

Abstract

This study was aimed to evaluate the presence of Insect Vectors that transmit Banana Wilt Associated Phytoplasma (BWAP) on the banana plants in the Antiragen and Orori village gardens around the Mutzing station at Markham District in Morobe Province of Papua New Guinea. This research was conducted to find out which insects can be the hosts to Banana wilt associated phytoplasma. At first, insects were caught at night and their heads were crushed, later the DNA was extracted from insect sample and viewed under ultraviolet (UV) light following QIAGEN Quick Start Protocol. The LAMP assay was then performed on the 16S rRNA gene of this mollecutes for exceptional and specific detection. The results indicated that insects such as Blattidae (Dictyoptera), Eumastacoidea (Orthoptera) and Carculionoidae (Coleoptera) are vectors of banana wilt associated phytoplasma. Further, extensive studies should be conducted on Banana wilt associated phytoplasma to determine the vectors and their proper classification as novel taxa. To the best of our research team’s knowledge, it is the first report of the vector insects that host this plant pathogen in Papua New Guinea. From this research study it was concluded that insect vectors are responsible for inoculating the pathogen onto the host plant when they feed on the plant sap. In order to identify exotic insect host’s phytoplasma, it was recommended to use specific primers for Banana wilt associated phytoplasma, so that the disease management strategies are more viable.

Key words: Banana wilt associated phytoplasma, LAMP assay, 16S rRNA, Primers, DNA

Introduction

- The performance of banana and plantains are prone to biotic and abiotic factors.
- Draught resistant banana varieties are affected by Banana Wilt Associated Phytoplasma (BWAP).
- In 2008 to 2012 survey by NAQIA and ADFF found out phytoplasma affecting ABB cooking bananas.
- More studies are required on insect vectors of BWAP (Davis et al, 2012).
- Markham People greatly depends on these types of banana and loss by this disease would greatly impact their lives.
- 2008 outbreak of BWAP in Bogia and Markham have similar strain to Bogia Coconut syndrome (Davis et al, 2012).
- Studying and analyzing insect vectors shall pave the way forward for phylogeny, identification and development of combating method for BWAP disease (Miyazaki et al, 2018).

