Pineapple mealybug wilt-associated virus (PMWaV) on Sipahutar pineapple, in North Tapanuli, Indonesia

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Abstract. Pineapple Mealybug Wilt-associated Virus (PMWaV) is one of the main diseases that affect pineapples all over the world, including Sipahutar, North Tapanuli, Indonesia. Currently, there are no studies or information on PMWaV disease as well as the species of mealybugs found in sipahutar pineapples. Therefore, this study aims to identify the mealybugs causing PMWaV in sipahutar pineapple. Data were obtained from 3 (three) villages, namely Siabalabal II, Onan Runggu, and Sabunganni Huta V, to assess the disease incidence and collect mealybugs. The results showed that the disease incidence of PMWaV in the 3 villages were between 15.6 - 21.6%. The highest disease incidence was found in Siabal-abal II (21.6%), followed by Onan Runggu (21.3%), and Sabunganni Huta V (15.6%). The mealybugs found on pineapple sipahutar with PMWaV symptoms in the 3 surveyed villages were identified as Dysmicoccus brevipes.

1. Introduction
Pineapple scientifically known as Ananas comosus L. (Merrill) and Smooth Cayenne is a tropical fruit plant with consumable fruits, which is significantly planted and cultivated worldwide. Globally, Indonesia is the 9th largest producer of pineapples with approximately 1.39 million tons exported to various parts of the world, such as South Korea, Saudi Arabia, Spain, the United States and Singapore, yearly. Several studies have shown that the potential for pineapple production in Indonesia can be further improved by minimizing the limiting factors.

One of the limiting factors globally affecting pineapple cultivation is wilt disease, which is caused by Pineapple mealybug wilt-associated virus-1 (PMWaV-1) and -2(PMWaV-2). According to previous studies, this disease has led to numerous losses to the majority of the world’s pineapple industry, including Hawaii and Cuba by 35% and 40%, respectively [1-5].

In Indonesia, this disease has also spread to many pineapple plantations, such as Blitar, Subang, Simalungun, and Bogor by 90%, 60-70%, 50-60%, and 50%, respectively [6]. Furthermore, studies have shown that the average fruit weight of plants affected by wilting was 35% lower than the virus-free plants and 30% lower than those infected with PMWaV-1 [4].

There are two different types of mealybugs on pineapples, namely pink and grey mealybugs. According to [7] pink mealybug reproduced parthenogenetic, which causes wilting symptoms, while the
grey sexually reproduces green spots [7]. Furthermore, Beardsley [7] reported that the pink and grey mealybugs are also known as *Dysmicoccus brevipes* and *D. neobrevipes* with differing number of antenna segments and body color. For instance, the pink and grey mealybugs have 8 and 10 antenna segments, respectively. North Sumatra is one of the districts in North Tapanuli Regency, which acts as the center for sipahutar pineapple [8]. Similar to other pineapples producing areas, this sipahutar experience cases of disease of PMWaV, thereby leading to wilt infection, which damages the plant roots. Furthermore, the Plants attacked by the virus become wilted, with the production of small fruit, reddish leaves and possible death. However, farmers’ limited knowledge and information regarding this disease have increased its widespread. Therefore, this study aims to analyze the mealybugs associated with PMWaV in sipahutar pineapple. The results are expected to lower and prevent the spread and effect of the disease.

2. Materials and methods

2.1. Survey

The survey was carried out in three villages that are pineapple producers, and cultivators in Sipahutar District, namely Siabalabal II, Onan Runggu III, and Sabunganni Huta V. Before, to this study each village is identified by one pineapple garden with the survey process carried out by observing the similarities in the disease symptoms during the cultivation process in the three locations and by calculating the occurrence of the disease from each location using the following formula:

\[
\text{Disease Incidence (DI)} = \frac{n}{N} \times 100\%
\]

Where: \( n \) is the number of plants with symptoms of illness, \( N \) is the number of symptomatic plants.

2.2 Mealybug sampling

The purposive random method was used to obtain white mealybugs from the 3 villages in Sipahutar and put into yellow envelope bags by cutting the plant shoots for further processing.

