

Research Article

Determination of Mealybug Population Density for Transmission of Pineapple Mealybug Wilt Disease in Central Uganda

B. Bua ¹, M. E. Mawa,¹ J. Ayiga,¹ and A. Ocwa ^{1,2}

¹Department of Agriculture Production, Faculty of Agriculture, Kyambogo University P.O. Box 1, Kyambogo, Kampala, Uganda

²Institute of Land Use, Engineering and Precision Farming Technology, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, 138 Böszörményi Street, Debrecen 4032, Hungary

Correspondence should be addressed to B. Bua; bbua@kyu.ac.ug and A. Ocwa; ocwaakasairi@gmail.com

Received 26 July 2023; Revised 30 December 2023; Accepted 27 January 2024; Published 12 February 2024

Academic Editor: Othmane Merah

Copyright © 2024 B. Bua et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Pineapple mealybug wilt disease (PMWD) is a complex and most destructive viral disease constraining pineapple production world over. Pineapple mealybug wilt disease is transmitted by mealybugs (*Dysmicoccus* species). Currently, in Uganda, the population of *Dysmicoccus brevipes* that can transmit PMWD is not known. Yet, closing this knowledge gap would provide a better understanding of PMWD etiology and development of sound management strategies. Experiment was laid out in a completely randomized design (CRD) with four replications in the screenhouse at the Faculty of Agriculture, Kyambogo University during the period 2017 and 2018. Pineapple variety Smooth Cayenne was inoculated with five levels of viruliferous mealybugs including one (1), five (5), ten (10), fifteen (15), and a control (uninoculated). Data collection commenced on symptoms appearance and continued on a 15-day interval for a period of two months. Data were collected on the incidence and severity of PMWD. All the data collected were analysed using Genstat computer programme. Results showed that the number of mealybugs significantly ($p < 0.05$) affected the incidence and severity of PMWD during both trials. Although one viruliferous mealybug was able to transmit PMWD virus, the efficacy of transmission was recorded with the highest number of mealybugs per plant. It was, therefore, concluded that the higher the number of viruliferous mealybugs colonising a pineapple plant, the higher the incidence and severity of PMWD. The result of this study, therefore, adds to the pool of knowledge on the understanding the population density of mealybugs transmitting PMWD as well as provide insights to improvement of mealybug vector monitoring as a key component of integrated PMWD management.

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is among the most commercialised edible fruits in the Bromeliaceae family in the tropical and subtropical countries around the world [1–4]. Globally, pineapple production was estimated at 28 million metric tonnes in 2020 [5]. Notable amongst, the major global producers are Philippines (2.7 million metric tonnes), Costa Rica (2.6 million metric tonnes), and Brazil (2.5 million metric tonnes) [5]. In Africa, the major producers are Nigeria, Kenya, Democratic Republic of Congo (DRC), Ivory Coast, Guinea, and South Africa [6]. In Uganda, pineapple production is predominantly concentrated in the

central region around the Lake Victoria basin crescent with Smooth Cayenne being the predominant variety grown [7, 8]. However, pineapple production in Uganda and elsewhere is under threat from a diversity of constraints including diseases such as pineapple mealybug wilt disease [5, 7, 9, 10]. Indeed, PMWD is a complex, significant, and the most destructive viral disease constraining pineapple production world over [10–14]. PMWD is transmitted by two mealybugs species, namely, *Dysmicoccus brevipes* (pink mealybug) and *Dysmicoccus neobrevipes* (gray mealybug) [15–18]. For instance, in Colombia, a survey indicated a 32% prevalence of PMWD due to *Dysmicoccus brevipes* [10]. Similarly, in Indonesia, a prevalence of PMWD of 15.6–21.6% was attributed to

Dysmicoccus brevipes [19]. In Peru, incidence of 70%–90% was reported in hybrid MD-2 and cultivar Hawaiiiana of pineapple [20].