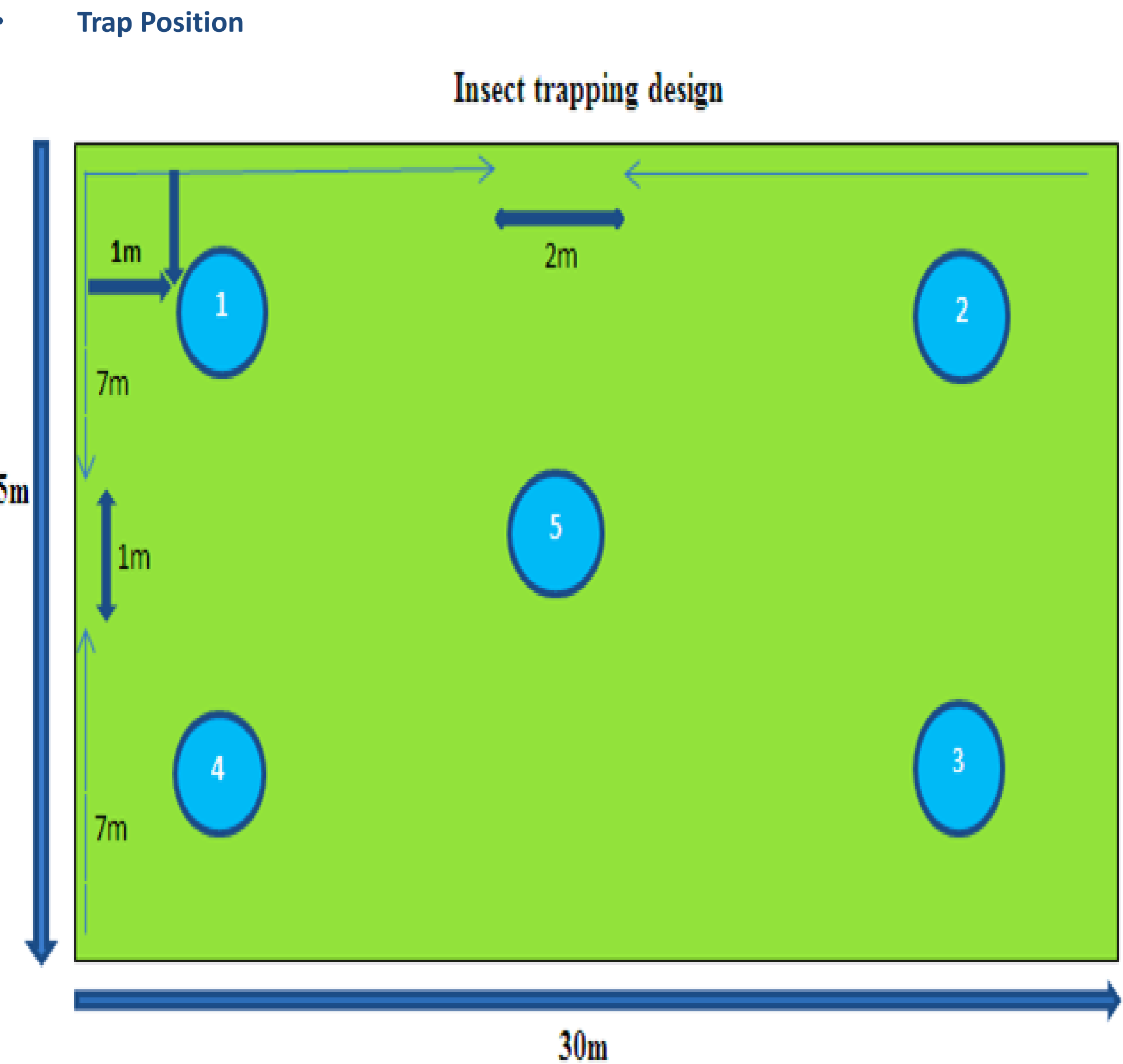
Methodology

The insects were collected at Antiragen (06°14’45.6 S; 146° 12’48.3 E) and Orori (06° 19’39.6 S; 146° 34.3 E). These two sites are located close to Mutzing Station which is a 360 kilometer drive from Lae City. The observed altitude of the station is 335.3 meters above sea level. The equatorial coordinates and the altitude of these chosen sites were obtained with Garmin GPS 72H.

