded. Data was analyzed by adopting least significant difference test (LSD) at 5% level.

Inoculation was done with a procedure to mimic the natural field conditions [14]. For it, two plants shown 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. Proper care for watering and weeding was taken After planting observations on disease incidence and severity, were recorded every week for 5 weeks. Disease incidence was calculated with following formula

$$I = n/N \times 100\%$$

where, I = disease incidence (%), n = number of plants shown symptoms, and N = total number of plants observed.

Disease severity was calculated using modified method of Pataky et al. [15] as mentioned below

$$S = \Sigma (n_i x v_i)/(N \times V) \times 100\%$$

Where, S = severity of disease, n_i = number of plants from each category of attack, v_i = value score for each category of attack, N = total number of plants observed, and V = 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 symptomsand
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 [16]:

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

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 [17]:

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

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

Observations of the stomata was carried out to determine whether the number and size of stomata affected the MDM intensity. The observations were made 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 maize cobs were picked up. Then maize cobs were separated, harvested, dried, and weighed.

2.3 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. 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 pit 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. Plant nutrition was done as described above. All concerned data were analyzed as done in previous experiment.

In the second experiment, maize variety of P-27 (F1 and F2) was used all other parameters kept same as previous experiment.

3. RESULTS AND DISCUSSION

3.1 *Peronosclerospora* Species Causing MDM in Lampung

The morphological characteristics and morphometry of *Peronosclerospora* are presented in Table 1. The results of this study 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 of the Province of Lampung, respectively. 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 Fig. 1. This agreed with the results earlier workers [4]. *P. australiensis* infecting maize in Australia [18] 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 *P. sorghi* (Fig. 2). No *P. maydis* isolate was observed to produce any oospore. This agreed with the morphological characteristics reported by CIMMYT [8] 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 that in some isolates produced oval and ovoid to cylindrical (Table 1; Fig. 1 g). Similar results were obtained by Bock et al.[19] who found different morphology of *P. sorghi* isolated from different geographic locations and hosts in Africa. In addition, the size of spherical conidia varied. 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 it was 21.9 µm. This range was different from that of conidial size proposed in CIMMYT [8]. This identification was not in total agreement with Widiantini et al. [5] who proposed that conidia with oval shape to be *P. maydis*.

Therefore, the presence of the *Peronosclerospora* species was reported tentative. It is suggested that further studies are to be conducted to identify the species based on the results of morphological and DNA sequence observations [7]. 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.[18].

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.

3.2 Effect of Maize Variety and its Progeny on MDM

On both varieties, i.e. P-27 and NK-22, disease severity, AUDPC and r on F1 plants was greater than those of F2 plants, except NK-22 where both disease severity and r were not significantly different (Table 3). The smallest AUDPC occurred on P-27 F2, and the greatest AUDPC was on NK-22 F1 plants. The greatest r occurred on NK-22 F1 plants, and the smallest r occurred on P-27 F2 plants. The graphic of AUDPC of NK-22 and P-27 both F1 and F2 could be seen in Fig 3 (a,b). Maize variety seems to influence disease intensity (2,15,21).

Table 1. Morphology and morphometry (µm) of conidiophores and conidia of *Peronosclerospora* from diseased maize plants

Field location	Plant	Conidiophorelength average (range) (µm)	Branching level	Conidiashape	Widthaverage (range) (µm)	Lengthaverage (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	Spherical,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, Sub spherical	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*