Pineapple Mealybug Wilt Disease is associated with mealybug wilt-associated virus PMWaV-1, 2, and 3 as important viral pathogens [12, 19, 21–24]. The infection by these viruses can be single or mixed [20, 21, 25], hence unleashing varying effects. According to [26], PMWaV-1 is correlated with growth reductions of plant crop and yield reductions in the ratoon crops, whereas PMWaV-2 infection and mealybugs feeding are necessary for the development of PMWD. In Peru, both single and mixed infections by PMWaV-1, 2, and 3 were detected in symptomatic samples [20]. Pineapple mealybug wilt disease is a syndrome characterised by wilting, pinkness and/or redness of leaves, drying of the leaf tips, inward leaf curling, stunting, root decaying, and collapse of the whole plant [5, 27, 28]. PMWD symptom development depends on factors such as population of mealybugs and access acquisition time (AAT). In addition, symptom development due to mealybug exposure is also reported to depend on pineapple hybrid, origin of planting material, and growing location [29]. Unless controlled, severe PMWD infection is reported to cause yield loss of 35% [30] or between 25% and 100% [23]. According to [12] in China, a PMWD incidence of 60% led to 20% production and economic losses. However, the magnitude of yield loss depends on time of symptom development and stage of disease onset among other factors [30]. Moreover, understanding the comparative efficiency of virus transmission and factors that affect virus transmission competence is paramount [31]. Currently, in Uganda, the mealybug population threshold that can transmit PMWD is not known. Yet, closing such knowledge gaps would provide a better understanding of PMWD etiology and the development of sound control mechanisms [17]. According to an earlier report by [32], *Dysmicoccus brevipes* is the most prevalent species of mealybugs in Uganda, with infestation ascribed to cropping systems, soil management practices, and farm type. With the variability in the infestation behaviour, understanding *Dysmicoccus brevipes* population density that can transmit PMWD is vital. Therefore, this study was conducted to determine the effect of mealybug population density on the symptomatology of PMWD in central Uganda.

2. Materials and Methods

2.1. The Experimental Site. The experiment was conducted in a screenhouse at the Faculty of Agriculture, Kyambogo University, Uganda, during 2017 and 2018. Kyambogo University is located 8 km east of Kampala Capital City Centre along the Kampala–Jinja highway by road and lies on the coordinates 0° 20'54.0"N 32° 37'49.0"E [33].

2.2. Test Plant Material Collection and Detection of Latent Infection. Pineapple suckers of Smooth Cayenne variety used in this study were collected from the districts of Mukono, Kayunga, Luwero, and Masaka in Central Uganda. Suckers were physically inspected and those found to be free from pineapple mealybug and pineapple mealybug wilt disease

symptoms were transported to Kyambogo University for laboratory analysis. Later, suckers were assayed for latent disease infection using polymerase chain reaction (PCR) with specific primers targeting the associated viruses PMWaV-2 (primer set 223/224) and PMWaV-1 (primer set 225/226) [34]. The symptomatic diseased plants with mealybugs were used as the inoculum source.

2.3. The Experimental Design, Treatments, Potting, and Management. The experiment was laid out in a completely randomized design (CRD) with four replications per treatment during both trials. The treatments included inoculation of pineapple plants with five levels of viruliferous mealybugs, namely, one (1), five (5), ten (10), fifteen (15), and the control (uninoculated). Forest soil sterilized by direct heating was filled in pots (6 kg) and suckers planted. The test plants were placed in individual cages in the screenhouse to restrict dispersal of mealybugs by ants and wind. Caging also limited potential mealybug predators (Figure 1). The summary of the steps involved in the study is highlighted in Figure 2. Other insects were controlled by maintaining good sanitation in and around the screenhouse. Watering and weeding of pineapple suckers were done as and when necessary.

2.4. Inoculation. The pink mealybugs obtained from diseased pineapple plants were given access acquisition period (AAP) of seven (7) days as described by [15]. A sub-population of the mealybug vectors was randomly assayed for their viruliferous potential by PCR (Figure 3) [15, 34]. The viruliferous mealybugs were transferred onto the test plants using a fine paint brush. Monitoring for the inoculation access period (IAP) was done prior to the commencement of data collection.

2.5. Data Collection and Analysis. Data were collected after 15 days of the inoculation access period (IAP) and continued at an interval of 15 days for a period of two months [15]. Incidence was assessed as the number of disease-infected plants expressed as percentage of the total number of plants per unit area. Severity was calculated from the average number of chlorotic spots from three leaves per plant and scored using a modified scale of 0–5, where 0 = no chlorotic spot, 1 = 1–5 chlorotic spots, 2 = 6–13 chlorotic spots, 3 = 14–20 chlorotic spots, 4 = 21–26 chlorotic spots, and 5 = 27 and above chlorotic spots on the leaves [35]. Data collected were subjected to one-way analysis of variance (ANOVA) of the Genstat Computer Programme 14th edition [8]. Significant differences between the means were separated using the least significant test (LSD) at 5% probability level.