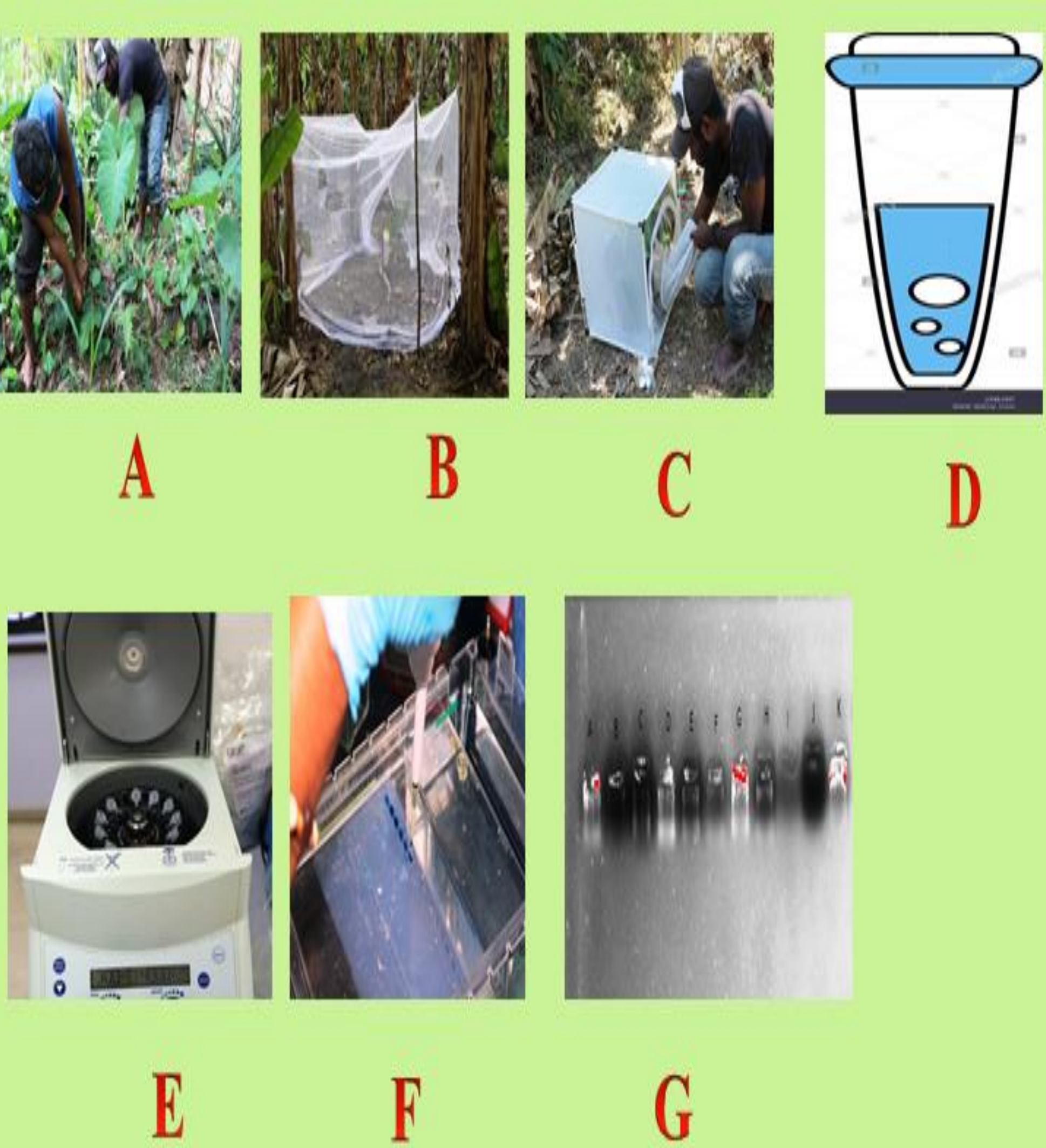
The insects were captured overnight and during early morning hours and promptly taken to the Ghodake Biotechnology Laboratory for morphological identification and phytoplasma detection (Cassis et al., 2016). The deceased ones were kept in the fridge at -20°C, while the live ones in the cages were introduced to the sucrose solution feeding medium readily prepared in the Eppendorf tube.

The sucrose solution feeding medium consisted of 0.5M NaOH, EDTA, 10mM Tris-HCL buffer pH 7.4 and 5% sucrose in 200 µl TE buffer. The extraction of the DNA from the head tissues of the insects was done using protocol by Moreau, S. (2014) and the Quick-Start Protocol-DNeasy ‘Blood & Tissue Kit (cat.nos.69504 and 69506) QIAGEN, 2011.