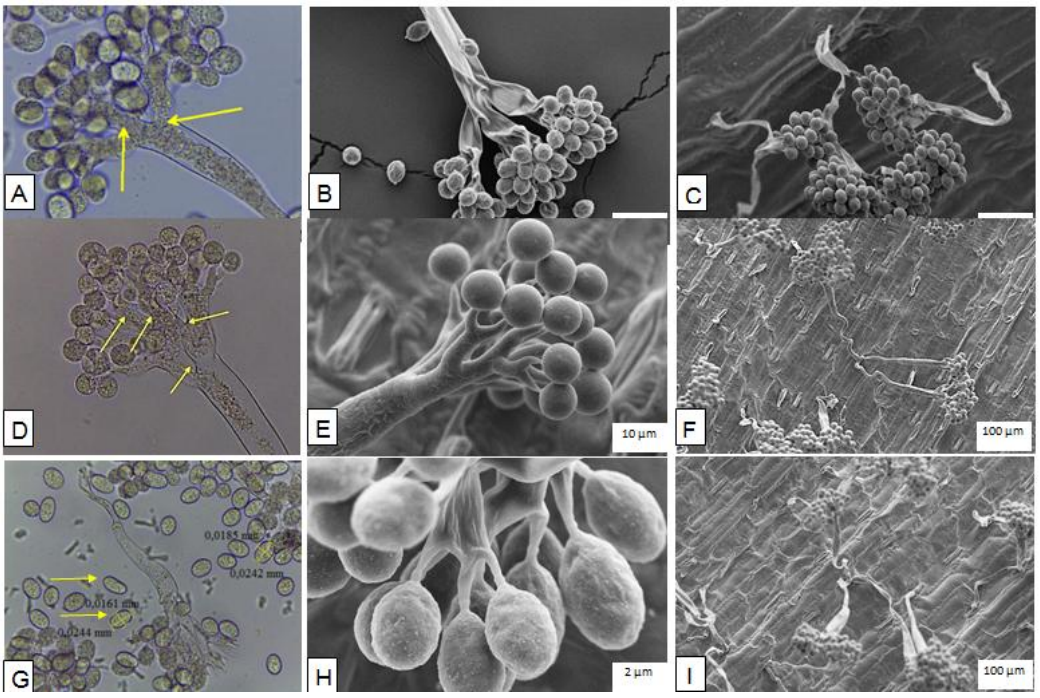


Fig. 1.Conidia and conidiophores of *Peronosclerospora*: *P. sorghi* (A, B, C), *P. maydis* (D, E, F), and *P. philippinensis* (G, H, I) observed under light microscope (A, D, G) or scanning electrom microscope (B, C, E, F, H, I)

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

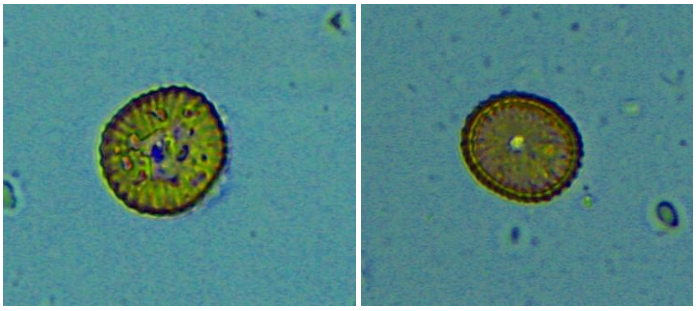


Fig. 2. Oospores of *Peronosclerospora*:*P. sorghi* (A) and *P. philippinensis* (B)

The number of stomata was different among varieties, while the length and width of stomata holes were not significantly different (Table 4). It seems that stomata did not affect the occurrence or severity of disease. The number of stomata on P-27 F1 plants was significantly lower than that on P-27 F2 plants. In addition, the length and width of stomata on P-27 F1 plants were not significantly different from those on P-27 F2 plants (Table 4). However, P-27 F1 plants showed higher disease severity and AUDPC than P-27 F2 plants (Table 3). Furthermore, the number of stomata and their size on NK-22 F1 plants were notdifferent from those on NK-22 F2 plants (Table 4); however, AUDPC on NK-22 F1 plants was higher than that in NK-22 F2 plants (Table 3. This results were different from those reported by Pudjiwati et al. [20] that, in conjunction with the maize breeding program, low density trichomes and stomatal density characters increase resistance to MDM. More observations are need to determine the relationship of stomata and disease intensity.

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 of F1 plants (Table 3). When released, P-27 was resistant to MDM, while NK-22 was susceptible. However, both varieties were heavily affected by the disease. In addition, metalaxyl that was effectively control MDM beforehand becoming ineffective [2]. In future, more breeding programs should be conducted to find more alternative lines or varieties to control the disease. Wisser et al. [21] found that the genetics of resistance of maize was very complex and suggested that certain breeding schemes may be more suitable for management of certain diseases. Rashid et al.[2] implemented several programmes/schemes to evaluate or to screen the resistance of maize cultivars or lines to MDM.

Table 3. Disease severity, AUDPC, infection rate (r), and production of maize F1 and F2 of NK-22 and P-27

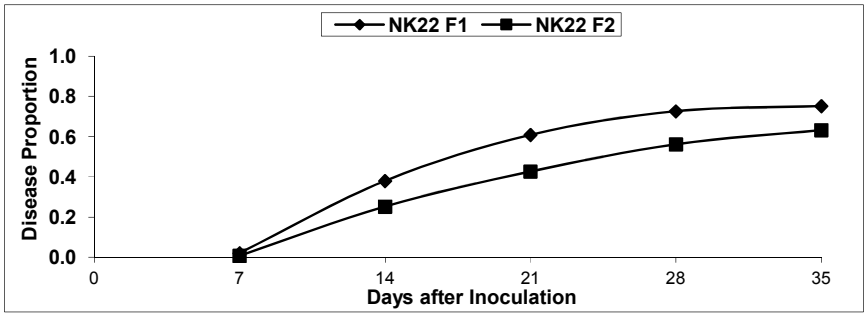
Veriety	Disease severity (%)	AUDPC	r	Production (g)
P-27 F2	11 a	0.04 a	0.02 a	6.17 b
NK-22 F2	63 b	0.19 ab	0.06 ab	3.96 ab
P-27 F1	59 b	0.25 bc	0.06 ab	1.33 a
NK-22 F1	75 b	0.30 c	0.07 b	3.29 a