3. Results and Discussion

The incidence of PMWD was significantly ($p < 0.05$) affected by the number of mealybugs during both trials (Table 1). During both trials, the highest and lowest incidences of PMWD at 15DAI were recorded in the plants inoculated with 15 mealybugs and 1 mealybug, respectively. A similar



FIGURE 1: Cages showing pineapple plants inoculated with different levels of mealybugs to prevent mealybug dispersal by ants and wind at the Faculty of Agriculture, Kyambogo University, 2017 and 2018.

trend was observed at 30DAI, 45DAI, and 60DAI during both trials (Figures 4(a) and 4(b)). The severity of PMWD was significantly ($p < 0.05$) affected by the number of mealybug (Table 2). During both trials, the highest and lowest PMWD severity was recorded in the plots with 15 mealybugs and 1 mealybug, respectively. A similar trend was observed at 30DAI, 45DAI, and 60 DAI during both trials (Figures 5(a) and 5(b)). Overall, the severity of PMWD corresponded with the increase in incidence. Although, the results showed that mealybug population density responsible for the transmission of PMWD is variable, higher incidences and severities were associated with higher number of mealybugs. Besides, it is also worth noting that it is not only the higher number of mealybugs that is responsible for the transmission of PMWD but also that they must be viruliferous. According to earlier studies, higher incidences of PMWD were associated with high mealybugs populations which are viruliferous [15, 36]. Similarly, Bua et al. [7] reported that although PMWD was prevalent in all the fields surveyed, only 20% of the fields had 100% incidence implying that PMWD is not a fast spreading disease or mealybug vectors have low transmission efficiency. This could be true because the mealybugs which vectors PMWD are immobile and, therefore, require to be moved from one plant to another or from field to field. However, in this study, the need for the movement from one plant to another was deliberately done by artificially inoculating the plants with the required number of mealybugs. Hence, the strong association reported between the mealybugs and PMWD may support these assertions. The strong association reported between pineapple mealybug wilt-associated virus and PMWD where both gray and pink pineapple mealybugs (*D. neobrevipes* and *D. brevipes*) were identified as vectors of the virus explains why only viruliferous mealybugs are responsible for PMWD transmission [15].

Exposure to the higher number of viruliferous mealybugs had a significant effect on the incidence and severity of PMWD transmission as well as infections as opposed to the lower number. This was because inoculation with a high number of mealybugs resulted into transmission of high viral load capable of significantly causing PMWD. In generally, the results showed higher transmission efficiency of the virus with

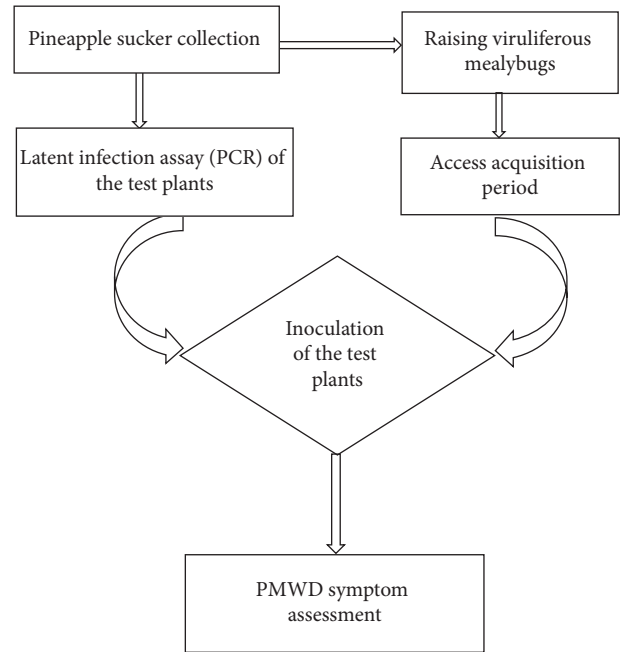


FIGURE 2: A flowchart showing how the viruliferous mealybugs were reared, assayed, and used for inoculation of pineapple plants in the screenhouse at the Faculty of Agriculture Kyambogo University, 2017 and 2018.