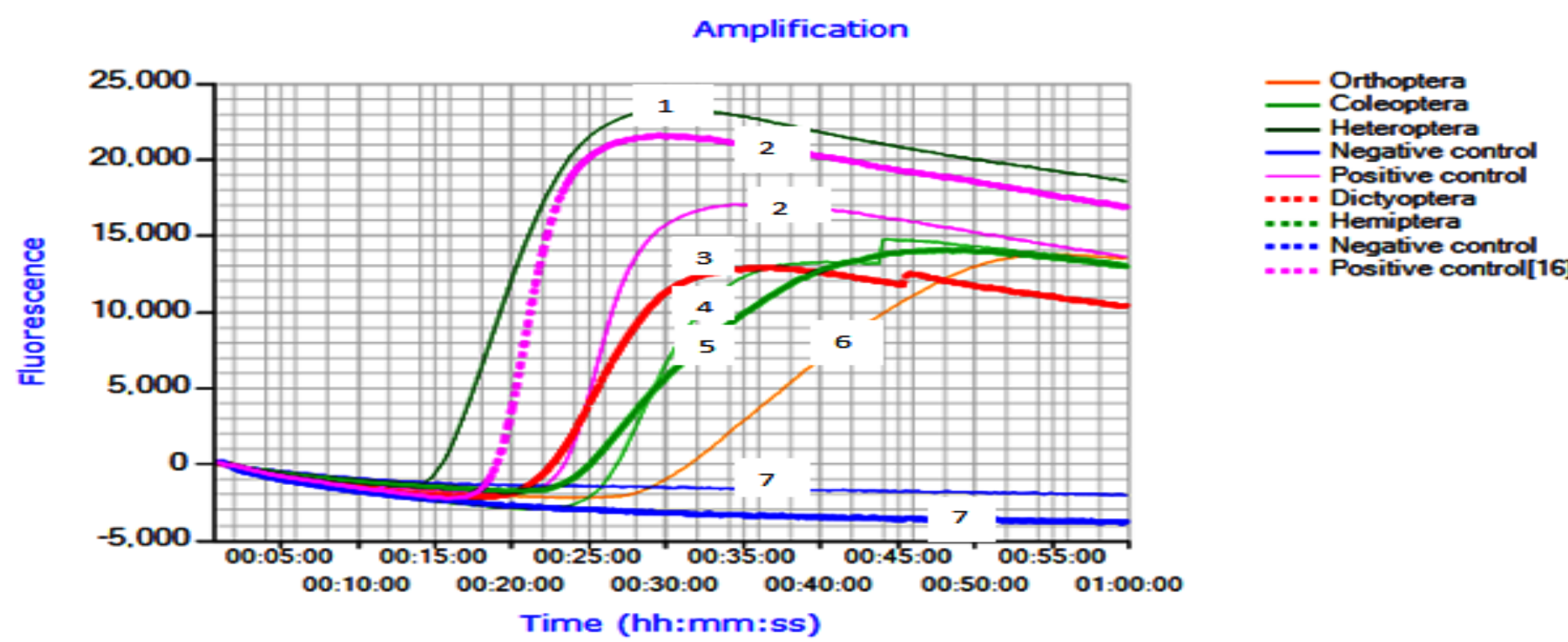
The LAMP technique was employed (Notomi et al., 2000). The DNA extracts were run in the Gene II qPCR. The running time was set at 60 minutes at Preheat: 40°C, Amplification: 65°C and Anneal: 98°C to: 80°C. The primers used for the LAMP assay; F3 BCS4, B3 BCS4, FIP BCS4, BIP BCS4, LoopF BCS4 and LoopB BCS4 (Mayazaki et al., 2018)



Procedural summary



Result



Loop-mediated isothermal amplification in real time showing phytoplasma detected on the insect heads collected in the field. (1) = Heteroptera (*Irantha sp.*); (2) = positive control; (3) = Dictyoptera (blattidae)- *Margattea spinosa*; (4) = Coleoptera (curculionoidae) - *Longitarsus nigrocillus*; (5) = Hemiptera (pentatomoidea); (6) = Orthoptera (eumastacoidea)- *Cardiodactylus novaeguineae*; and (7) =Negative control.

- The five insects detected as phytoplasma positive after LAMP assay. The images of these insects are included for clear understanding



Orthoptera (Eumastacoidea).

Common name: Grasshopper

GPS coordinate: 06°14’ 45.6 S; 146° 12’ 48.3 E



Dictyoptera (Blattidae).

Common name: Cockroach

GPS coordinate: 06°14’ 45.6 S; 146° 12’ 48.3 E



Hemiptera (Pentatomoidea).

Common name: Unknown

GPS coordinate: 06°14’ 45.6 S; 146° 12’ 48.3 E



Coleoptera (Eurculionoidae).

Common name: Weevil

GPS coordinate: 06° 19’ 39.6 S; 146° 34.3 E



Heteroptera.