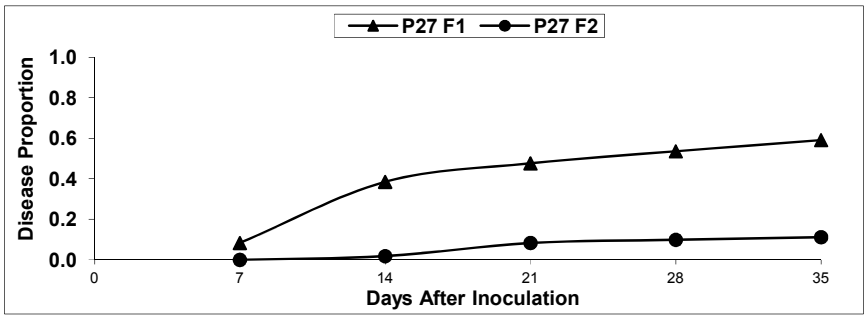
Note. Numbers followed by same letter are not different according to LSD (<5%)

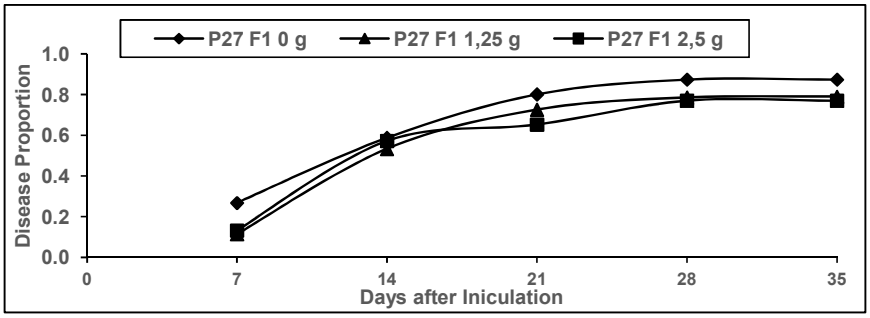
Table 4. The number of stomata and their size formed by NK-22 dan P-27 F1 dan F2 maize

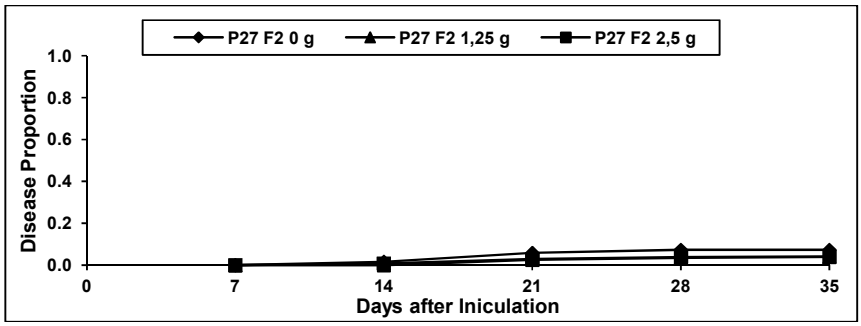
Treatment	Number of Stomata per mm^2	Length of Stomata hole (µm)	Width of Stomata hole (µm)
NK-22 F1	71.50 b	32.89 a	13.10 a
NK-22 F2	67.75 ab	34.36 a	13.96 a
P-27 F1	62.75 a	34.39 a	12.61 a
P-27 F2	81.75 c	32.21 a	12.99 a

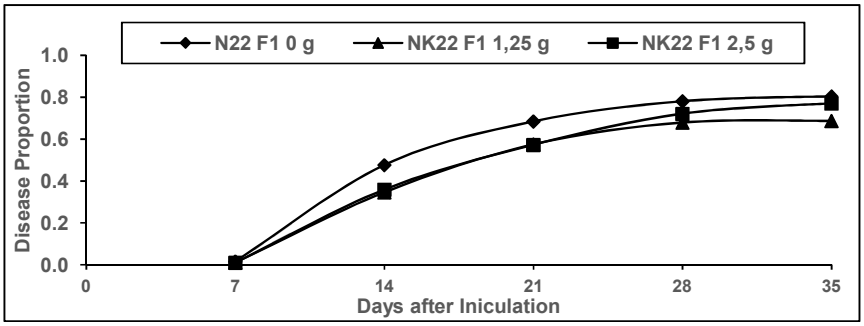
Note. Numbers followed by the same letter are not different according to LSD (<5%)