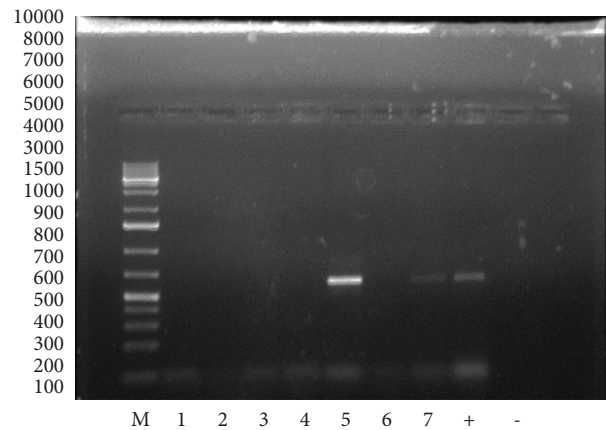


FIGURE 3: PCR amplification of the viruliferous mealybug subpopulation used for inoculating pineapple plants at the screenhouse, Faculty of Agriculture, Kyambogo University, 2017 and 2018. Note. M is the DNA gene ruler, lanes 1–7 are PCR-amplified samples using primer set 225/226, lane marked (+) is the positive control, and lane marked (–) is the negative control. Subpopulation from sample 5 was used for pineapple plant inoculation.

increasing number of mealybugs thus reflecting high virus retention (VR) due to sufficient virus acquisition access period. This implies that the inoculation acquisition period (IAP) of the fifteen mealybugs was shortened. These results, therefore, have corroborated the earlier findings which demonstrated that the mealybug population of 10–20 per plant resulted in higher transmission efficiency of PMWD [15]. In fact, the ingestion of adequate virus by a large number of vector population increases transmission of the virus, hence developing the disease

TABLE 1: Summary of ANOVA (mean square errors) for percentage incidence of PMWD on pineapple plants with different levels of mealybug inoculum at the Faculty of Agriculture, Kyambogo University, 2017 and 2018.

		Days after inoculation			
		15DAI	30DAI	45DAI	60DAI
<i>Trial one (November–December 2017)</i>					
Source of variation	Degrees of freedom				
Treatment	4	2238.28***	2781.25***	5097.66***	5519.53***
<i>Trial two (March–April 2018)</i>					
Source of variation	Degrees of freedom				
Treatment	4	2190.23**	2431.89**	4998.74**	5224.17**

DAI, days after inoculation. ***Significant at <0.001. **Significant at 0.01.

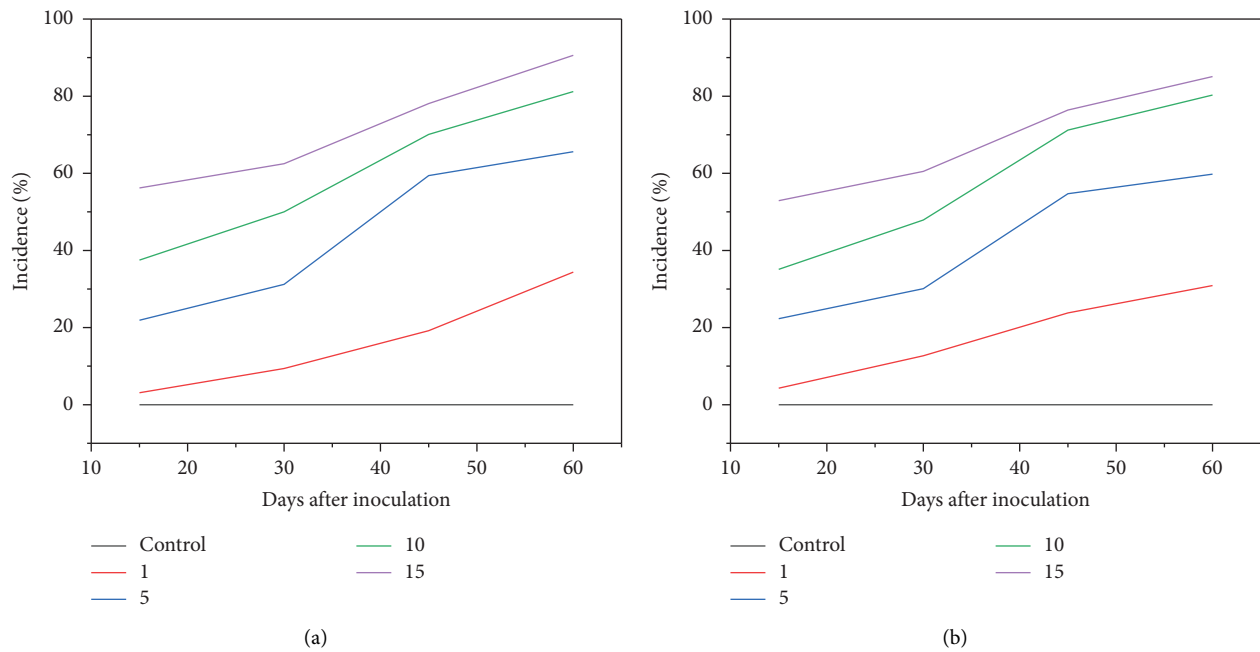


FIGURE 4: Percentage incidence of PMWD on pineapple plants grown in the screenhouse with different levels of mealybug inoculum at the Faculty of Agriculture, Kyambogo University, 2017 and 2018. (a) Trial one. (b) Trial two.

