UNIVERSITY OF CAPE COAST

PREVALENCE OF MEALYBUG WILT OF PINEAPPLE DISEASE AND THE ASSOCIATED VIRUSES

JOSEPH NYARKO

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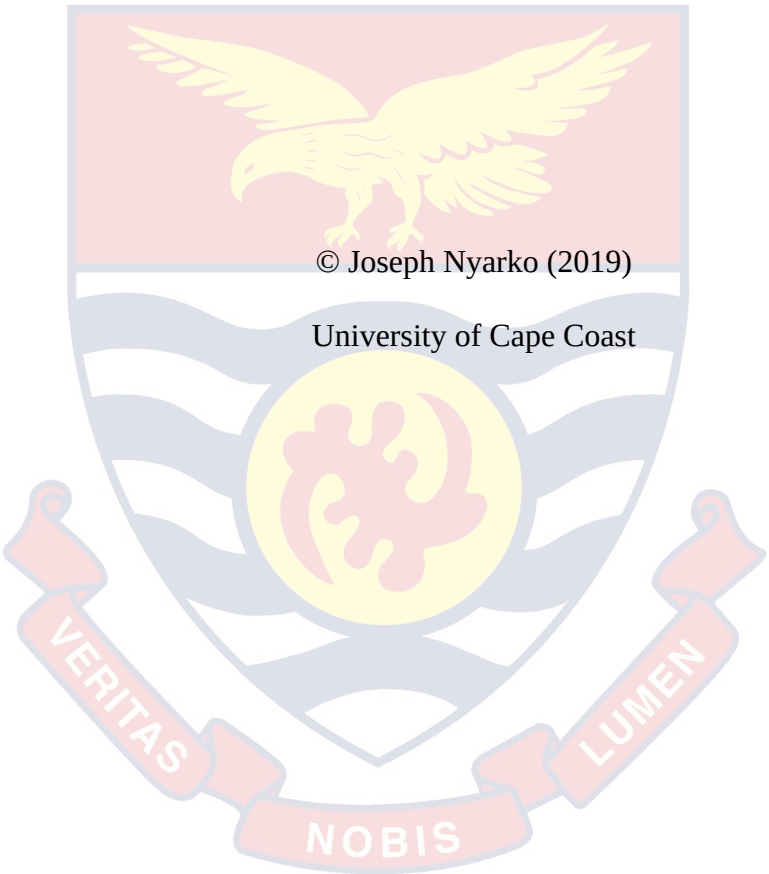

UNIVERSITY OF CAPE COAST

PREVALENCE OF MEALYBUG WILT DISEASE OF PINEAPPLE AND THE ASSOCIATED VIRUSES

BY

JOSEPH NYARKO

Thesis submitted to the Department of Crop Science of School of Agriculture of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Crop Science

AUGUST 2019

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:..... Date:.....

Mr. Joseph Nyarko

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:..... Date:.....

Prof. Elvis Asare-Bediako

Co-Supervisor's Signature: ..... Date:.....

Dr. Grace Van der Pujie

ABSTRACT

The study assessed the awareness of mealybug wilt of pineapple (MWP) farmers and their disease management practices in the Komenda-Edina-Eguafo-Abirem (KEEA), Abura-Asebu-Kwamankese (AAK), and Ekumfi districts in the Central Region of Ghana. The study also surveyed the incidence and severity of the MWP disease in pineapple fields across the three districts in 2018. Household data were collected using structured questionnaire from 180 respondents and analysed using descriptive and inferential statistics. Incidence and severity scores of MWP disease were determined from twenty (20) pineapple farms selected from each of the three districts. The field data was subjected to analysis of variance (ANOVA) and the means separated with least significant difference (LSD) method at $P<0.05$. The majority of the farmers (88.9%) had knowledge on the existence of the disease in their farms and its effect on yield. Majority of farmers used their own planting materials (59.4%), practice monocropping (67.8%), do not apply any fertilizer (78.9%), do not manage the disease on their farms (58.4%), fallow plots (63.9%) and mother plots (55.6%). Incidence of MWP disease differed significantly between pre- and post-induction growth stages and among the three districts ($p<0.05$), ranging from 2.20±0.46 to 9.45± 1.10%. Soil fertility status of the farms was inherently low. Five qRT-PCR assays with pineapple mealybug wilt associated virus (PMWaVs) species specific primers successfully detected five species of PMWaVs, namely PMWaV-1, PMWaV-2, PMWaV-3, PMWaV-4 and PMWaV-5, with abundance of mixed infections. Phylogenetic analyses with 15 sequenced isolates from the study and some published sequences from GenBank, confirmed the presence of PMWaV-1, -2 and -3 in the Central region.

KEY WORDS

Farmers

Incidence

Mealybug wilt of pineapple

Prevalence

RNA extractio

Virus

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DEDICATION

I dedicate this work to my daughter, Hadassah Nyamekye Teyki Nyarko

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LIST OF ACRONYMS

AAK	Abura-Asebu-Kwamankese District
ANOVA	Analysis of Variance
CGR	Crop Growth Rate
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	The Food and Agriculture Organization
GEPA	Ghana Export Promotion Authority
GSS	Ghana Statistical Service
HSP 70	Heat Shock Proteins
IPM	Integrated Pest Management Approach
ISEM	Immunosorbent electron microscopy
KEEA	Komenda-Edina-Eguafo-Abrim district
LAMP	Loop-mediated Isothermal Amplification
LSD	Least Significance Difference
MEGA	Molecular Evolutionary Genetics Analysis
MoFA	Ministry of Food and Agriculture
MWP	Mealybug Wilt of Pineapple
NGS	Next-Generation Sequencing
PMWaVs	Pineapple Mealybug Wilt-associated Viruses
RNAs	Ribonucleic Acid
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
RT-qPCR	Quantitative Reverse-Transcription Polymerase Chain Reaction

CHAPTER ONE

INTRODUCTION

Background to the study

Pineapple (*Ananas comosus* L. Merrill), a Bromeliaceae, is the third most important fresh fruit crop after citrus and banana worldwide (Usman *et al.*, 2013). Pineapple is widely thought to have originated in Southern Brazil and Paraguay, where its wild relatives occur (Morton, 1987). The crop is presently cultivated throughout the world in tropical and subtropical regions.

Pineapple contributes to over 20 % of the world's production of tropical fruits and about 70% of the pineapple is consumed as fresh fruit in producing countries (Medina and Garcia, 2005). In 2014, global pineapple production exceeded 24 million tons with its world trade representing more than US$7 billion. Brazil, Philippines, and Thailand are the leading producers worldwide whilst in Africa, Côte d'Ivoire, Nigeria, Ghana, and Kenya are the main pineapple producing countries (Ministry of Food and Agriculture (MoFA), 2006).

In Ghana, the pineapple sector is the most developed horticultural sector (Kleemann and Abdulai, 2012; Kuwornu and Mustapha, 2013). The industry remains the active labour force and the biggest industry, employing up to 42 percent in 2010 of the labour force (Ghana Statistical Service, 2012). Pineapple production is a source of income for thousands of people ranging from small-holder farmers to large-scale farmers and market women. The crop provides raw material to feed industries, leading to the establishment of cottage industries. Currently, a fruit juice processing factory is being established at Ekumfi Nanaben to process pineapple produced in the area. This is in response to the

"one district one factory" policy by the government of Ghana. Pineapple is a non-traditional export crop cultivated mainly in the areas of Central, Greater Accra, Eastern and Volta regions of Ghana, in small and medium scale. Overall, pineapple production contributed more than USD 283,000,000 in foreign exchange to the economy of Ghana between 1990 and 2013 (GEPA, 2014).

Pineapple is mostly used in the form of fresh fruits, canned chunks, or slices, and about 95% of canned pineapple is produced from the smooth cayenne variety. Pineapples are also used as an ingredient in a variety of food such as pizza, condiments, sweets, yoghurt, ice cream, cakes, and pastries. (Bartholomew *et al.*, 2003; Rohrbach *et al.*, 2002; d'Eeckenbrugge and Leal, 2003; Medina and Garcia, 2005). It contains vitamins A, B, B6, C, pantothenic acid, manganese, copper, dietary fibre and also the best source of copper, folate, and a rich source of the protein-digesting enzyme bromelain.

Statement of the Problem

Notwithstanding the economic importance and health benefits of pineapple crops, its production has progressively declined over the years. This decline in pineapple production could be due to numerous factors including post-harvest constraints, poor soil fertility, poor agronomic practices, lack of high-quality propagules, pests, and diseases (Thresh, 1983; 2003).

Mealybug wilt of pineapple (MWP) disease is among the major diseases of pineapple in the world which causes up to 100% yield losses (Sether and Hu, 2002). It is caused by a pineapple mealybug wilt-associated virus (PMWaV), a member of the family *Closteroviridae*, and the genus Ampelovirus. Pineapple mealybug wilt-associated virus-1 (PMWaV-1), PMWaV-2, PMWaV-3, PMWaV-4, and PMWaV-5 are the five distinct species identified in Hawaii,

Australia and Cuba from diseased pineapple fields (Sether *et al.,* 2001, 2005; Gambley *et al.,* 2008). These viruses are transmitted by two species of mealybugs namely the gray pineapple mealybug (*Dysmicoccus neobrevipes*, (Beardsley), and the pink pineapple mealybug (*Dysmicoccus brevipes* (Cockerell), and also by man through the planting of infected planting materials (suckers or slips crowns). These mealybugs have a symbiotic association with the ants. The ants help the mealybugs in the foundation of mealybug settlements and consume the honeydew created by the mealybugs and can suppressively affect the natural enemies of mealybugs (Jahn, 1992; Petty and Tustin, 1992; Rohrbach *et al.,* 1998; Jahn *et al*., 2003). MWP disease symptoms are portrayed by serious tip dieback, descending curling, reddening, and wilting of the leaves which can prompt a complete breakdown of the plant (Sether and Hu, 2002). However, these problems create high demand for pineapples on the local and international markets due to the inadequate or lack of healthy planting materials to mark the current demand both locally and worldwide.

Justification

Effective management of MWP disease is quite important to improve the production of pineapple and also save the pineapple industry in Ghana. There is however limited information on effective management of MWP disease in Ghana. Information on the temporal and spatial prevalence and severity of the MWP disease and farmers' perception and management of the disease are important prerequisites for developing effective strategies for managing the disease in pineapple crops.

Main Objective

The aim of the study is to collect information that will be useful in developing an effective management strategy against MWP disease

Specific objectives of the study are to:

1. Conduct a household survey to determine farmers' perception of MWP disease and their disease management practices.
2. Determine the incidence and severity of the MWP disease in pineapple crops in the Central region of Ghana.
3. Detect and identify the viruses responsible for MWP disease in the Central region.

CHAPTER TWO

LITERATURE REVIEW

Factors Affecting Pineapple Production

Numerous factors affect the production and yield of pineapple. The principal factors include agronomic, abiotic, and biotic factors (Baruwa, 2013).

Agronomic Factors

Like any other crop, pineapple needs good cultural practices to guarantee high yields. Some of the cultural practices that could guarantee high yield include proper soil preparation, proper planting with excellent planting equipment, weed control, intercropping, and harvesting at the appropriate time (Kuwornu and Mustapha 2013; Iwuchukwu *et al.*, 2017).

Traditional (smallholder) farmers hardly follow the recommended agronomic practices for pineapple production. This makes them unaware of the existence of improved varieties. The use of unimproved varieties together with inadequate size and age of planting material and incorrect plant population, depth, and time of planting, are among the reasons why yields under most traditional systems are low. This is because since the use of good planting material that is free from diseases are important aspects of pineapple production; the planting material must be harvested from healthy mother plants provided the suckers are not within 1 m2 perimeter of an infected mother plant (Iwuchukwu *et al.*, 2013, 2017; Sarpong *et al.*, 2017).

In traditional systems, land preparation starts before the onset of the rainy season and consists of clearing the vegetation and burning it. On sandy soils there is little land preparation; farmers merely slash weeds and plant pineapple suckers in relatively undisturbed soil. Pineapple is mainly

intercropped with cassava, plantain, maize among others. However, these crops are known to reduce the yield of pineapple (Donkoh and Abgoka, 1995). Pineapple is now mainly cultivated as a single crop (monoculture) due to the growing demand for it. In pineapple farms where this practice persists has increased the level of soil erosion (Kuwornu and Mustapha, 2013; Iwuchukwu *et al.*, 2017).

Abiotic factors

Major areas for pineapple cultivation are found to have a wide range of edaphic and climatic conditions between 30o N and 30o S latitude, with some areas considered marginal for various reasons (Bartholomew and Malézieux, 1994). Pineapple is grown in regions from sea level to altitude of 1,100 m, mostly considered marginal for other crops and so long as the area is free from frost and has high atmospheric humidity and an average rainfall of 760-1,000 mm (Ficciagroindia, 2007). The most important abiotic factors that affect pineapple production are temperature, rainfall, and solar radiation.