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


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


P27 F1 0 g
P27 F1 1,25 g
P27 F1 2,5 g
Disease Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
Days after Iniculation


P27 F2 0 g
P27 F2 1,25 g
P27 F2 2,5 g
Disease Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
Days after Iniculation


N22 F1 0 g
NK22 F1 1,25 g
NK22 F1 2,5 g
Disease Proportion
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
Days after Iniculation

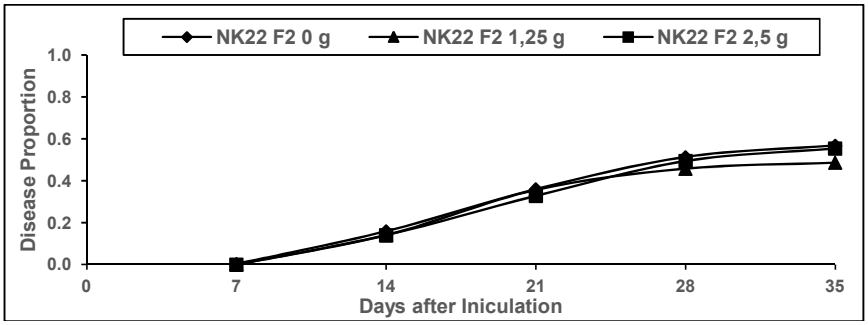


Fig. 3. Graphics of AUDPC from 7 to 35 days after inoculation: variety of NK-22 F1 and F2 (A); variety of P-27 F1 and F2 (B); F1 of P-27 treated with 0; 1.25 and 2.5 g metalaxyl (C); F2 of P-27 treated with 0; 1.25 and 2.5 g metalaxyl (D); F1 of NK-22 treated with 0; 1.25 and 2.5 g metalaxyl (E); F2 of NK-22 treated with 0; 1.25 and 2.5 g metalaxyl (F)

3.3 Efficacy of Metalaxyl to Control MDM

The results of the second experiment 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 phenomena that metalaxyl was not efficient to control MDM [5,10,11,12].

The problem of fungal pathogen resistance towards fungicides is well known since long ago. Molecular techniques developed to detect it [22] should also be tried to detect the incidence of *Peronosclerospora* resistance to metalaxyl. This could contribute to formulate integrated control strategy of MDM.

Metalaxyl and other phenylamide fungicides are site-specific and inhibit the polymerization of r-RNA. Frequent use of these fungicides leads to resistance population of 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 [23].

In addition, nk-22 f1 was more susceptible than nk-22 f2 to as shown by audpc on all nk-22 f1 and nk-22 f2 plants. Apparent infection rate was

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

Treatments		Disease severity (%)	AUDPC	r (unit/day)
Variety	Metalaxyl Concentration (g)			
NK-22 F2	2.50	49 a	0.14 a	0.05
	1.25	55 ab	0.18 a	0.06
	0.00	57 ab	0.19 a	0.06
NK-22 F1	1.25	77 c	0.30 b	0.09
	2.50	69 bc	0.28 bc	0.07
	0.00	80 c	0.34 c	0.09

Note. Numbers 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

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

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

not different among treatments (Table 5). This was in line with results of the first experiment.

The results of the third experiment showed that P-27 F1 plants were more susceptible than P-27 F2 plants as shown in all three variables, i.e. disease severity, AUDPC, and r (Table 6). Metalaxyl generally was not effective to control downy mildew on Pioneer plants, except that P-27 F1 treated with 1.25 g metalaxyl per kg seed reduced AUDPC if compared by F1 plants untreated with the fungicide. Apparent infection rates on P-27 F1 plants on all metalaxyl levels were greater than those on P-27 F2 plants. The graphic of AUDPC of NK-22 and P-27 both F1 and F2 plants treated with metalaxyl could be seen at Fig. 3 (c,d,e,f). It is interesting to note that metalaxyl effectivity was influenced by species of pathogen causing MDM. MDM caused by *Peronosclerospora maydis* was controlled if resistant varieties were used in combination with metalaxyl application at a dose of 5 g or 7 g/kg of maize seeds. However, metalaxyl application at a dose of 2 g to 7 g/kg of seeds did not control MDM on susceptible variety [24]. However, metalaxyl application at a dose of 2-7 g/kg of maize seeds was effective to control MDM on both resistant and susceptible varieties caused by *Peronosclerospora philippinensis* [25].

4. CONCLUSIONS

Based on the results of this investigation, it is concluded 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 are 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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