Common name: Unknown

GPS coordinate: 06°14’ 45.6 S;

146° 12’ 48.3 E

Summary of LAMP Annealing of insects’ hosts of BWAP

Graph Name	Well Number	Well Name	Peak Value (mm:ss)	Temperature (°C)
Wells A				
AMP Time	2	Orthoptera brown	30:41	87.6
AMP Time	4	Coleoptera	27:30	86.6
AMP Time	5	Heteroptera	17:15	87.3
AMP Time	6	Negative control		
AMP Time	7	Negative control		
AMP Time	8	Positive control	25:15	86.8
Wells B				
AMP Time	9	Dictyoptera	25:45	87.1
AMP Time	12	Hemiptera	24:38	87.0
AMP Time	14	Negative Control		
AMP Time	15	Negative Control		
AMP Time	16	Positive control	20:30	87.0

Discussion

- Cicadellidae, Fulgoridae, and Psyllidae carries mycoplasma-like organism (MLO) (Banttari & Zeyen, 1979). Presently it is called mollicutes (Lee et al, 2000).
- Causal agent identified via analysing the 16S rRNA gene using real-time PCR (Davis et al, 2015).
- The detection BWAP on insect vectors relies mainly on molecular biological methods such as analyzing the 16S rRNA gene (Miyazaki et al., 2018; Lee et al., 2016; Kruh et al., 2014 and Davis et al., 2012;).
- However; we have analyzed the 16S rRNA of the insects collected from Antiragen and Orori village gardens especially the v2, v3 and v6 hypervariable regions with the specific primers suggested by Miyazaki et al. (2018) and detected Orthoptera (Eumastacoidea), Coleoptera (Eurculionoidae) and Dictyoptera (Blattidae) as new hosts of BWAP. If the *gyr β* was analyzed together with 16S rRNA gene would have been able to identify more new hosts as suggested by Rodrigues et al. (2003).

Conclusion

Hand pick method and mosquito nets trap are ideal for trapping insects. Other insects taxon can be hosts of BWAP as the results showing Orthoptera, Coleoptera, Heteroptera, Dictyoptera and Hemiptera. Therefore other insects apart form three known superfamilies' can also host mollecutes that cause BWAP.

References

1. Cassis, G., Reid, C., Symonds, C., Mathews, H., Namyatova, A., Jackson, G., Lam, S. & Burleigh, M. (2016). *Entomology methods and diagnostics manual* (Vol. 4). Port Moresby: University of Papua New Guinea.
2. Davis, R., Kokoa, L., Jones, M., Mackie, J., Constable, F., Rodoni, C., Gunua, G. & Rosse, B. (2012). A new wilt disease of banana plants associated with phytoplasmas in Papua New Guinea (PNG). *Australasian Plant Disease Notes*, 7(1), 91–97.
3. Davis, R., Henderson, J., Jones, L., McTaggart, A., Dewyer, C., Tsatsia, F., Fanai, C. & Rossel, J. (2015). First record of a wilt disease of banana plants associated with phytoplasmas in Solomon Islands. *Australasian Plant Dis. Notes*, 1-6. doi:10.1007
4. Lu, H., Wilson, W., Ash, G., Woruba, S. & Fletcher, M. (2016). Determining putative vectors of the Bogia Coconut Syndrome phytoplasma using loop-mediated isothermal amplification of single - insect feeding media. *Science Report*, 6, 35801. doi:10.1038
5. Kruh. L., Weintraub, P., Daube, N., Robinson, W., Perlman, S. & Zchori-Fein, E. (2013). Novel Rickettsiella Bacterium in the Leafhopper *Orosius albicinctus* (Hemiptera: Cicadellidae). *Applied and Environmental Microbiology*, 79(14), 4246-4252. doi:10.1128
6. Miyazaki, A., Shigaki, T., Koinuma, H., Iwabuchi, N., Rauka, G., Kembu, A., Saul, J., Watanabe, K., Nijo, T., Maejima, K., Yamaji, Y. & Namba, N. (2018). Candidatus Phytoplasma noviguineense', a novel taxon associated with Bogia coconut syndrome and banana wilt disease on the island of New Guinea. *International Journal of Systemic and Evolutionary Microbiology*, 68, 170-175: www.microbiologyresearch.org
7. Notomi. T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. & Hase, T. (2000). Loop Isothermal Amplification of DNA. *Nucleic Acid Research*, 28(12), 1-7.
8. Rodrigues, J., Stenico, M., Gomes, J., Lopes, R. & Tsai, M. (2003). Detection and Diversity Assessment of Xylella fastidiosa in Field-Collected Plant and Insect Samples by Using 16S rRNA and gyrB Sequences. *Journal of Applied and Environmental Microbiology*, 69(7), 4249-4255. doi:10.1128