Temperature

Pineapple grows favourably under annual temperature ranging from a minimum to maximum temperatures of 15-200C and 25-320C respectively with the optimum being close to 300C during the day, and 200C at night (Nakasone and Paull, 1998). However, different optimum temperatures are required for different growth stages, for instance, root elongation required an optimum soil temperature of 290C, 320C for leaf elongation, 20-300C for fruit weight, and 290C for growth development (Nakasone and Paull, 1998). Higher temperatures are associated with a greater crop growth rate (CGR) and high photosynthetic rate and the high sensitivity of photosynthesis to temperature points to the need

for genotypes more tolerant to low temperature, which could be used in the highland tropics and subtropics. Temperature also affects sprouting, leaf size, leaf formation, storage root formation, and, consequently, general plant growth. Frosts and night temperatures below 7-100C for a few hours for several weeks cause leaf-tip necrosis and fruit injury and also at an extreme temperature leaf burnt, yellowing, and wilt (Nakasone and Paull, 1998; Williams *et al.*, 2017).

Rainfall

The rainfall requirement in areas where pineapples are grown ranges from 600 mm to over 3500 mm annually, with optimum from 1000-1500 mm for good commercial production (Nakasone and Paull, 1998). Pineapple plants can withstand long periods of drought (xerophytic), as the leaves have water-storage parenchyma that serves as a water reservoir. Pineapple requires a potential evapotranspiration rate of 4.5 mm daily. They can also survive in soils with water holding capacity rarely exceeding 100 mm, the water supply for these crops could be exhausted within 3-4 weeks (Nakasone and Paull, 1998). In cases where the crops were subjected to prolonged water stress, plants were not able to obtain the desired size needed for flower induction.

Solar Radiation

Pineapple is a crop that requires high solar radiation to photosynthesize more efficiently. Its development is affected by the shading of the crop. There is a direct association between fruit weight and solar radiation intensity thus yield decreases by about 10% with every 20% decrease in solar radiation (Nakasone and Paull, 1998). Consequently, shading at higher plant densities leads to a linear decrease in fruit weight and curvilinear decrease in yield (Nakasone and Paull, 1998; Williams *et al.*, 2017).

Intense sunlight, especially during fruit maturation can lead to sun scalding of the fruit. In order to avoid these disorders, several methods including shading the crop with newspaper and weeds, spraying a reflective coating on the fruit, and painting the side exposed to the afternoon sun with lime paste were used. Low solar radiation can lead to limited photosynthesis, thus, most photosynthates are utilised for shoot growth, which affects the development of storage root significantly, as a result of the shoots been stronger sink than roots and could lead to yellowing of the pineapple crops (Nakasone and Paull, 1998).

Soil Fertility and pH Requirements

Pineapple can grow in hot-to-moderate temperatures and are sensitive to waterlogged soils. Pineapple requires good drainage and aeration with a general pH ranging from 5.0 to 6.0. The flavour quality of the fruits of pineapple grown in light soils is considered superior to those grown on good drainage and aerated soils. However, pineapple can be grown on sandy and loamy soils that are rich in humus. Hence, the ideal soil condition for pineapple growth could be found in tropical lowlands, hot dry, and hot humid ecosystems (Ficciagroindia, 2007).

Among the cultural practices adhered to in pineapple, production is fertilizer application. According to Evans et al. (2002), Nitrogen (N) and potassium (K) are the two components applied in large quantities to pineapple on the farms, and relatively lower quantities of iron (Fe), phosphorus (P) and calcium (Ca). There are various reports on pineapple nutrition indicating that the total amount of N applied ranges from about 4 to 8 g per plant (300 to 600 kg/ha at a density of 75,000 plants/ha) (Paulle and Duarte, 2011; Leon and Kellon, 2012).

In commercial pineapple production, a small amount of N is applied before planting and the remainder is used as a foliar application, usually at fortnightly intervals after the plants are established. The amount of K applied usually ranges from 225 to 450 kg/ha, and it is usually applied to the soil before planting and later maybe side dressed whiles 20 ppm in the soil is considered adequate for P as reported by Leon and Kellon, (2012). Fertilizer requirements for Pineapple can be obtained by analysing elements immobilised in the various plant parts. Thus, large amounts of N and K are found in the plant, fruit, and slips. In ratoon fields, which develop on suckers on the mother plant, nutrients removed by the first fruit crop must be replenished. The approximate amounts of nutrient requirements are 175 kg N, 27 kg P, 336 kg K, 47 kg Ca, and 27 kg Mg per hectare (Paulle and Duarte, 2011).

Biotic Factors

The biotic factors that affect pineapple production in Africa include pests, diseases, and weeds. These constraints have contributed to significant yield losses in pineapple production (Rohrbach and Mau, 2002). There are many insects and pathogens recorded on the pineapple plant and out of these, only a fraction is of economic importance (Donkoh and Abgoka, 1995; Gumi and Aliero, 2012).

Pest of Pineapple Production

Numerous pests attack pineapple crop but the severity of damage varies with location and vector population. The occurrence of these pests also depends on the environmental conditions, the susceptibility of the cultivar, and the presence or absence of the organism. A high population of pineapple pests at

the various stages in the pineapple life have varying impacts (Rohrbach *et al.*, 2002).

There are pests of pineapple that are found both above and below the ground. Below the ground pests that feed on the roots of pineapple include symphylids and grubs. Pests that feed on the shoots and fruit of pineapple include mites, scales, Lepidoptera larvae, fruit borers, and midges. The most important pests of pineapple, in terms of damage and yield reduction, are mealybugs and symphylids. Several of the arthropod pests cause uneconomic damage problems to mature fruit, rendering the fruitless desirable in the fresh market and also reduce the quantities in yield per hectare.

Pineapple scales

The pineapple scale, [*Diaspis bromeliae* (Kerner)] from the order Hemiptera (*Coccoideae*) is a very little living organism (1–2 mm) and found on upper leaf surfaces of pineapple leaves and fruit around the world (Broadley *et al.*, 1993). Scale insects are most often sedentary as adults while the first instars are mobile and known as crawlers' and their movement is by wind (Beardsley and Gonzales, 1975; Jahn and Beardsley, 2000). Adult female scales are quite often stationary and for all time attached to the plant. Adult male more often has wings (depending on their species) yet never feed, and die in a day or two. A symptom of an attack is rust coloured spots and yellow spots may form on leaves when scale densities are low (Broadley *et al.*, 1993). The insect is found beneath a discharge, which aids as a shield. The scale of the defensive protective layer is made up of a partially waxy discharge of the insect and incomplete shed skins.

Scale insects can be found on the fruit and leaves of pineapple and are most severe on ratoon crops (Rohrbach and Johnson, 2003). In planting material

(crowns, slips, or suckers), huge populace densities of scale can result in the desiccation of the planting material and render the material useless (Broadley *et al.*, 1993). Scale insects at high population densities are capable of killing pineapple plants. When scales reach high populations on the fruit, unwanted and undesirable cracks may form between fruitlets.

Scale insects can be controlled by cleaning the planting material. Chemical dips can also be used but initial attention should be directed to the selection of clean, scale-free planting material. Biological control with natural enemies is successful in many pineapple growing areas (Rohrbach and Johnson, 2003). Tiny wasps and ladybirds can be used as biological control, and ladybird beetles can be quite successful if the scale population is low at planting (Rohrbach and Johnson, 2003).

Pineapple weevils

The pineapple weevils in the order Coleoptera and the family *Curculionidae* are the most widely recognised insects that are disseminated around the world. Their eggs are oval, dull, white, and semi-transparent. The female weevils lay eggs in an opening inside the plant part, mainly, the base of the crown or base of the shoots. It attacks plant parts like a crown, flower stalk, fruit, and leaf. They feed on leaves causing necrotic edges.

Once a while the fruit they attack gets rotten (Salas and O'Brien, 1997). It influences the typical development of the fruit as a result of causing the absence of crown which leads to exudation of a gelatinous material that is protective of the insect. It shows symptoms of marks on leaves, leaf browning, and causing deterioration of the base of focal leaves due to the feeding of adults (Larson and Frank, 2000). They can be managed by planting in shade-free areas

or application of pesticides such as Malathion or Diazinon can help reduce the effect of the pest.

Pineapple thrips

Pineapple thrips (*Holopothrips ananasi*) in the order Thysanoptera (*Phlaeothripidae*) is a significant pest of pineapple which causes serious damages to pineapple (Cavalleri and Kaminski, 2007). There are about 39 species of thrips worldwide in and around pineapple fields (Rohrbach and Johnson, 2003). They are little (1.5 mm long), brown insects with light yellow hind wings that show up as a yellow line down the back of the body when the insect is very still. Mature thrips have transparent wings with an edge of hairs around the outside edge emerging in a similar plane as the wing. They feed mainly on the flower and the crown of fruit resulting in concentric ring patterns on the crown leaves.

Thrips can move extraordinary distances with the wind, and high humidity is significant for their action. Thrips fundamentally feed on the plant sap by damaging the leaves. They feed by piercing individual cells and sucking the content and the in cells lose their ordinary colours, and when numerous contiguous cells are damaged, the tissue shows up as whitish spots or silvery spots or streaks. The primary sign of the damage is a silver-flecked leaf surface which in serious cases turns brown. These leaves cannot adequately photosynthesise and they show little dark spots on the leaves.

Removal of weeds and crop rotation with mulching of crops reduces the invasion of thrips extensively since thrips are most prevalent during the dry season. Furthermore, irrigation may also decrease the thrips population. The economic threshold for small scale farmers is when 20 percent of the plants are

infested with thrips. Plants that have a natural repellence to thrips, for example, citronella, garlic, and pyrethrum are additionally planted as obstructions (Bartholomew and Malézieux, 1994; Rohrbach and Johnson, 2003).

Pineapple red mite

The pineapple red mite, in the order Dolichotetranychus floridanus and the family *Acarina* is bright orange to red coloured and larger. They are constantly found on the white basal segment of the leaves, especially on the crown of the plant. Severe damage leads to the appearance of dark brown abrasions which may cover almost the basal white tissue leads to necrosis and death of the leaves.

The adult and nymph cause damage to leaves and fruit by sucking the sap. Plants that are infested early in their growth remain small and fruit production is either curtailed or non-existent. Intensely infested plants may die before producing fruit. Feeding brings about drying and breaking of epidermal tissue which allows fungal and bacterial infection of the plants and causes tissue rot, scarring, and tissue malformation (Petty *et al.*, 2002).

Effective management activities include the planting of mite-free plant materials, monitoring of flower initiation, and early fruit harvest. Routine application of pesticides and minimal fertilizer treatments are critical for successful control of the population densities of *D. floridanus* (Bartholomew and Malézieux, 1994; Rohrbach and Johnson, 2003).

Pineapple mealybug complex

Mealybugs, which belong to order Hemiptera and the family *Pseudococcidae is the* most common and important insects of pineapple. The two most important mealybug species found on pineapple are *Dysmicoccus*

brevipes and *Dysmicoccus grassii*, the pink and grey mealybugs, respectively. These two were initially thought to be different strains of the same species, (*D. brevipes)* but Beardsley (1959) later discovered that the two were different species and hence proposed the name *D. neobrevipes* for the gray mealybug. However, according to Mau and Kessing (2007), D. *brevipes* (Cockerell) (the pink pineapple mealybug) reproduces only by parthenogenesis.

These pineapple mealybugs are small, elliptical in shape, soft-bodied sucking insects with 17 pairs of wax filaments (Tanwar *et al.*, 2008). They are delicate-skinned insects with waxy secretions, which give their body surfaces a chalky appearance. They are mainly observed as colonies excess of 20 individuals (Bartholomew *et al.*, 2003).

The life cycle of *D. brevipes* according to Ito (1938) indicates that the insect goes through three larval stages before becoming an adult. The life span (first instar to death as an adult) varies from 78 to 111 days, averaging 95 days. The larvae, called "crawlers", are the primary dispersal stage in all mealybug species. They have flattened bodies with long hairs that aid in their dispersal by wind. They remain protected underneath the body of the mother for a short time before developing a waxy covering. The larvae which moult three times before reaching the adult stage goes through first, second, and third instars or larval stages which last 10 to 26 days, 6 to 22 days, and 7 to 24 days, respectively. The total larval period varies from 26 to 55 days, averaging about 34 days. The larvae only feed as a first instar and in the early part of the second instar (Mau and Kessing, 2007).