TABLE 2: Summary of ANOVA (mean square errors) for severity of PMWD on pineapple plants with different levels of mealybug inoculum at the Faculty of Agriculture, Kyambogo University, 2017 and 2018.

		Days after inoculation			
		15DAI	30DAI	45DAI	60DAI
<i>Trial one (November–December 2017)</i>					
Source of variation	Degrees of freedom				
Treatment	4	5.80***	8.50***	15.17***	16.55***
<i>Trial two (March–April 2018)</i>					
Source of variation	Degrees of freedom				
Treatment	4	5.91**	7.20**	14.95**	15.83**

DAI, days after inoculation. ***Significant at <0.001. **Significant at 0.01.

symptom [37]. However, it was also observed that symptoms development due to exposure to large number of mealybugs depended on the pineapple hybrid, origin of planting material, and growing locations [29]. Several authors have reported varying incidences of PMWD transmitted by pink mealybug (*Dysmicoccus brevipes*) [10, 19]. For instance, in Colombia, it was demonstrated that PMWD incidence of 32% was transmitted by *Dysmicoccus brevipes* [10], while in Indonesia and

Cuba, incidence of 15.6–21.6% and 2.4–99.9% was transmitted by *Dysmicoccus brevipes* depending on the location of the field [23]. However, in Ghana, severity varied with varieties and Smooth Cayenne was the most affected [4]. This clearly depicts the role played by hybrid and location in influencing the transmission of PMWD by *Dysmicoccus brevipes*. Overall, this study presents the first comprehensive results establishing the strong nexus between the number of mealybugs and PMWD

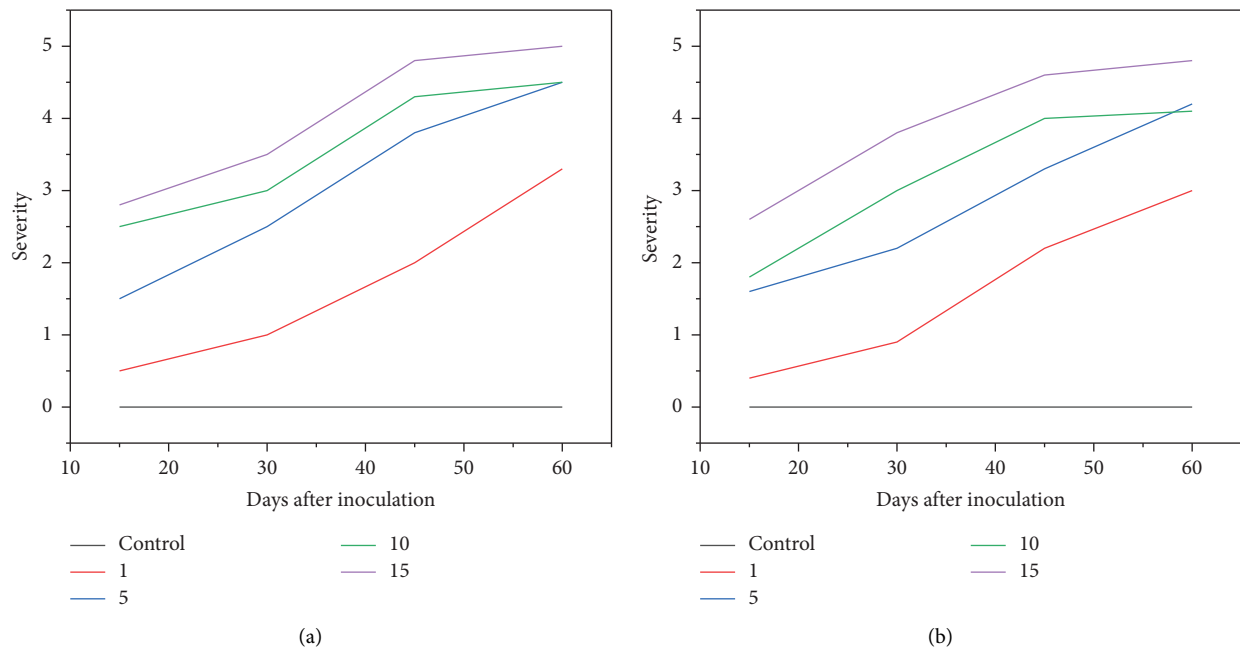


FIGURE 5: Severity of PMWD on pineapple plants grown in the screenhouse with different levels of mealybug inoculum at the Faculty of Agriculture, Kyambogo University, 2017 and 2018. (a) Trial one. (b) Trial two.