2.3 Mealybug identification

Mealybug identification was conducted by making microscopic preparations using the modified from previous studies [9]. They were fixed by inserting them in a test tube containing ± 2 ml of 95% alcohol, followed by a 5 minutes heating in a water bath. Furthermore, the alcohol was transferred with the mealybugs into a dish containing syrup, with dorsal thorax pierced with a needle to produce a hole in the dorsal part. Maceration was conducted by inserting white mealybugs in a 10% KOH solution and boiling it to transparency, then pouring it into a dish and removing the insect’s body contents. The mealybugs were then washed twice with distilled water before it is immersed in 50% acid alcohol solution for 10 minutes. Fuchsin acid solution was added to 50% acid alcohol solution with the preparations incubated in glacial acetic acid overnight. Dehydration was gradually carried out by soaking in 80% alcohol for 5 minutes, followed by 100% alcohol for 10 minutes, then carbol xylene solution for 2 minutes, and finally in 100% alcohol for 10 minutes. Subsequently, along with the dehydration treatment, staining with fuchsin acid was also carried out for 12 hours and immersed in clove oil for 10 minutes before, it is prepared in a glass object with Canadian balsam adhesive. Identification was performed by comparing the samples with reference books [9-10].

3. Results and discussions

3.1. Survey and mealybugs collection

The results showed that the disease incidence of PMWaV wilt was detected in three survey locations with the most common being yellow-red and curl at the tips of plant leaves. It was found that pineapple leavers with severe symptoms (leaf curl and leaf tips dry), consists of live mealybugs colonized
pineapple plants attacked by viruses. The disease incidence in the 3 locations was between 15.6 - 21.6%, with the highest in Siabal-abal II (21.6%), followed by Onan Runggu (21.3%) and Sabunganni Huta V (15.6%) as shown in Table 1 and Figure 1.

Table 1. Disease incidence (%) of pineapple wilt disease on surveyed locations in Sipahutar District.

| Surveyed locations              | Disease incidence (%) |
|---------------------------------|------------------------|
| Siabal-abal II Village          | 21.6                   |
| Onan Runggu Village             | 21.3                   |
| Sabunganni Huta V Village       | 15.6                   |

Pineapple mealybug wilt (PMW) is a disease characterized by reddening of the leaves, severe tip dieback, wilting and death of mature plants associated with PMWaV-1 and PMWaV-1–3 [11]. PMWaV-1 was detected in mature plants with symptoms of stunted growth and irregular chlorotic spots on all leaves. In the study area, the decline in yields was not identified, however, the presence of PMWaV-1 in the region had the ability to affect production assuming vector management and water supply are neglected due to the virus and limited irrigation, thereby producing negative (6.7 and 4.2%, respectively) and additive effects (13.4%) on pineapple fruit size and weight [12].

3.2. Mealybugs identification

Figure 1: Symptom of Pineapple mealybug wilt associated PMWaV, presence of mealybugs in the plants (red cycle), and the mealybug in 3 surveyed villages, namely Siabal-abal II Village (A, D, G); Onan Runggu III Village (B, E, H); and Sabunganni Huta V Village (C, F, I).
The identification results of mealybug samples obtained from the three villages in Sipahutar District indicated that the species of mealybug was *Dysmicoccus brevipes*, also known as Pink pineapple mealybug. Furthermore, the *D. brevipes* is oval in body shape, with several translucent pores on the rear femur and tibia, 8 antenna segments, and two discoidal pores round the eye. The ostiole is properly developed without thickening with the presence of circulus and 17 pairs of cerarii. In addition, there is a cerarii with 3-4 setae located in the posterior abdominal, head segments and prothorax. There is a pair of anal lobes with two large ribs on both. According to a previous study, mealybugs with these characteristics belong to the Pseudococcidae family, Dysmicoccus genus, D.brevipes species, as shown in Figure 2 [9].

Only one species of mealybugs, namely *D. Brevipes*, was identified in the sample locations. Meanwhile, the absence of species of grey mealybug (*D.neobrevipes*) is related to the distribution area of mealybugs. This is also due to the less diverse attribute of the sampling locations. Therefore, it is necessary to carry out further survey and identify mealybugs from other pineapple cultivation centers in North Tapanuli Regency.

**Figure 2:** The *Dysmicoccus brevipes* species of mealybugs characteristics in 3 surveyed villages: A) Siabal- abal II Village, B) Onan Runggu III Village, dan C) Sabunganni Huta V Village

4. **Conclusions**

The disease incidence of PMWaV wilt in the Sipahutar district was between 15.6 - 21.6% in the three villages, with the highest disease incidence found in Siabal-abal II (21.6%), followed by Onan Runggu (21.3%), and Sabunganni Huta V (15.6%). The mealybugs found in pineapple plants infected with PMWaV in these three villages were identified as *Dysmicoccus brevipes*.

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Acknowledgements
The authors would like to thank you to Dr. Dewi Sartiami from IPB University, Bogor for her technical assistance. This study was funded by Ministry of Research and Technology/ National Agency for Research and Innovation (Doctoral Grant 2020. Contract No. 167/UNS.2.3.1/PPM=DPRP/2020).