The mealybugs have various host ranges of which the pink pineapple mealybugs attack more than 140 plant species throughout the tropical and

subtropical parts of the world whereas grey pineapple mealybugs have a narrow host range, with smaller geographical distribution, infesting approximately 50 plant species (Williams and Willink, 1992). In addition to bromeliads, some important hosts of pink pineapple mealybugs recorded are banana, *Musa paradisiaca* L., sugarcane, *Saccharaum officinarum* L., Annona, celery, citrus, coffee, cotton, Euphorbia, Gliricidia, Hibiscus, Hilo grass, mulberry, Natal soursop, nutgrass, orchid pineapple, Straussia (Mau and Kessing, 2007). The grey pineapple mealybugs have a more restricted host plant range, which includes century plant, *Agave sisalana* L. (Agavaceae), in addition to pineapple and the bananas (Jahn and Beardsley, 2000).

The effect of these insects on pineapple production is that *Dysmococcus* species are the vectors for transmitting the PMWaVs. The pink pineapple mealybugs commonly feed on the roots, leaves, stems, fruit, and crowns of pineapple, whereas gray pineapple mealybugs infest only the aerial roots, stems, fruit, and crowns of pineapple (Jahn and Beardsley, 2000; Paull and Rohrback, 1985). Mealybugs initially show up on roots and make it hard to handle in its initial stages. The roots stop developing and result in a breakdown of the tissue of roots. They are also found on the aerial parts of the plant, mostly in the leaf axils and on the forming fruits. Mealybugs feed on plant sap in the phloem of their host plants.

As the population builds up, mealybugs turn out to be increasingly destructive since the bugs suck the sap from leaves bringing about wilting manifestations. The accumulation of honey excreta in large quantities promotes the growth of sooty mould which impedes the photosynthesis in the leaf bringing about rotting and dripping of fruits. The secretion of honeydew by the

mealybug pulls in ants and favour the growth of sooty mould. When fruits are severity infested, they become completely covered with white, waxy covering making it unfit for selling. Leaves seem pale green to yellow streaks and tips become brown. The predominant symptoms the wilting of leaves which begins from the leaf tips. Mealybug causes quick wilt displaying yellow or red leaves under substantial invasion or moderate wilt in minor attack because of root damage. (Rohrbach and Johnson, 2003).

Ants are known to transport homopterans and they have a symbiotic relationship with the mealybugs and these include ants transporting mealybugs from plant to plant between and within fields, ants protecting mealybugs from natural enemies. Ants protect mealybugs from adverse weather by building earthen shelters around them and moving them to protected places. Ants also stimulate the increased feeding by mealybugs and also remove honeydew produced from mealybugs, thereby preventing fungi from attacking mealybugs (Rohrbach *et al.*, 1988). According to Rohrbach et al. (1988), the ants feed on the honeydew and could benefit mealybugs by preventing the accumulation of honeydew on the mealybugs themselves and also immature mealybugs get stuck in honeydew and dying.

Management of pineapple mealybug (Taniguchi *et al.*, 2005) hence reported that controlling the ants will control the mealybug colonies, which is the predominant means for checking the wilt. Ant populations can be monitored using trap stakes baited with peanut butter/ soybean oil, pitfall traps, honey vial traps, or pineapple juice traps. Chemical treatments applied to the entire field or along the perimeter of a field are a very effective control for ant (Rohrbach *et al.*, 1988; Reimer and Beardsley 1990; Rohrbach and Mau 2002). However, it

was shown that if ants are controlled, predators keep mealybugs under control (Taniguchi *et al.*, 2005). Ants may also be managed through cultural methods such as row placement, control of weeds along field borders, tillage, and elimination of volunteer plants during the intracycle period (Jahn *et al.*, 2003). Pre-planting treatments of suckers are successful in controlling pineapple mealybugs, suckers can be treated with pesticides such as insecticide or botanical extracts in organic agriculture.

Mealybug has numerous natural enemies that suppress its population and its spread can be limited by destroying the ant population which protects mealybugs. All the plant deposits or residues in a field infested with mealybugs ought to be collected and incinerated. Weeds present on the field should be cleared since if present help in the multiplication of mealybug by serving as food resources. The use of sterile equipment in an un-infested field is desirable in the control of pineapple mealybugs (Rohrbach and Mau, 2002).

Diseases of Pineapple Production

There are many diseases affecting pineapple production worldwide, the most prominent ones include fungal diseases, bacterial diseases nematode, and viral diseases (Evans *et al.*, 2002).

Fungal Diseases of Pineapple

Fungal pathogens of pineapple encompass soil-borne and aerial diseases that infect the pineapple during production, as well as pathogens that cause postharvest problems of pineapple fruits; these include butt rot, heart rot, root rot, and fresh fruit rot

Butt rot

Butt rot is a fungal disease caused by *Ceratocystis paradoxa* in the family *Ceratocystidaceae***.** The fungus is widespread in the tropics and found on pineapple, banana, cacao, coconut, and sugarcane (Hubert *et al.*, 2014; Rohrbach and Johnson, 2003). Butt rot is most serious on pineapple planting material and infection may lead to rot of the entire planting piece or the entire planting material pile. Butt rot is associated with a soft rot and blackening of the basal part of the pineapple stem tissue (Rohrbach and Johnson, 2003). The disease incidence may reach 100% with plants becoming stunted due to the loss of stem tissues that contain carbohydrate reserves and the initial roots.

Management of butt rot entails good agricultural practices; planting material should be properly cured before planting. Curing the planting material on the mother plants provides good air circulation and minimises exposure to inoculum in the soil. Pre-plant fungicidal dips may similarly be used (Wijeratnam *et al.*, 2006; Wijensighe *et al.*, 2011).

Heart rots

Heart rots are caused by several Stramenopiles (Oomycetes) including *Phytophthora nicotianae*, *P. cinnamomi*, and *P. palmivora.* Heart rots are associated with wet environmental conditions. Plant loss due to heart rot can reach 100% depending on the soil type, pH, and rainfall (Rohrbach and Schmitt, 2003). Heart rots caused by *P. nicotianae* and *P. palmivora* occur at warmer lower elevations compared to *P. cinnamomi* that occurs at cooler higher elevations (Rohrbach and Schmitt, 2003).

Infected plants will initially show a failure of young leaves to elongate, progressing to the leaves showing yellow colour, which is easily pulled from

the plant. Ultimately the entire centre and growing point of the pineapple rot away. Infection occurs from the sporangia produced from chlamydospores that are spread to plants by splashing soil or wind dispersal.

Management of heart rot involves cultural practices, the application fungicides, and cultivar selection. Improving soil drainage through raised beds or digging ditches to enhance drainage reduces disease incidence. Removal of pineapple trash, through tillage or burning, generally decreases disease incidence. Fungicide applications, especially fosetyl aluminum, are effective pre-plant dips and post-plant foliar applications (Sipes and Wang, 2017). Some pineapple cultivars have been identified with resistance to *Phytophthora* and may provide a source for future breeding work (Rohrbach and Schmitt, 2003).

Root rots

Root rots of pineapple may be caused by one of several Stramenopiles. *Phytophthora cinnamomi, P. nicotianae,* and *Pythium spp.* are the causal agents of root rot in pineapple. Initial symptoms of root rot are a reduction or elimination of growth with subsequent reddening of the leaves, yellowing, and death of leaf edges. With *P. cinnamomi* that causes heart and root rot, the root rot stage results in decreased plant development, yields, and, in cooler situations, can result in a complete loss of the ratoon crop (Rohrbach and Schmitt, 2003).

Root rots are mostly extreme when soils are cold and ineffectively depleted. If the environment and soil conditions become dry after the infection, affected plants may seem reddish as though under serious drought stress. Plant anchorage in the soil is poor after the loss of roots. Root rot can be controlled by improving soil water management through the use of raised beds, deep

cultivation and improving surface water drainage. Applications of the fungicide fosetyl aluminum, as a pre-plant crown dip or spray application, can effectively control Stramenopiles (Paul and Rohrbach, 1985).

Bacterial diseases of pineapple

Bacterial diseases that are known to cause harm to pineapple fields include marbling disease, bacterial heart rot and pink disease.

Marbling disease

Marbling disease of pineapple occurs in the hot, humid lowland tropics. Its symptoms include a brown granular appearance in the fruit. It is caused by *Acetobacter peroxydans* and *Pantoea ananas*. Marbling bacterial infection occurs through flower opening (Rohrbach *et al.*, 1988). The bacteria are ubiquitously existing on the plant and disease depends upon entry into the pineapple flower. The bacteria remain latent in the flower until about one month before the fruit matures (Rohrbach *et al.*, 1988). Management is by limiting bacterial introduction into the flower by insects, wind, or splash.

Bacterial heart rot

Pineapple bacterial heart rot is caused by an agent *Dickeya* sp. (*Erwinia chrysanthemi*). The bacteria infection is characterised by soaking water in areas of young leaves in the centre whorl. These water-soaked areas exhibit brown streaks and blister-like lesions and within a few days, the meristem is dead (Kaneshiro *et al.*, 2008). Ants, wind, and wind-blown rain introduce the bacteria into the stomata of nearby pineapple plants (Kaneshiro *et al.*, 2008). Injury from mite feeding or chemical burns can also provide entry for the bacteria (Rohrbach and Johnson, 2003). Sanitation is significant in preventing the entry of the bacterial into new areas and in preventing low incidences of bacterial heart rot.

Infected plants should be destroyed or removed from the field (Rohrbach and Johnson, 2003).

Viral diseases of pineapple

The viral diseases that cause serious harm to pineapple plants include yellow spots and Mealybug wilt of pineapple.

Yellow spots

The yellow spot of pineapple is a common viral disease across pineapple producing areas in the world. It is caused by the tomato spotted wilt virus (TSWV). Thrips (vector) transmits TSWV. Infection initially starts with a slightly raised yellowish spot with a darkened centre and later, spots develop in a line that progresses to a basil leaf and stem rot. Infection usually occurs on young pineapple plants but the crown of a developing fruit may also become infected. The disease mostly results in the death of the pineapple plant. Eliminating weeds that serve as TSWV reservoirs and are hosts to thrips, reduce inoculum, and vector presence, thus protecting the pineapple crop (Rohrbach and Johnson, 2003; Joy and Sindhu, 2012).

Pineapple Mealybug Wilt Disease (MWP)

Overview

Mealybug wilt of pineapple (MWP) disease is among the most prevalent and devastating pineapple diseases in the world (Rohrbach and Johnson, 2003). It is the major cause of financial loss in commercial production of pineapple and it is a bane to commercial production of pineapple in those areas where the disease is not properly managed (Hughes and Samita, 1998; Petty and Tustin, 1992).

It is a complex disease involving mealybugs, ants, and a virus. The disease is caused by pineapple mealybug wilt associated virus (PMWaVs), a Closterovirus that spread through active feeding of mealybug (Sether and Hu, 2002). It was originally revealed that wilt disease was caused by ants. However, Illingworth (1931) identified the relationship among ants and mealybugs as well as the cause of MWP disease and reported that the mealybugs are carried by the ants and that the mealybugs were thought to have injected an agent during feeding and to suppress the virus tolerance of the pineapple plant (Sether and Hu, 2002; Jahn *et al.*, 2003).

In the production of pineapple, MWP has a long history. MWP was a limiting factor that contributed to yield decrease in all pineapple-growing areas around the world. Closterovirus from infected pineapple plants was identified over 50 years after mealybugs were associated with MWP disease (Gunasinghe and German, 1989). MWP was first referred to as "fast" wilt (Rohrbach *et al.*, 1988), however, Carter in 1910 described the two types of MWP disease as slow wilt and rapid wilt, and now fast wilt is widely known as mealybug wilt (Carter, 1932).

The literature indicates that symptoms appear in slow wilt after mealybugs have been feeding on the crops for several months. Leaves on the inside turn brown and dry while leaves on the outside drop. With 'quick wilt', symptoms are noted within two months after feeding by a large number of mealybugs. Within six months the symptoms of plants vary from very light, dull green to pale blue or purple colour in the internal leaves; the leaves lose stiffness, the tips of the leaves colour change to brown and dry; and when six months old, the leaves turn red leaves and finally turn to purple. Some plants

show signs of recovery, however, the crops that recover, the leaves dry up and die and do not produce fruit, or form small fruit in both fast and slow wilt. Illingworth (1931) reported that wilt-affected pineapples continue to spread the disease from the leaves to the roots, causing root damage.

Effect of MWP disease on pineapple

MWP disease may be caused by one or more viruses, but there is no evidence of this yet. Several viruses detected in pineapple crops have been studied, reportedly causing yield losses ranging from trace to nearly 100% (Sether and Hu, 2002). It is also reported that the disease is very serious in pineapple production and that if a plant shows any signs of wilt, the suckers or slips crowns should not be used as planting material, and where possible, the wilting plants should be pulled and destroyed. Also, selecting planting materials at a distance of the one-meter radius of an infected mother plant, as a source of planting material, should be avoided. Thus, choosing planting material from the PMWaV-free mother plant is very essential (Joy and Sindhu, 2012). Joy and Sindhu (2012) indicated that if less than 3 percent of pineapple plants show wilt symptoms, those affected are pulled out and destroyed. They argued that if in a field more than 3 percent wilt symptom is detected, that individual plants are destroyed and a mealybug control spray programme is also implemented. Furthermore, if more than 10% of crops in a field show symptom of PMWaVs early, the plants should be destroyed and planting material should not be taken from that field, even though wilt control appears to be efficient (Joy and Sindhu, 2012).