transmission in Uganda. However, considering that the study was conducted in the screenhouse, validation under field conditions is recommended due to the complexity of the interactions between factors in the field. Although, earlier, it was reported that the etiology of PMWD is not fully understood, it was concomitant with the ants that protect mealybugs, certain viruses, and environmental factors [38]. Moreover, *Dysmicoccus brevipes* as one of the vectors has a wide host range of 100 plants genera of 53 families, hence a threat to other crops [38–40]. These findings partially contributes to solving this dilemma by bridging knowledge gap concerning transmission threshold.

4. Conclusion

This study presents the first comprehensive record of the mealybug population density that can transmit PMWD in Uganda. Although one viruliferous mealybug was able to transmit PMWD virus, the efficacy of transmission was higher with higher mealybug numbers. The findings, therefore, suggest the need for immediate implementation of control measures upon detection of mealybugs in pineapple plantations even in absence of PMWD symptoms. This result contributes to the pool of knowledge for the improvement of PMWD management since detection of *Dysmicoccus brevipes* signals the potential outbreak of PMWD. Moreover, *Dysmicoccus brevipes* monitoring is the principal component of integrated management of PMWD. However, considering that the study was conducted in the screenhouse, validation of mealybug population density that transmits PMWD under field conditions is imperative since environmental conditions affect survival of mealybugs and ants, which are the fundamental components of the PMWD transmission cycle.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The funder did not have any influence in data collection, article writing, or publication process.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The funding for this study was provided by the Ministry of Education and Sports through the Uganda National Council for Higher Education to the first author. The authors are grateful to the administration of the National Crop Resources Research Institute (NACRRI), Namulonge for the use of laboratory facilities, and the farmers for providing pineapple materials used in this study.

References

- [1] M. Dhananjay, "Eco-friendly management of mealybug and wilt in pineapple," *The Journal of Plant Protection Sciences*, vol. 1, no. 1, pp. 40–43, 2009.
- [2] L. Hernández, P. L. Ramos, M. Rodríguez, I. Peña, and J. M. Pérez, "First report of Pineapple mealybug wilt associated virus-3 infecting pineapple in Cuba," *New Disease Reports*, vol. 22, no. 1, p. 18, 2010.
- [3] Z. Sapak, A. N. Mohd Faisal Mahadeven, N. F. Mh, S. Norsahira, and M. Z. Aw, "A review of common diseases of