Taxonomy and geographical distribution.

MWP occurs worldwide in all the major pineapple production areas (Sether and Hu, 2002). A report in the early twentieth century was that once the relationship between mealybugs and MWP was established phytotoxins were released by the insect when feeding. This vector comprises two types of mealybug, the pink pineapple mealybug, (*Dysmicoccus brevipes*) (Cockerell), and the grey pineapple mealybug, (*D. neobrevipes*) (Beardsley), (Illingworth, 1931; Sether *et al.*, 1998). These mealybugs have a symbiotic relationship with ants. Subsequent research implicated a latent transmissible factor, which is most likely as a virus, as the cause of MWP disease (Ito, 1962).

Gunasinghe and German (1989) detected in filamentous virions typical of a member of the family *Closteroviridae* (subsequently referred to as *closterovirids*), and dsRNA, a replicative intermediate of many genera of plant viruses. Viruses related to MWP disease are associates of the genus Ampelovirus, family *Closteroviridae* (Gunasinghe and German, 1989; Hu *et al.*, 1992). However, there is very little available information on the MWP disease and its causes in Ghana, and Africa as a whole.

The mealybugs association with wilt disease.

Mealybugs were first reported to be the main pests of pineapple. Illingworth (1931) provided proof that mealybug-feeding causes wilt disease symptoms in pineapple. Feeding by the long-tailed mealybug results in wilt symptoms in the laboratory, but was not associated with mealybug wilt epidemics in pineapple fields (Jahn *et al.*, 2003; Rohrbach and Johnson, 2003). Sether and Hu (2002) also found that wilt only develops in plants infected with a closterovirus that are also exposed to mealybug feeding.

Association of ants with mealybugs and MWP

Ants are important pests not because they damage the pineapple directly but because they are related to mealybugs and MWP. There are at least 28 distinct types of ants that are related to mealybugs on pineapples (Jahn *et al.*, 2003; Rohrbach and Johnson, 2003). The most common genera of ants related to pineapple mealybugs worldwide are the Pheidole and Solenopsis (Jahn and Beardsley, 2000).

Studies by many scientists sought to establish the association between ants, mealybugs, and MWP even before the virus was implicated in the disease etiology. It was uncovered that there is a mutualistic relationship between ants and mealybugs, with the ants playing a key role in dispersing mealybugs from an alternate host or older pineapples to newer plantings of pineapple. The ants offered protection to mealybugs against their natural enemies discouraging the parasitoid from infecting the mealybugs, which in return feed on the honeydew rich in amino acids and sugars secreted by mealybugs. Removal of the honeydew from mealybugs by ants also prevents fungi from attacking the mealybugs (Rohrbach and Johnson 2003).

According to Sether and Hu (2002), there is a direct association between high incidences of MWP disease and high populations of mealybugs. Mealybug is capable of transmitting PMWaVs and the ants help carry mealybugs from one plant to another plant within a field, and from different fields (Illingworth 1931; Rohrbach and Schmitt, 2003; Sether and Hu 2002). Thus, it has been reported that if ants are controlled, predators will keep mealybugs under control (Jahn *et al.*, 2003; Taniguchi *et al.*, 2005).

Association of viral particles to MWP

The association of viral particle and MWP has been severally explained, hence, there was a proposed theory via Carter (1960) that mealybug wilt was a toxemia reaction. Thus, the saliva of mealybugs is lethal to the plant, and based on these observations there was a relationship between mealybug populations, effective feeding on pineapple plants, the time spent feeding, and the onset of wilting symptoms.

Further studies revealed that the feeding of a large population of mealybugs generally results in wilt symptoms, hence healthy plants ended up infected when mealybugs were transferred to them from symptomatic plants. Thus, the presence of "transmissible factor" was strongly suggested in the etiology of MWP disease (Carter, 1960 Rohrbach, and Johnson, 2003; Jahn *et al.*, 2003; Taniguchi *et al.*, 2005).

According to Ito (1962), a dormant virus could be transmitted and could multiply in vegetatively propagated pineapple plants, and also those that recovered from MWP symptoms were immunised to future disease in his field experiments. Unfortunately, serological techniques to detect different strains of the virus were not available to confirm at that time (Jahn *et al.*, 2003).

Diversity of the pineapple mealybug wilt associated viruses

Many scientific researches have shown that the virus was once named "pineapple closterovirus" (PC or PCV) and was later renamed as the "pineapple mealybug wilt associated virus" (PMWaV). These viruses were perceived as a complex virus isolate, having long, flexuous, and rod-shaped virus particles. Based on this particle morphology and the presence of multiple, high molecular weight, double-stranded RNAs (dsRNAs) in MWP-symptomatic plants, viruses

were confirmed to be present in plants infected with MWP. MWP-symptomatic pineapple plants in Australia, Hawaii, and Cuba (Gunasinghe and German, 1986; Hu *et al.*, 1992).

The genera of these viruses are separated based on virion morphologies, genome organisation, and vector-transmission properties. It was also proposed that these viruses from the family *Closteroviridae* and genus Ampelovirus and other genera in the family *Closteroviridae* are *Closterovirus, Crinivirus*, and *Velarivirus* (Agranovsky, 1996) be further separated into two clades. This is based on the distinction in phylogeny and genome organisation between GLRaV-3 (type individual of genus Ampelovirus) to which PMWaV-2 is closely related and other Ampeloviruses such as PMWaV-1 and PMWaV-3 (Gambley *et al.*, 2008; Sether *et al.*, 1998, 2009).

Generally, these PMWaVs are currently recognised as a complex of viruses belonging to five recognised species, designated as pineapple mealybug-wilt associated viruses thus PMWaV-1, PMWaV-2, PMWaV-3, PMWaV-4, and PMWaV-5. The identification of more than four related but genetically distinct viruses in pineapple is similar to Grapevine leaf roll-associated viruses identified in grapevines (Gambley *et al.*, 2008; Melzer *et al.*, 2001, 2008). Gambley et al. (2008) also explained the relationship of PMWaV-1, PMWaV-2, or PMWaV-3 with MWP disease in Australia. Furthermore, PMWaV-2 was not observed to be common in Australian plantings. Moreover, double infections with PMWaV-1 and - 3 or single infection with PMWaV-3 were observed to be related to MWP symptom development. Another species (PMWaV-5) that is most closely identified with PMWaV-1 has likewise been

found in Australia (Ullman *et al.*, 1989; Gambley *et al.*, 2008; Melzer *et al.*, 2001, 2008).

Transmission and interaction of PMWaVs

Studies in acquisition and transmission of PMWaVs from infected plants to healthy plants were made possible through the development of monoclonal antibodies (MAbs) for PMWaVs and the use of tissue-blot immunoassays (TBIAs) and immunosorbent electron microscopy (ISEM) with PMWaVs-antibodies. Recently, the development of a sensitive and reliable reverse-transcription polymerase chain reaction (RT-PCR) assays allow the evaluation of the acquisition and transmission by infected mealybugs. These technologies have enabled the ready detection of PMWaVs in MWP-affected pineapple plants. Transmission of PMWaVs from MWP-affected plants to healthy plants is influenced by several factors such as environmental conditions, mealybug populations, pineapple genotype, and activities of man during the planting of the suckers. Some pineapple plants could remain asymptomatic even though they are infected with PMWaVs (Hu *et al.*, 1992; Melzer *et al.*, 2001 Gambley *et al.*, 2008; Sether *et al.*, 2009). Dey et al. (2018) reported that active mealybug feeding on PMWaV-2 infected plants developed MWP disease, which means that PMWaV-2 species alone without the others could cause MWP. They additionally showed that almost all pineapple plants with MWP symptoms on the field had PMWaV-2 infection but diseases with PMWaV-1 or PMWaV-3 were not related to MWP developing symptoms.

In a similar report, it was indicated that PMWaVs were transmitted by two pineapple mealybug species namely *D. brevipes* and *D. neobrevipes.* The relationship among infection with PMWaV-2, active mealybug feeding, and

MWP symptoms development shows that there was some component from insect source or perhaps from an endogenous organism present inside the insect together with PMWaV-2 that is transferred into the plant during mealybug feeding. The incidence and levels of PMWaVs infection of pineapple are also found to differ based on the level of tolerance or susceptibility of the pineapple cultivar, the source of planting materials (the mother field cleaned from MWP disease), growing locations and the activities of man which include the selection of planting materials (Melzer *et al.*, 2001; Sether *et al.*, 2005; Gambley *et al.*, 2008).

Detection of pineapple mealybug wilt associated viruses (PMWaVs)

Contextual study in the field of plant pathology has increased our level of knowledge of diseases. However, currently, our understanding of the disease has progressed with the technologies available. There were reports on evidence since the early 60s that show that virus is involved in MWP etiology, however, they could not be demonstrated until the approach of electron microscopy. In the 1990s, there was great advancement of the knowledge of the association and distributions of the virus in the field with the development of serological techniques that were used in the detection of PMWaV-1 and 2. It was used in the rapid screening of thousands of pineapple plants grown worldwide due to the robustness of the specific monoclonal antibodies developed against these PMWaVs (Melzer *et al.*, 2008; Gambley *et al.*, 2008). However, due to the very low titers of PMWaVs in plants in the 1980s, a specific reverse transcription-PCR (RT-PCR) techniques were developed to detect the presence of PMWaVs in plants with or without symptoms. RT-PCR has been employed to screen large numbers of pineapple samples, and it is also used to verify assays after using

ELISA (Enzyme-Linked Immunosorbent Assay) or TBIA (Tissue Blot Immunoassay) samples.

In recent years, quantitative (real-time) qPCR assays have been developed to allow accurate quantification of PMWaVs titers in different pineapple plants. According to Dey et al. (2018), TBIA allowed hundreds of samples to be processed directly in the field with little preparation an indication that it is very practical for the screening of large-scale PMWaVs. However, it cannot detect the virus when the concentration is low. TIBA, the sample can be prepared and blotted in the field and then shipped to a laboratory for testing instead of transporting infected planting material for testing. Loop-mediated isothermal amplification (LAMP) techniques have also been developed and useful for PMWaVs detection.

Furthermore, single-tube dual primer PCR (STDP-PCR) using nested PCR primers was developed to identify low titers of PMWaV-2 that are underneath the levels identified with RT-PCR (Fujiwara and Ikeshiro, 2017; Piyasak and Peerasak, 2010; Dey *et al.*, 2012, 2015; Adams *et al.*, 2009). Studies show next-generation sequencing (NGS) is an extremely amazing tool that can detect the identities of virus(s) present, this may help during the process of clarifying the etiology of diseases, where the viruses are complex, for example, GLRaVs and PMWaVs. It is used in finding the identities of diseases of unknown etiology. For an instance, Coetzee *et al.* (2010) reveal how a whole viral profile in diseased vineyards was created using NGS and also identified a new GLRaV-3 variant not previously known as earlier reported by Adams et al, (2009) and Al Rwahnih et al. (2009).

Management of mealybug wilt in pineapple

Chemical approaches

Virus diseases cannot be controlled once the plant is infected. There are no synthetic chemicals available to control MWP. Therefore, every effort should be made to prevent the introduction of virus diseases into the farm. Chemical approaches can be effective in managing MWP in pineapple production through the controlling of the vector mealybug and its associated ants.

However, it is not easy to control mealybugs by chemical means. Thus, it is difficult to completely cover a pineapple plant with an insecticide, since mealybugs tend to hide deep in leaf axils, under the sepals of flower or inside of closed flower cups, where they are protected from insecticidal sprays. Mealybugs have thick and waxy coats which make insecticide penetration difficult. Mealybugs sometime developed resistance to many of these chemicals over time. However, most effective and widely used class of insecticides used in reducing mealybugs populations and have been effective in reducing the incidence of PMWaVs infected pineapple plants are of neonicotinoids class and this includes Thiomethoxam, Imidacloprid, and Dinotefuron (Carter *et al.*, 1996; D'Eeckenbrugge *et al.*, 2011).

Biological Approaches

The use of biological controls for managing the disease in pineapple growing areas aim at controlling the mealybug and the ants. However, these reductions will occur when an ant's population are controlled since the growth of mealybug populations is influenced by the myriad of natural enemies found in pineapple fields (Gonzalez Hernandez *et al.*, 1999). For instance, reports by,

Carter et al. (1996) reveal that many parasites and predators were introduced to Hawaii to control pineapple mealybugs. These include *Lobodiplosis pseudococci* Felt (Diptera: *Cecidomyiidae*), *Nephus bilucernarius* Mulsant (Coleoptera: *Coccinellidae*), and *Anagyrus ananatis* Gahan (Hymenoptera: *Encyridae*). However, none of these parasites and predators provided adequate control of mealybugs in the presence of ants (Carter *et al.,* 1996). Hence, efforts to control pineapple mealybugs biologically without the management of the ants, have proven to be unsuccessful (Rohrbach *et al.*, 1988).