- pineapple: the causal pathogens, disease symptoms, and available control measures,” *Food Research*, vol. 5, no. S4, pp. 1–14, 2021.
- [4] T. M. Sarpong, E. Asare-Bediako, and L. Acheampong, “Perception of mealybug wilt effect and management among pineapple farmers in Ghana,” *Journal of Agricultural Extension*, vol. 21, no. 2, pp. 1–16, 2017.
- [5] A. E. Larrea-Sarmiento, A. Olmedo-Velarde, X. Wang et al., “Genetic diversity of viral populations associated with ananas germplasm and improvement of virus diagnostic protocols,” *Pathogens*, vol. 11, no. 12, p. 1470, 2022.
- [6] F. Hossain, “World pineapple production: an overview,” *African Journal of Food, Agriculture, Nutrition and Development*, vol. 16, no. 4, pp. 11443–11456, 2016.
- [7] B. Bua, J. Karungi, and G. Kawube, “Occurrence and effects of pineapple mealybug wilt disease in central Uganda,” *Journal of Agricultural Science and Technology A*, vol. 3, pp. 410–416, 2013.
- [8] J. Oculi, B. Bua, and A. Ocwa, “Reactions of pineapple cultivars to pineapple heart rot disease in central Uganda,” *Crop Protection*, vol. 135, Article ID 105213, 2020.
- [9] R. A. Alvarez, R. R. Martin, and D. F. Quito-Avila, “First report of *Pineapple mealybug wilt associated virus-1* in Ecuador,” *New Disease Reports*, vol. 31, no. 1, p. 15, 2015.
- [10] I. Moreno, R. Tarazona-Velásquez, Y. Campos-Patiño, K. A. Rodríguez-Arévalo, and T. Kondo, “Prevalence of *Dysmicoccus brevipes* (Cockerell, 1893) (Hemiptera: pseudococcidae) in MD2 pineapple crop areas in Colombia,” *Pesquisa Agropecuária Tropical*, vol. 51, 2021.
- [11] A. A. Khan, G. M. Avesi, S. Z. Masud, and S. W. A. Rizvi, “Incidence of mealy bug *dysmicoccus brevipes* (Cockerell) on pineapple,” *Journal of Zoology*, vol. 22, pp. 159–161, 1998.
- [12] N. Yu, Z. Luo, H. Fan et al., “Complete genomic sequence of a Pineapple mealybug wilt-associated virus-1 from Hainan Island, China,” *European Journal of Plant Pathology*, vol. 141, no. 3, pp. 611–615, 2015.
- [13] D. Massé, N. Cassam, B. Hostachy et al., “First report of three pineapple mealybug wilt-associated viruses in queen victoria pineapples in reunion island,” *Plant Disease*, vol. 105, no. 3, p. 715, 2021.
- [14] I. Moreno, K. A. Rodríguez-Arévalo, R. Tarazona-Velásquez, T. Kondo, and T. Kondo, “Occurrence and distribution of pineapple mealybug wilt-associated viruses (PMWaVs) in MD2 pineapple fields in the Valle del Cauca Department, Colombia,” *Tropical Plant Pathology*, vol. 48, no. 2, pp. 217–225, 2023.
- [15] D. M. Sether, D. E. Ullman, and J. S. Hu, “Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.),” *Phytopathology*, vol. 88, no. 11, pp. 1224–1230, 1998.
- [16] H. Yan-biao, L. Ying-hong, R. Zhan et al., “The occurrence of two species of pineapple mealybugs (*Dysmicoccus* spp.) (Hemiptera: pseudococcidae) in China and their genetic relationship based on rDNA ITS sequences, *Caryologia*,” *International Journal of Cytology, Cytosystematics and Cytogenetics*, vol. 67, no. 1, pp. 36–44, 2014.
- [17] J. C. Green, M. A. Rwahnihi, A. Olmedo-Velarde et al., “Further genomic characterization of pineapple mealybug wilt-associated viruses using high-throughput sequencing,” *Tropical plant pathology*, vol. 45, no. 1, pp. 64–72, 2020.
- [18] P. A. Guerra, E. H. de Souza, E. C. de Andrade, D. A. S. Max, R. S. de Oliveira, and F. V. D. Souza, “Comparison of shoot tip culture and cryotherapy for eradication of ampeloviruses associated with Pineapple mealybug wilt in wild varieties,” *In Vitro Cellular and Developmental Biology- Plant*, vol. 56, no. 6, pp. 903–910, 2020.
- [19] A. J. Hutahayan, A. R. Tantawi, M. C. Tobing, and L. Lisnawita, “Pineapple mealybug wilt-associated virus (PMWaV) on Sipahutar pineapple, in North Tapanuli, Indonesia,” *Institute of Physics Conference Series: Earth and Environmental Science*, vol. 782, no. 4, Article ID 042062, 2021.
- [20] E. C. Carrasco-Lozano, G. A. Carrillo-Ordóñez, D. Gamarraga-Gamarrá et al., “Pineapple mealybug wilt-associated viruses 1, 2, and 3 are associated with mealybug wilt disease of pineapple in Peru,” *Journal of Plant Pathology*, vol. 105, no. 2, pp. 581–586, 2023.
- [21] C. F. Gambley, V. Steele, A. D. W. Geering, and J. E. Thomas, “The genetic diversity of ampeloviruses in Australian pineapples and their association with mealybug wilt disease,” *Australasian Plant Pathology*, vol. 37, no. 2, pp. 95–105, 2008.
- [22] L. Carnielli, W. A. Amorim, A. Vaz, P. M. B. Fernandes, and J. A. Ventura, “Molecular diagnosis of *Fusarium guttiforme* and Pineapple mealybug wilt-associated virus,” *Bone marrow concentrate Proceedings*, vol. 8, no. S4, p. P121, 2014.
- [23] L. Hernandez-Rodriguez, P. Ramos-Gonzalez, G. Garcia-Garcia et al., “Geographic distribution of mealybug wilt disease of pineapple and genetic diversity of viruses infecting pineapple in Cuba,” *Crop Protection*, vol. 65, pp. 43–50, 2014.
- [24] J. S. Hu, D. M. Sether, M. J. Metzger et al., “Pineapple wilt associated virus and mealybug wilt of pineapple,” *Acta Horticulturae*, vol. 666, pp. 209–212, 2005.
- [25] J. Nyarko and E. Asare-Bediako, “First report of Pineapple mealybug wilt-associated virus-1 and-3 in Ghanaian pineapple,” *New Disease Reports*, vol. 40, no. 1, p. 18, 2019.
- [26] D. M. Sether and J. S. Hu, “The impact of Pineapple mealybug wilt-associated virus-1 and reduced irrigation on pineapple yield,” *Australasian Plant Pathology*, vol. 30, pp. 31–36, 2001.
- [27] D. M. Sether and J. S. Hu, “Closterovirus infection and mealybug exposure are necessary for the development of mealybug wilt of pineapple disease,” *Phytopathology*, vol. 92, no. 9, pp. 928–935, 2002a.
- [28] A. J. Hutahayan and S. P. Msi, “Identification of distribution the pineapple mealybug wilt disease in the pineapple plant in north tapanuli,” *International Journal of Environment, Agriculture and Biotechnology*, vol. 2, no. 5, pp. 2473–2480, 2017.
- [29] K. K. Dey, J. C. Green, M. Melzer, W. Borth, and J. S. Hu, “Mealybug wilt of pineapple and associated viruses,” *Horticulturae*, vol. 4, p. 52, 2018.
- [30] D. M. Sether and J. S. Hu, “Yield impact and spread of pineapple mealybug wilt associated virus-2 and mealybug wilt of pineapple in Hawaii,” *Plant Disease*, vol. 86, no. 8, pp. 867–874, 2002.
- [31] C. W. Tsai, A. Rowhani, D. A. Golino, K. M. Daane, and R. P. Almeida, “Mealybug transmission of grapevine leafroll viruses: an analysis of virus–vector specificity,” *Phytopathology*, vol. 100, no. 8, pp. 830–834, 2010.
- [32] S. Kabi, J. Karungi, L. Sigsgaard, and J. M. Ssebuliba, “*Dysmicoccus brevipes* (Cockerell) occurrence and infestation behaviour as influenced by farm type, cropping systems and soil management practices,” *Agriculture, Ecosystems and Environment*, vol. 222, pp. 23–29, 2016.
- [33] B. Bua, R. Owiny, and A. Ocwa, “Response of onion to different organic amendments in Central Uganda,” *Journal of Agricultural Science and Technology B*, vol. 7, no. 2, pp. 79–85, 2017.
- [34] D. M. Sether, A. V. Karasev, C. Okumura et al., “Differentiation, distribution, and elimination of two different