The natural enemies of ants have centered on the control of *Solenopsis* spp. to control the mealybugs. Additionally, biological control agents such as *Anagyrus ananatis*, can help in maintaining the populations of mealybug below economic thresholds (Rohrbach and Mau 2002; Hughes *et al.*, 2002; Jouvenaz *et al.*, 1981).

Sanitation

Sanitation is the primary means of controlling virus diseases since old pineapple plants are one of the best-known reservoirs of PWMaVs. Older pineapple plants can support the reproduction of mealybugs and PMWaVs replication (Carter *et al.*, 1996; Dey *et al.*, 2018). Since old plants or weeds are not ideal hosts for mealybugs, the mealybugs produced on these plants are likely to be migratory morphs that are more likely to leave the field after emergence in search of better quality hosts by the help of the ants, wind and the activities of man. Therefore, the infected field should be rogued immediately to prevent the spread of the pathogens (Trienekens *et al.*, 2004; Anon, 2005).

Alternative host plants should be destroyed and planting pineapple next to cassava, maize, banana, sugarcane, celery, citrus, coffee, cotton, Euphorbia,

Gliricidia, Hibiscus, or other vegetables should be avoided since they are susceptible to these diseases (Mau and Kessing, 2007). Pineapple propagules can be covered with insect-proof nets in the mother field, which would protect the propagules from mealybugs attack. Volunteer weeds should be removed before major planting is done (Trienekens and Willems, 2007).

Use of resistant cultivars

The use of PMWaV - resistant pineapple cultivars, when available, is the best approach to reduce losses due to infection by PMWaVs. Resistant or improved commercial cultivars are available in a limited number of genotypes. Progress in the introgression of PMWaVs resistance has been slow. This is due to linkage with poor fruit quality, complex inheritance patterns, and the difficulty of transferring the resistance to commercial cultivars due to the presence of interspecific barriers between the wild and domesticated pineapple species (Anon, 2005). The lack of advanced screening techniques for PMWaVs limits the search for true sources of resistance. However, tissue culture technique in association with thermotherapy can be used to clean PMWaVs - infected plants, thus use to control MWP.

Integrated pest management approach

Integrated pest management approach which combines different pest and disease management methods such as farm sanitation (Carter *et al*, 1996; Dey *et al.,* 2018), proper agronomic practices (Anon, 2005) and use of resistant varieties (Anon, 2005) in combination with pesticides are very useful for effective management of MWP. Insecticidal baits are most widely recognised as an effective way to control ants in pineapple fields (D'Eeckenbrugge *et al.*, 2011; Cherrett, 1986; Hughes *et al.*, 2002; Ko *et al.*, 2013).

CHAPTER THREE

MATERIALS AND METHODS

Introduction

The study involved a household survey to identify farmers' perception of the MWP diseases, their agronomic practices which influence the epidemiology of the diseases and its impact on their income. The study also covered a field survey to assess the incidence and severity of MWP disease in the study area. It also determined the prevalence of pineapple mealybugs wilt disease and its relationship with the mealybugs, ants, and soil fertility status. The study further detected and characterised virus (es) responsible for MWP disease in the Central region (using qPCR with species-specific primers).

The Study Areas

The study was conducted in three districts of the Central region, a leading pineapple production centre in Ghana. The three districts (Komenda-Edina-Eguafo-Abrim (KEEA), Abura -Asebu-Kwmankese (AAK) and Ekumfi) were purposively selected based on the fact that at least one of the three varieties of pineapple (Smooth Cayenne, MD2 and Sugar Loaf) was cultivated in these districts, and also, farmers cultivated at least half an acre of pineapple in each community per district in a planting season. The location, mean annual temperature, mean relative humidity/ rainfall, and the vegetation types of the three districts during the study are indicated in Table 1.

Table 1: Data on Location, Climate and Vegetation types of the three districts collected from Ghana meteorological service during the study

District	Altitude	Longitude/ Latitude	Relative humidity (%)	Temperature (°C)	Rainfall (mm)	*vegetation type
KEEA	31.1	01^{O} 15' W 05^{O} 06' N	86	26-35	9201	coastal zone
AAK	31.1	01^{O} 20' W 05^{O} 05' N	86	22-30	1940.2	deciduous forest
Ekumfi	15.2	00^{O} 37' W 05^{O} 22' N	81	24-28	631.2	coastal savanna

(Source: * MoFA, 2011; Ghana Meteorological Service, 2018)

Perception of Farmers on the Effect of Mealybug Wilt of Pineapple

Population and sampling

The population of the study was smallholder farmers in the pineapple growing area in the Central Region of Ghana.

Reconnaissance survey

I had interaction with the regional and district directorates of the Ministry of Food and Agriculture (MoFA) and also with the extension officers of the districts to get to know the study area**.** Through my interactions with the officials from the regional directorates of the Ministry of Food and Agriculture (MoFA), secondary data such as the common pineapple varieties grown in the districts were collected. Further interactions with pineapple producers and retailers were also made. Other information like the major pineapple growing

areas/communities in each district were collected from the officials and that was also used as a guide in choosing the communities and districts.

Selection of villages /communities

In each of the three districts, four communities were selected and, in each community, fifteen pineapple growing households were selected using purposive sampling methods (Littell *et al.*, 2006). A total of 180 respondents were interviewed. The farmers interviewed consisted of those who had pineapple farms at the time of the study and those who had pineapple farms in the previous year.

Instrumentation and data collection

The research utilised primary data collected using interview schedules by self-administration and also an observation made on the field during the survey. A structured interview schedule with both open- and closed-ended questions was prepared. The questions were written in English and administered in both English and local languages (Akan). The survey questionnaire was made up of four categories of questions that were aimed at identifying the following: 1. The demographic characteristics of respondents; 2. Characteristics of farms affected; 3. What the farmers know about the MWP disease; 4. What they are doing to cope with the disease; 5. What activities may influence the MWP infection levels on their farms; 6. Effect of MWP on their socio-economic development (see appendix 1).

Determination of Incidence and Severity of the MWP Disease in Pineapple Crops in the Central Region

Study area

Field survey for incidence and severity of MWP disease was conducted in pineapple farms (sugar loaf, smooth cayenne, and MD2) in the major pineapple producing areas in Central regions which include Komenda-Edina-Eguafo-Abirem, (KEEA), Abura-Asebu-Kwamankese (AAK) and Ekumfi districts.

Disease assessment

The disease assessment was done at both pre-forcing and post-forcing stages of the 2018/2019 planting season. The disease assessment was carried out in the same communities covered during the household survey. Five farms were selected per community, and in each farm, four MWP-affected plots were purposively selected and two hundred and fifty plants each were asses diagonally and scored for incidence and severity. A total of 1000 plants were assessed per farm thus 250 plants per plot (PIP, 2004). The incidence and severity of the disease were computed using the following formulae:

$$\text{Disease incidence (\%)} = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ observed} \times 100$$

The severity of MWP disease in each field was assessed based on the 0 -5 symptom severity scale developed by Broadley *et al.* (1993) and PIP (2004) (Table 2).

Table 2: Visual scale for assessing the severity of pineapple mealybug wilt (MWP) disease

Disease score	Description
0	No Symptoms (healthy)
1	A slight reddening of the leaves about halfway up the plant. This normally starts in small patches of plants -The Isolated wilt stage.
2	Definite and sudden change in leaf colour from red to pink and the leaf margins turn yellow and roll under, starting at the leaf tips.
3	The leaf tip die-back and affected leaves become limp and droop.
4	The affected leaves dry up for much of their length.
5	Entire plant completely withers and all leaves pulled off from the heart.

(Source: Broadley *et al.*, 1993; PIP, 2004)

Assessing populations of mealybug and ants and incidence of MWP disease on pineapple crops

Estimation of the number of pineapple mealybug and ants per plant and the extent of severity and the incidence of the MWP disease were carried out by selecting 50 MWP disease-affected plants at random per 0.40 ha of each pineapple field following X pattern. Two leaves were pulled from the middle part of each plant and the bases of these leaves examined for mealybug and ant infestations (PIP, 2004). The plants were then gently uprooted with a shovel. Both the roots and the soil around the roots were then examined for both

mealybug and ant infestations. The number of mealybugs and ants found on each part was then counted and recorded. The number of mealybugs per plant was calculated as the mean number of mealybugs on each plant ± standard error of the mean.

For this 50 MWP- symptom plants were selected at random, the incidence and severity of MWP were determined as previously described, to find the relationship between the estimated number of the ants and mealybugs and the extent of disease infections (PIP, 2004).

Soil sampling

Soil samples were collected from all the farms surveyed to find the relationship between the soil fertility status of nutrients (N, P, K, organic carbon contents, pH, bulk density, moisture content, and texture) in soils from each farm and disease incidence and severity. Surface samples (0 -15 cm) were collected from different spots of each farm in the zigzag pattern using a 5 cm diameter coring cylinder auger. At each site, the collected soils were thoroughly mixed and sub-sampled to form a composite sample after all plant debris had been removed. The samples were air-dried and sieved through a 2 mm mesh sieve. The fine earth (< 2 mm) fraction was used for laboratory analyses.

Soil analyses.

Total N concentration in the soil was determined using the micro Kjeldahl method described by Rowell (1994). Available phosphorus was determined using the method of Bray and Kurtz. A soil extract was obtained with 1.0 M NH4OAc (pH 7.0) and exchangeable K concentration in the extract was determined using flame photometry (FAO, 2008). Soil pH was determined by the use of a glass electrode of a pH meter in the soil suspension after the soil

had been shaken for 15 min using a mechanical shaker (Rowell, 1994; McKinney, 1923).).

Data Analyses

The household survey data was cleaned and coded into Statistical Package for Service Solution (SPSS version 20) and then analysed using descriptive statistics (frequency distributions, and percentages), and inferential statistics (chi-square test).

Data on percentage incidence and severity scores of PMW disease from the various fields were subjected to the analysis of variance (ANOVA) and the means were separated using the least significant difference (LSD) method at a 5% probability level. Based on the incidence and severity data independent sample t-test analysis was conducted to determine the relationships of MWP disease on pineapple crops between the pre-induction and post-induction stages. A Scatter plot was also carried out using MS EXCEL to find the relationships between incidence and severity scores at both pre- and post-induction growth stages.

Data on soil fertility status (organic matter, nitrogen, phosphorus, organic carbon, potassium, and CEC), soil pH, and moisture content were subjected to ANOVA and the mean separated by the least significant difference (LSD) method at 5% level of probability. Pearson's correlation coefficients were calculated to determine the relationships between incidence and severity of MWP disease and soil pH, moisture content, organic matter, nitrogen, phosphorus, organic carbon, potassium, and CEC and using GenStat version 12 (VSN International). Apart from the household survey data and the scatter plot,

all other statistical analyses were conducted using GenStat version 12 (VSN International)

Genetic Diversity of Pineapple Mealybug Wilt Associated Viruses (PMWaVs)

Virus isolates using RT-qPCR

Collection of pineapple leaf samples

Twenty four symptomatic leaf samples were collected randomly from the fields during the field survey and the presence of the viruses (PMWaV**s)** were detected by real time-quantitative polymerase chain reaction (RT-qPCR) using species-specific primers. Before the samples were taken, they were clean with 70% ethanol and then placed on ice until analysed.

RNA extraction

Viral RNAs were extracted from leaf tissues of each sample using Quick-RNATM Plant Miniprep Kit (ZymoResearch Corp.) according to the manufacturer's instructions. Pineapple leaf of 0.1 g was ground in a 2 mL ZR BashingBead™ Lysis Tube with 800 µL RNA Lysis Buffer that consists of guanidine thioacyanate and phenol, and it was centrifuged for 1 min.

A volume of 400 µL of the supernatant was transferred into a Zymo-Spin 111CG Column in a collection tube and centrifuged for 30 seconds. Subsequently, a volume of ethanol (95-100%) was added to the flow-through in the collection tube, mixed well, and then tightly covered to avoid splashing and was shaken vigorously using a vortex machine for 15 seconds. The resulting mixture was then transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 rpm for 15 min at 4oC and then 400 µL RNA Prep Buffer was added to the column and centrifuged for 30 seconds. RNA

Wash Buffer (700 µL) was also added to the column and centrifuged for 30 seconds.

A volume of 400 µL RNA Wash Buffer was added to the column and centrifuged for 2 minutes to ensure complete removal of the wash buffer. The column was carefully transferred into an RNase free tube. This was followed by centrifugation at 10,000 rpm for 10 min at 4oC and a volume of 50 µL RNase-Free Water was added directly to the column matrix and centrifuged for 30 seconds. The supernatant was removed and the RNA pellet was washed by adding 1 mL of 75% ethanol per 1mL of Trizol used in the sample preparation. This was mixed gently by inverting the samples a few times.

The samples placed in a new Collection Tube (Zymo-Spin™ III-HRC Filter) and 600 µL Prep Solution was added and centrifuged at 8,000 x g for 3 minutes. The eluted RNA was then transferred into a prepared Zymo-Spin™ III-HRC Filter in an RNase-free tube and centrifuged at exactly 16,000 x g for 3 minutes.

Reverse transcription-polymerase chain reaction (RT-qPCR)

Luna Universal One-Step RT-qPCR Kit (BioLabs Inc.) was used for the RT-qPCR amplification of heat-shock protein 70 genes of PMWaVs, according to the manufacturer's instructions. Briefly, an initial reaction volume of 12.6 µL containing 10 µL of 2× Luna Universal One-Step Reaction Mix, 1 µL of 20x Luna WarmStart RT Enzyme Mix, 0.8 µL of 10 µM reverse primer, 0.8 µL of 10 µM forward primer, was prepared and placed in qPCR tubes. Total RNA template (< 1 µg) was added to the mixture in the qPCR tubes and nuclease-free water was added to make up a final reaction volume of 20 µL. The qPCR tubes were then spun in a centrifuge for 1 min at 2, 500-3, 000 rpm to remove the

bubbles. The tubes were then incubated in a pre-warmed thermocycler (Applied Biosystems StepOnePlus) according to the program reaction conditions indicated in Table 3, and SYBR scan mode setting on the real-time instrument (thermocycler). The primer sequences are shown in Table 4.

Table 3 qRT-PCR reaction conditions

CYCLE STEP	TEMP	TIME	CYCLES
Reverse Transcription	55°C*	10 minutes	1
Initial Denaturation	95°C	1 minutes	1
Denaturation	95°C	10 seconds	40-45
Extension	60°C	30 seconds** (+ plate read)	
Melt Curve	60-95°C*	Various	1

*A 55°C RT step temperature is optimal for Luna WarmStart Reverse Transcriptase.

To ensure best performance and full WarmStart activation avoid using a temperature of < 50°C.

* For Applied Biosystems real-time instruments use a 60 second extension step.

* Follow real-time instrument recommendations for melt curve step.

Gel Electrophoresis

The amplification products were assessed by electrophoresis in 1.5% agarose gels in TBE buffer (89 mM Tris-borate and 2 mM EDTA, pH 8.3) and stained with ethidium bromide using a 2 kb ladder. The gel was then visualised in UV light in a gel documentation system and the gel photograph was then documented for further analysis.

Table 4: Primers used for RT-qPCR detection of PMWaVs species

Primer name	Primer sequence (5' – 3')	Reference
PMWaV-1	F: ACA GGA AGG ACA ACA CTC AC R: CGC ACA AAC TTC AAG CAA T	Melzer *et al.*, (2008); Sether *et al.*, (2009).
PMWaV-2	F: CAT ACG AAC TAG ACT CAT ACG R: CCA TCC ACC AAT TTT ACT AC	Melzer *et al.*, (2008); Sether *et al.*, (2009).
PMWaV-3	F: ATT GAT GGA TGT GTA TCG R: AGT TCA CTG TAG ATT TCG GA	Melzer *et al.*, (2008); Sether *et al.*, (2009).
PMWaV-4	F: GGT ACA GGC CCG ATA AA R: ACT TGG GCG TCG TA	Melzer *et al.*, (2008); Sether *et al.*, (2009).
PMWaV-5	F: ACCGGGAGCTAACAGAGAAV R: CACTCACTTGCTGACCG	Melzer *et al.*, (2008); Sether *et al.*, (2009).

(Source: Melzer *et al.*, 2008; Sether *et al.*, 2009)

Table 5: Pineapple mealybugs wilt associated viruses isolates from GenBank

Name of isolates	GenBank accession Number	Geographic region, country	Genomic region	Authors
PMWaV-1				
KT322148	KT322148.1	Thailand	Partial genome	*Srikumphung and Chiemsombat, (2015)
HQ129930	HQ129930.1	Cuba	Partial genome	Hernandez and Ramos, (2012)
KT322152	KT322152.1	Thailand	Partial genome	*Srikumphung and Chiemsombat, (2015)
EU769113	EU769113.1	Taiwan	Partial genome	Shen *et l.*, (2009)
HG940514	HG940514.1	Thailand	Partial genome	*Koohapitagtam,(2014)
MH704740	MH704740.1	USA	whole genome	Green *et al.*, (2018)
HE583225	HE583225.1	Thailand	Partial genome	*Koohapitagtam and *Hongprayoon, (2011)
EF620774	EF620774.1	Thailand	Partial genome	*Chiemsombat and *Maneechote, (2007)
KC800714	KC800714.1	Mexico	Partial genome	*Ochoa-Martinez *et al.*, (2013)
KJ872494	KJ872494.1	China	whole genome)	Yu *et al.*, (2015)
AF414119	AF414119.3	USA	whole genome)	Melzer *et al.*, (2008)
JX645771	JX645771.1	Cuba	Partial genome	*Hernandez-Rodriguez, (2012)
			PMWaV-2	
KT322167	KT322167.1	Thailand	Partial genome	*Srikumphung and *Chiemsombat, (2015)
FN825676	FN825676.1	Cuba	Partial genome	*Hernandez *et al.*, (2010)
EU769115	EU769115.1	Taiwan	Partial genome	Shen *et l.*, (2009)
MH704741	MH704741.1	USA	whole genome	Green *et al.*, (2018)
HE583226	HE583226.1	Thailand	Partial genome	*Koohapitagtam and *Hongprayoon, (2011)
NC043105	NC043105.1	USA	Partial genome	Melzer *et al.*, (2001)
EU016675	EU016675.1	Thailand	Partial genome	*Chiemsombat *et al.*, (2007)
JX645772	JX645772.1	Cuba	Partial genome	*Hernandez-Rodriguez, (2012)
PMWaV-3				
GU563497	GU563497.1	Cuba	Partial genome	*Hernandez *et al.*, (2010)
MH704742	MH704742.1	USA	whole genome	Green *et al.*, (2018)
NC_043406	NC043406.1	USA	whole genome	Sether *et al.*, (2009)
JX508636	JX508636.1	Cuba	Partial genome	Hernandez-Rodriguez *et al.*, (2012)

(Source: GenBank isolates**)** * Unpublished

Cleaning and sequencing of PMWaV-1, PMWaV- 2 and PMWaV-3

Purified reverse transcription (RT)-qPCR products of fifteen isolates which consist of PMWaV-1, PMWaV-2, and PMWaV-3 were sequence according to Sether *et al.* (2009) and Gambley *et al.* (2008). The isolates were sequenced with both primers reverse and forward. Each of the isolates was sequenced to assess variation within a virus isolate and to ensure consistent and reliable sequence data. The cDNA products of each virus species were sequenced in both directions using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to the instructions of manufacturer:https://www.nimagen.com/products/Sequencing/Capillary-Electrophoresis/BrilliantDye-Terminator-Cycle-Sequencing-Kit/.The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053): http://www.zymoresearch.com /downloads /dl/file /id/52/d4052i.pdf. The cleaned products injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50 cm array, using POP7: https://www.thermofisher.com/order/catalog/product/4406016.

BioEdit v7.0.5 (Hall, 2005) and FinchTV analysis software were used to visualise the sequence chromatogram. And the quality of each nucleotide in the sequence was examined to detect and evaluate changes in nucleotides and for each amplicon construct consensus sequences. By examining the chromatograms of each sequence by eye, polymorphic sites were manually verified. Both primer and non-coding sequences from the alignments were also removed. Additional published sequences obtained from GenBank were verified and added to the data sets (Table 5).

Multiple sequence alignments were made using the ClustalW programme implemented in MEGA version 7.0 (Thompson *et al.,* 1994; Tamura *et al.,* 2011; Chenna *et al.,* 2003). When almost identical nucleotide sequences were acquired from the same plant sample for two or more clones, only one of them was chosen for multiple sequence alignment and phylogenetic analysis (Ala-Poikela *et al.,* 2005). Alignments were also manually altered to guarantee the right reading frames. The analyses included a total of fifteen PMWaV-1, PMWaV-2, and PMWaV-5 nucleotide sequences of HSP 70, and twenty-four published sequence from GenBank.

Sequence comparisons and phylogenetic analyses

For HSP 70 homologous genes of PMWaV-1, PMWaV-2, and PMWaV-3, the nucleotide and the deduced amino acid sequence identities were determined using BioEdit v7.0.5 (Hall, 2005). For HSP 70 homologous genes between PMWaV-1, PMWaV-2, and PMWaV-3 sequence alignments, MODELTEST (Posada and Crandall, 1998) implemented in MEGA version 7 program (Kumar *et al.,* 2016) was conducted to select the most suitable nucleotide substitution model using the Akaike Information Criterion (Akaike, 1974), the Bayesian Information Criterion (Fraley and Raftery, 2002) and the hierarchical probability ratio test. The best-fit nucleotide substitution model was then used for phylogenetic analyses using the maximum likelihood method used in MEGA 7 and the resulting phylogenetic trees were visualised for each of PMWaV-1, PMWaV-2, and PMWaV-3, as well as phylogenetic tree for combined PMWaV-1, PMWaV-2 and PMWaV-3nt sequences with bootstrap analysis done on 1000 trials. The neighbour-joining method also implemented in MEGA 7 was used for comparison.

Genetic diversity

The following genetic diversity indices for all samples of the HSP 70 homologous gene for each of the PMWaVs (PMWaV-1, PMWaV-2, and PMWaV-3) were measured using the DnaSP V.5.0 program (Librado and Rozas, 2009): haplotype diversity (h), nucleotide diversity (π), number of segregating sites (S) and the total number of mutations (Eta).

Determination of Genetic distance and selection pressure

For each of PMWaV-1, PMWaV-2 and PMWaV-3, the overall genetic distance (the number of base substitutions per site from averaging across all sequence pairs in a population) within HSP 70 homologous nucleotide sequence data sets were estimated using the Maximum likelihood model (Tamura *et al.,* 2004). Bootstrap method (1000 replicates) was used to obtain standard error estimates. The analyses were conducted in MEGA 7.

The HyPhy package Maximum Likelihood analysis of the natural codon-by-codon selection technique (Pond and Muse, 2005) implemented in MEGA 7 (Tamura *et al.,* 2011) was used to predict the number of synonymous substitutions inferred per synonymous site (dS) and the number of non-synonymous substitutions per non-synonymous site (dN). These estimates were produced using the joint Maximum Likelihood reconstructions of ancestral states under the defaults Muse-Gaut model (Muse and Gaut, 1994) and the General Time Reversible model (Nei and Kumar, 2000). The dN-dS test statistic was used to detect codons that were under positive.

The overabundance of non-synonymous substitutions shows a positive value for the test statistics. In this case, Kosakovsky and Frost (2005) and Suzuki and Gojobori's (1999) methods were used to calculating the probability

of rejecting the null hypothesis of neutral evolution (P-value). Values of P less than 0.05 are considered significant at a 5% level. The overall ratio dN/dS was also calculated from the mean values of dN and dS to compare the selection pressures acting on the HSP 70 genes of each PMWaV-1, PMWaV-2, and PMWaV-3 species. The gene is under positive (or diversifying) selection when the dN/dS ratio is > 1, negative (or purifying) selection when the dN/dS ratio < 1, and neutral selection when dN/dS ratio = 1.

Neutrality test

Tajima and Fu and Li's D and F statistics were used to test the hypothesis that PMWaVs diversity trends are consistent with the neutral molecular evolution theory (Kimura, 1983; Tajima, 1989; Fu and Li, 1993). The neutral theory of molecular evolution says that the great majority of molecular-level evolutionary modifications are caused by selectively neutral mutants shifting randomly (Kimura, 1983). 10,000 permutations estimated the importance of each test statistic.

CHAPTER FOUR

RESULTS

Household Survey

Demographic characteristics of the respondents

The results of the demographic and farm characteristics of the respondents are shown in Table 6. Out of the 180 pineapple farmers interviewed, 160 farmers representing 88.9% were males, whereas 20 representing 11% were females. Forty-five percent of farmers interviewed were between the ages of 29 years and 38 years. This was followed by 23.9% of farmers and 21.7% in the age range of above 50 years, and between 39 and 48 respectively whereas the least 17 (9.4 %) being those between the ages of 18 years and 28. The majority of the respondents (85%) do farming for a living whilst the others (15%) do other business in addition to farming.