- pineapple mealybug wilt-associated viruses found in pineapple,” *Plant Disease*, vol. 85, no. 8, pp. 856–864, 2001.
- [35] L. V. Madden, G. Hughes, and F. van den Bosch, *The Study of Plant Disease Epidemics*, APS Press, Washington, DC, USA, 2007.
- [36] C. Gary, J. Jahn, W. Beardsley, and H. González-Hernández, “A review of the association of ants with Mealybug wilt disease of pineapple,” *Proceeding of the Hawaiian Entomology Society*, vol. 36, pp. 9–28, 2003.
- [37] P. La Notte, N. Buzkan, E. Choueiri, A. Minafra, and G. P. Martelli, “Acquisition and transmission of *Grapevine virus A* by the mealybug *Pseudococcus longispinus*,” *Journal of Plant Pathology*, vol. 79, no. 1, pp. 79–85, 1997.
- [38] A. Larrea-Sarmiento, A. Olmedo-Velarde, X. Wang et al., “A novel ampelovirus associated with mealybug wilt of pineapple (*Ananas comosus*),” *Virus Genes*, vol. 57, no. 5, pp. 464–468, 2021.
- [39] R. K. Tanwar, P. Jeyakumar, and D. Monga, “Mealybugs and their management. technical bulletin 19, september,” *National Centre for Integrated Pest Managements Building, Pusa Campus, New Delhi*, vol. 110, 2007.
- [40] W. J. Morandi Filho, V. C. Pacheco-da-Silva, M. C. Granara de Willink, E. Prado, and M. Botton, “A survey of mealybugs infesting South-Brazilian wine vineyards,” *Revista Brasileira de Entomologia*, vol. 59, no. 3, pp. 251–254, 2015.