Generally, the majority of the farmers (37.2%) had been in pineapple production for the range of 6 to 10 years. About 26.7% of the farmers had been in the production for 1-5 years whereas 4.4% had less than a year experience in pineapple production, Also, 13.3%, and 7.8% and 10.6%, had been in pineapple production for 11-15 years, 15- 20 years and above 20 years respectively.

The majority (86.1%) of the respondents produce Sugarloaf variety, 8.9% grows MD2 whilst 5.0% grows Smooth cayenne variety.

Table 6: Demographic of respondents

Variables	Frequency	Percentage (%)
Gender		
Male	160	88.9
Female	20	11.1
Total	**180**	**100.0**
Age		
18 – 28 years	17	9.4
29 – 38 years	81	45.0
39 – 48 years	39	21.7
Above 50 years	43	23.9
Total	**180**	**100.0**
Occupation		
Farmer	153	85.0
Fisherman	9	5.0
Businessman	15	8.3
Trader	3	1.7
Total	**180**	**100.0**
Number of years of pineapple cultivation		
Less than 1year	8	4.4
1 - 5years	48	26.7
6 - 10 years	67	37.2
11 - 15years	24	13.3
15 - 20 years	14	7.8
Above 20 years	19	10.6
Total	**180**	**100.0**
Variety of pineapple under cultivation		
Sugar loaf	155	86.1
Smooth Cayenne	9	5.0
MD2	16	8.9
Total	**180**	**100.0**

Source: Field Survey, Nyarko (2018)

From Figure 1, over 32.2% of the respondents (58 farmers) had no education, 25% (46 farmers) had Junior High School Education, 11.7% (21 farmers) had Senior High School Education and MSLC whereas few farmers 8.9% (16) had primary education.

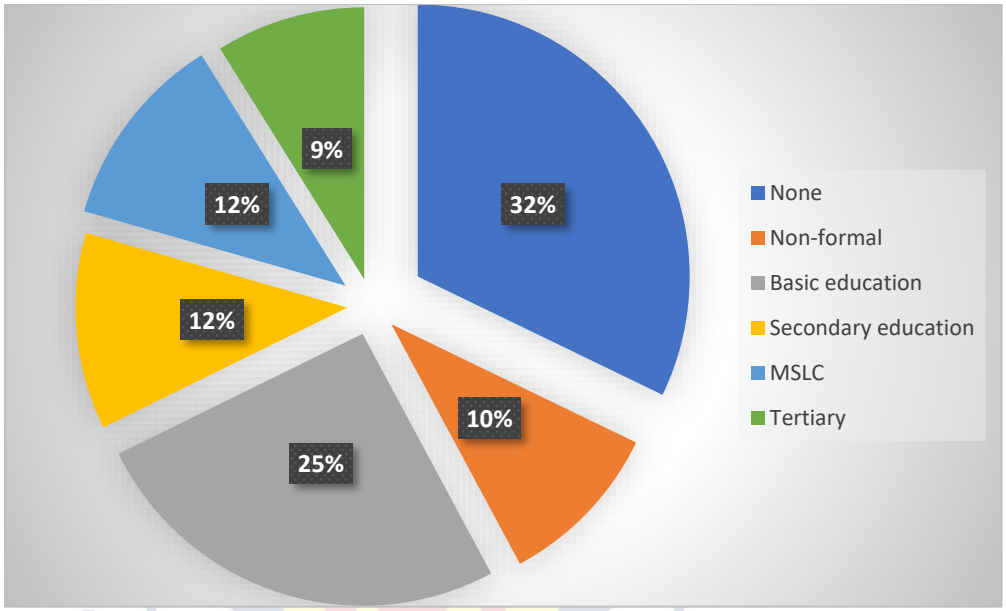


Figure 1: Level of education of respondent pineapple farmers

Farm characteristics of farmers

The majority of the farmers (58.9%) had small farm holdings that were between 0.4 and 1.2 hectares (Table 7). About 18.3% had farms holding less than 1 acre, whereas the other 16.7% have farm holdings between 1.6 -2.4 hectares.

With the types of land ownership among the respondents, 119 of the respondents representing 66.1% were renting lands whilst 32.8% either personally owned or inherited their lands. Most of the respondents (83.3%) hired labour to work on their farms whilst 17.7% relied on either family members or friends. Concerning their source of finance, most farmers (81.7%) responded that they generate their own money for farming activities. On the

other hand, 13.3% said they obtain funds from their customers, whilst 5.0% said they receive funds through banks and family members.

In responding to their source of planting materials, 107 farmers representing 59.4% said they obtain their planting materials from their own farms only, 24.6 % of them got their planting materials both from own farm and from their neighbour's farms whilst 16% of the respondents said they relied only on their neigbours for planting materials.

Table 7: Farm characteristics of respondents

Variables	Frequency	Percentage (%)
Size of land under cultivation		
Less than 0.4 hectare	33	18.3
0.4 and 1.2 hectare	106	58.9
1.6 -2.4 hectare	30	16.7
2.8 – 4.0 hectare	11	6.1
Total	**180**	**100.0**
Land tenure system		
Self-owned	59	32.8
Rent	119	66.1
Sharecropping	2	1.1
Total	**180**	**100.0**
Source of labour		
Hired	150	83.3
Family labour	16	8.9
Nnoboa	14	7.8
Total	**180**	**100.0**
Source of finance		
Self	147	81.7
Bank	6	3.3
Costumers	24	13.3
Family members	3	1.7
Total	**180**	**100.0**
Source of planting materials		
Own source	107	59.4
Other farms	29	16
Own source and Other farms	44	24.6
Total	**180**	**100.0**

Source: Field Survey, Nyarko (2018)

Figure 2 is a graphical presentation of the source of information on pineapple per location for the farmers. The majority of the respondents (52.8%) had their information on pineapple production from other farmers, 17.2% relied on input dealers, 17.8% depended on family and friends whilst the remaining 12.2% received information from the agricultural extension agents (Figure 2).

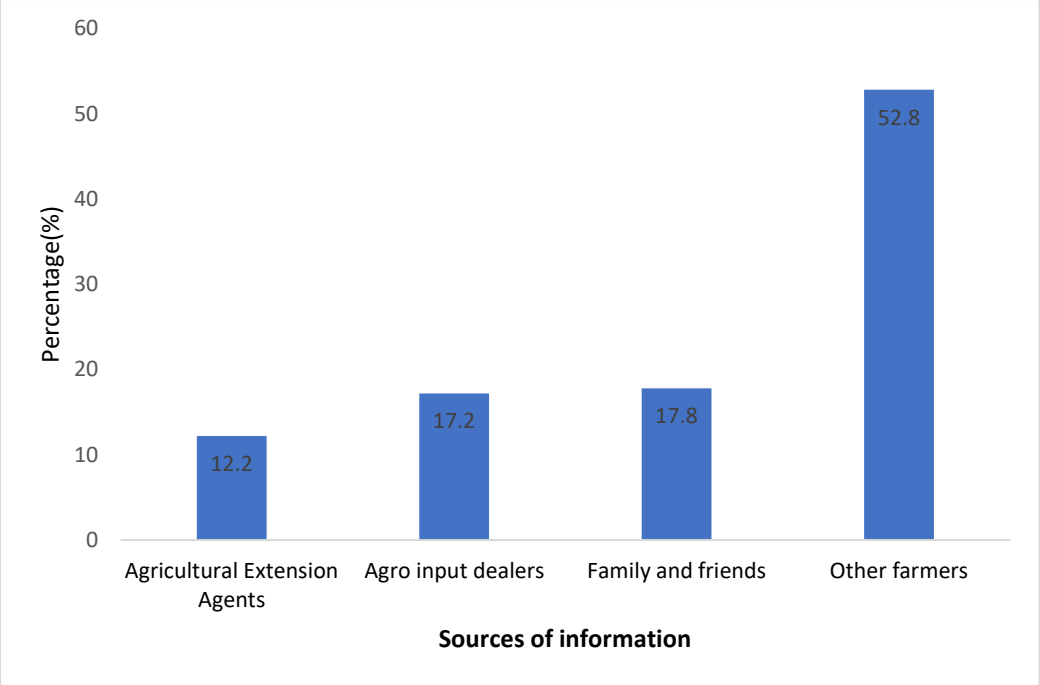


Figure 2: Source of information received by the pineapple farmers

Farmers' Agronomic practices

Table 8 shows the land preparation methods used by pineapple farmers. From the table, the majority of the farmers (68.4%) said they practice slash and burn, 8.3% adopt zero tillage, whilst 23.3% plough their land before planting. A response of farmers as to whether they keep fallow plots, 93.3% of them said they keep kept fallow plots whilst 6.7% said they do not keep fallow plots (Table 8). Among the 168 farmers that kept fallow plots 67.9% responded that they allowed the farms to fallow between 2 and 3 years, Others 22.0% and 10.1% of

the respondents allowed a maximum of 1year duration and 4 to 5 years duration respectively for their fallow (Table 8).

In respect of soil fertility management, 142 farmers representing 78.9% neither used chemical fertilizer nor organic fertilizer on their pineapple farms whilst 21.1% (38 farmers) used both types of fertilizer on their farms (Table 8). Among the farmers who applied both types of fertilizers, the majority (86.8%) used chemical fertilizer, whiles 13.2% applied organic manure. From table 8, it can be seen most farmers (52.6%) used chemical fertilizer because they said it was cheaper; 28.9% said it was more efficient whilst 18.4% said it is easier to apply. Regarding the type of chemical fertilizer, 52.6% used only NPK fertilizer, 2.6% used urea only, and 5.2% apply ammonia only whilst 39.6% used a combination of NPK, urea, and ammonia (Table 8).

The majority of the farmers (73.7%) used the foliar method in the application of fertilizer whilst 26.3% used the broadcasting method in applying fertilizer. When the farmers were asked to estimate the amount of chemical fertilizer used per acre, 20 farmers representing 52.6% said they use between 6 and 10 kg of fertilizer per acre, 13 farmers representing 34.2% apply 1-5 kg fertilizer per acre whilst 13.2% used less than 1 kg of fertilizer per acre. From table 8, it was observed that 52.6% of the farmers applied fertilizer three times, 26.3% applied fertilizer two times whilst 13.2% applied more than three times in a growing season. The majority of respondents (55.3%) indicated that they apply fertilizer 20 days after planting, 31.5% apply fertilizer 28 days after planting whilst 13.2% said they apply it seven days after planting.

Table 8 also shows that the majority of the farmers (67.8%) practice mono-cropping whilst 32.2% practice mixed cropping. Intercrops mainly used

by the farmers were cassava (43.1%), banana/plantain (32.8%), plantain and cassava (22.4%), and maize (1.7%). All the farmers (100%) indicated that they do not practice crop rotation. The majority of the respondents (91.7%) practice major season planting whilst only 8.3% plant during the minor season.

Most farmers (88.9%) indicated that they do not treat the soil before planting new suckers in the earlier seasons whilst only 20 farmers (11.1%) apply soil treatment. These twenty farmers (11.1%) said they treat the whole plot before planting new suckers in an area that had previously been cropped.

Table 8: Agronomic practices employed by respondents

Variable	Frequency	Percentage (%)
Method of land preparation		
Slash and burn	123	68.4
Zero tillage	15	8.3
Tractor plough	42	23.3
Total	**180**	**100**
Keeping of fallow plots		
Yes	168	93.3
No	12	6.7
Total	**180**	**100.0**
Duration of fallow plots / Fallow period		
1 year	37	22.0
2 -3 years	114	67.9
4- 5 years	17	10.1
Total	**168**	**100**
Type of soil amendment applied on pineapple farms		
Do not apply any soil amendment on the farms	142	78.9
Apply fertilizer on the farms	38	21.1
Total	**180**	**100.0**
Type of fertilizer used		
Chemical fertilizer	33	86.8

Table 8 Cont'D

Organic manure	5	13.2
Total	**38**	**100.0**
Reason for fertilizer type		
Cheaper	20	52.6
More efficient	11	28.9
Easy to apply	7	18.5
Total	**38**	**100.0**
Type of chemical fertilizer		
NPK	20	52.6
Urea	1	2.6
Ammonia	2	5.2
All the above	15	39.6
Total	**38**	**100.0**
Method of fertilizer application use		
Broadcasting	10	26.3
Foliar application	28	73.7
Drilling	0	0.0
Total	**38**	**100.0**
Estimate the quantity of chemical fertilizer usage per acre		
Less than 1 kg	5	13.2
1 - 5 kg	13	34.2
6 - 10 kg	20	52.6
Total	**38**	**100.0**
Number of times respondents apply fertilizer		
Once	3	7.9
2 times	10	26.3
3 times	20	52.6
Above 3 times	5	13.2
Total	**38**	**100.0**
Time of fertilizer application after planting		
1 week	5	13.2
2 weeks 6 days	21	55.3
4 weeks	12	31.5
Total	**38**	**100.0**
Farming practice used		
Monocropping	122	67.8
Mixed cropping	58	32.2
Total	**180**	**100.0**

Kinds of intercrops used by the respondents		
Banana/Plantain	19	32.8
Cassava	25	43.1
Maize	1	1.7
Banana/Plantain and Cassava	13	22.4
Total	**58**	**100.0**
Practice of crop rotation		
Yes	0	0.0
No	180	100.0
Total	**180**	**100.0**
Time respondents plant crops		
Major season	165	91.7
Minor season	15	8.3
Total	**180**	**100.0**
Treatment of the soil that has been planted in the earlier seasons before new suckers are planted		
Yes	20	11.1
No	160	88.9
Total	**180**	**100.0**
Soil treatment when replanting on land that has been planted before with an incidence of mealybug wilt.		
Spot treatment	0	0.0
Whole plot treatment	20	100
Total	**20**	**100.0**

Source: Field Survey, Nyarko (2018)

Farmers' awareness and knowledge of MWP diseases

The awareness of farmers and knowledge levels of MWP disease are shown in Table 9. The majority of the farmers (88.9%) indicated that they had observed the MWP disease in their farms whilst only 11.1% did not know of it. Most farmers were able to describe the various symptoms of the diseases like slight reddening of leaves (88.9%), a definite and sudden change in leaf colour (86.1%), tip die-back (88.9%), drying up of leaves (84.4%) and presence of mealybug underneath (88.9%).

From Table 9, out of the farmers who were aware of the disease, 120 (75.0%) of them knew the cause of the disease whilst 40 (25.0%) did not know the cause of the disease. Among the 120 farmers who said they knew the cause of the disease, majority of them 50(41.7%) attributed it to unfavourable climatic conditions, 37.5% attributed it to unfavourable soil conditions, whereas the others (20.8%) knew the disease was transmitted by an insect vector (mealybug).

Farmers' response to how they were able to observe other wilt diseases besides MWP, the majority (48.1%) of them said the leaf colour of the affected plants turns yellow, 16 farmers (10%) said the tips of the leaves become necrotic (dieback) whilst 67 farmers (37.2%) indicated both yellowing and tip necrosis. Regarding the incidence of the disease, most of them (63.8%) indicated that symptoms appear few days after fertilizer or pesticide application or forcing whiles (29.3%) farmers said the symptom do appear before any fertilizer or pesticide or forcing whilst few of them 6.9% also said it appeared any time.

In terms of the growth stage at which farmers observed the symptoms of the disease, 114 farmers representing 71.3% responded that they saw the symptoms of the disease during the induction stage, 16 farmers representing 10% said they saw the disease at the juvenile stage whereas 30 farmers representing 18.7 % said it was at both juvenile and induction stage.

Almost all the responses from the farmers indicated that they knew some of the major pests encountered on their pineapple farm. All (100%) the farmers said they saw ants on the field, 97.2% indicated that they normally see rodents, 83.3 % indicated that they normally see a snail on their farms whilst 94.4% each said they normally encounter mealybug and termite presence underneath the soil

or at the base of the fruits. With respect to the types of pest damage, all the farmers (100%) indicated that the pests either chew or perforate the leaves of the plants, whereas 93.3% of the farmers said the pests cause the wilt disease.

Most farmers (93.8%) indicated that there were relationships between ants and mealybug populations and the incidence of the MWP disease with only 6.2% indicating no relationships. The majority of respondents 74.4% said the higher the ants/mealybug the higher severity of the disease whilst 4.4% said that the ants/mealybug association does not affect the severity of the disease. Other farmers (21%) however said they did not know whether the ant/mealybugs populations have any effect on the level of the disease severity.

Table 9: Farmers' awareness of viral diseases

Variables	Frequency	Percentage (%)
Observed MWP disease on your farm?		
Yes	160	88.9
No	20	11.1
Total	**180**	**100.0**
Description of MWP disease		
Slight reddening of leaves	160	88.9
Definite and sudden change in leaf colour	155	86.1
The leaf tip dies back	160	88.9
Affected leaves dry up	152	84.4
Presence of mealybug underneath	160	88.9
Do you know the causes of MWP disease		
Yes	120	75.0
No	40	25.0
Total	**160**	**100.0**
What causes the MWP disease		
Unfavourable soil conditions	45	37.5
Insects attack (Mealybug)	25	20.8
Unfavourable climatic	50	41.7
Total	**120**	**100.0**
How wilt is observed other than MWP disease		
Yellowing of leaves	77	48.1
burn at the tip	16	10

Table 9 Cont'D

all the above	67	41.9
Total	**160**	**100.0**
Time other wilt symptoms appear		
Few days after fertilizer/agrochemical application /forcing	102	63.8
Before fertilizer/agrochemical application/forcing	47	29.3
At any time	11	6.9
Total	**160**	**100.0**
Stage disease is first encountered		
Juvenile stage	16	10.0
Induction stage	114	71.3
All the Above	30	18.7
Total	**160**	**100.0**
Season disease occurs		
Dry season	116	72.5
Wet season	9	5.0
Both seasons	35	21.9
Total	**160**	**100.0**
Season disease is very severe		
Dry season	148	92.5
Wet season	4	2.5
Both seasons	8	5.0
Total	**160**	**100.0**
Major pests encountered on your pineapple farm		
Ants	180	**100.0**
Mealybug	170	**94.4**
Snail	150	**83.3**
Rodent	175	**97.2**
Termite	170	**94.4**
Type of damage these pests cause to plants		
Chewing	180	**100.0**
Piecing the leaves	180	**100.0**
Wilt	168	**93.3**
Is there a relationship between ants and mealybug population and the incidence and severity of the mealybug wilt virus disease		
Yes	150	93.8
No	10	6.2
Total	**160**	**100.0**

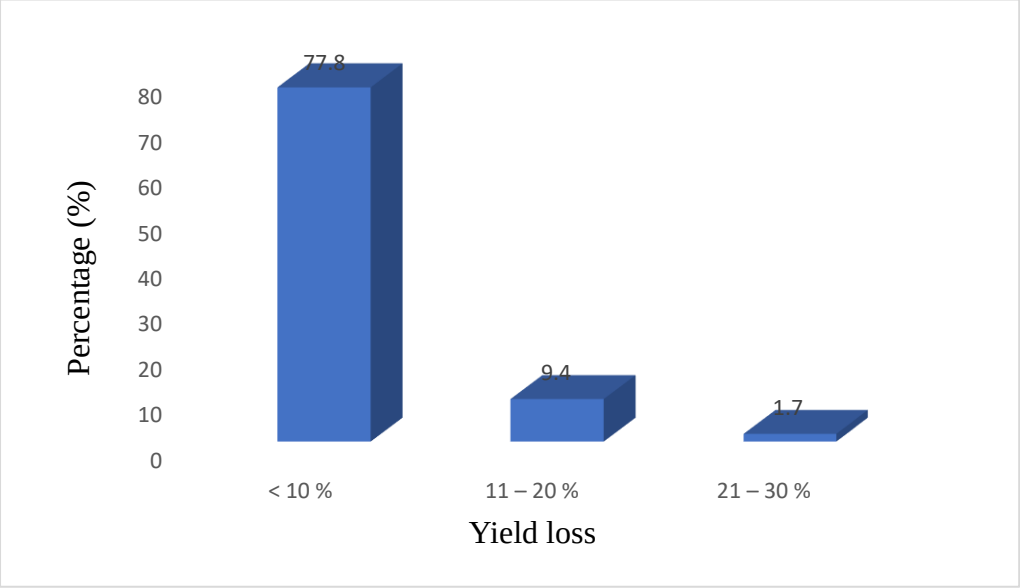
Table 9 Cont'D

Ants - mealybugs relation and the incidence and severity of the mealybug wilt virus disease		
The higher the ants/mealybug the higher the incidence and severity of the disease	119	74.4
The ants/mealybug has no effect on the incidence and severity of the disease	**7**	4.4
No Idea	34	21.3
Total	**160**	**100.0**

Source: Field Survey, Nyarko (2018)

Perceptions of farmers on the effect of the mealybug wilt of pineapple (MWP) disease on crop yield

Most responses (77.8%) received from the farmers indicated that the estimated yield loss was less than 10% due to MWP disease incidence, 9.4% indicated yield losses between 11% and 20%, whilst 1.7% of the respondents had yield losses between 21% and 30% due to MWP disease incidence in their farms (Figure 3).



Source: Field Survey, Nyarko (2018)

Figure 3: Farmers' perceptions on the effect of the mealybug wilt virus disease on crop yield

Distribution of MWP disease on the field

The majority of the respondents (55.0%) indicated that the MWP disease was restricted to certain portions of the fields in aggregated or clustered forms, 31.3% indicated the random spread of diseased plants whilst 13.7% indicated that the affected plants were uniformly distributed in the field (Figure 4).

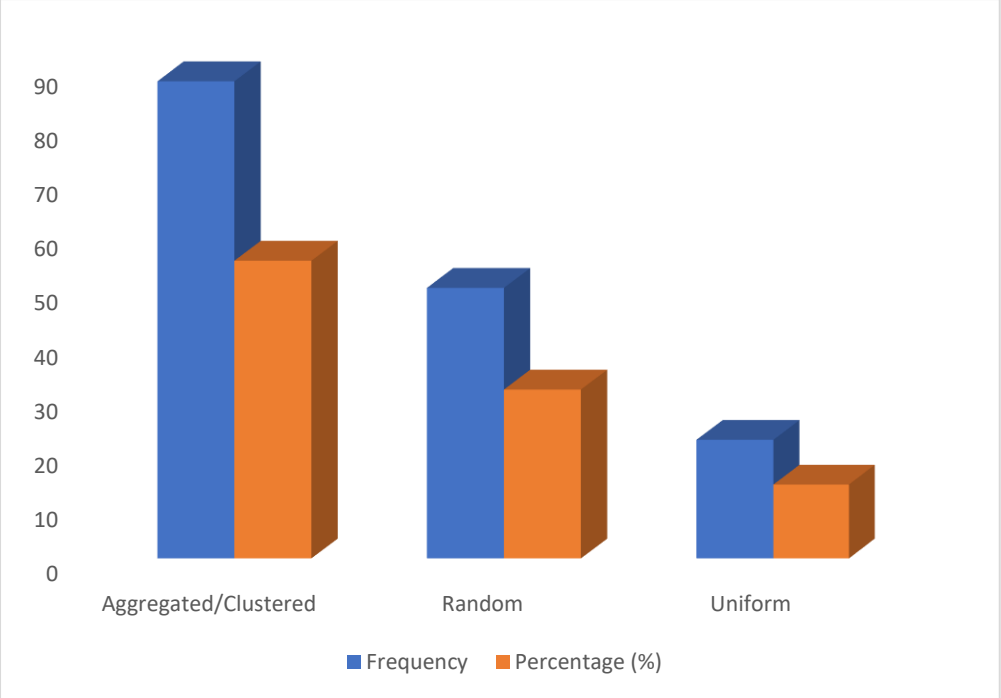


Figure 4: Distribution of MWP disease on the field

Plant ability to recover from MWP disease attack and its effect on the market

Table 10 shows the farmers' responses to the question of either plant could recover from MWP disease or not and its effect on the market value. The majority (84.4%) of respondents indicated that plants were not able to recover from MWP disease whilst 15.6% rather said the plants were able to recover from MWP disease. On the nature of the fruit harvested from infected plants, about 97.5% of the respondents said they were not marketable since they were very

small in size and also sour. Only 2.5% of the respondents said fruits from infected plants were marketable (Table 10).

Table 10: Showing plant ability to recover from MWP disease attack and effect on the market

Variables	Frequency	Percentage (%)
Plants that are attacked by the wilt disease can recover to bear fruits.		
Yes	25	15.6
No	135	84.4
Total	**160**	**100.0**
Nature of infected plant fruit		
Marketable	4	2.5
Non-marketable	156	97.5
Total	**160**	**100.0**

Source: Field Survey, Nyarko (2018)

Management of MWP disease by the respondent pineapple farmers

Table 11 shows the responses of farmers on their management of MWP disease on fallow plots. It was observed that most farmers (72.2%) did not control the disease on fallow plots; 16.7% removed infected plants from the fallow plots whilst 11.1% applied pesticides.

The answers of farmers to how they managed MWP disease on their mother plots revealed that most of them (55.6%) did not apply any control measure. However, 18.3% said they controlled the disease in their farms by applying insecticides whist 26.1% managed the disease by physical destruction or rogueing of infected mother plants (Table 11). In respect of the time of controlling the MWP disease, 90% of the farmers apply the control measure any time the disease appears on their farms whilst 7.3% and 2.7% manage the

disease in their mother plots at a three-monthly and six-monthly interval respectively.

Table 11 also shows that about 54.5% of the farmers' collected sucker/slips from all mother plants, provided the suckers/slips look healthy, 44.4% did not harvest suckers or slips from an infected mother plant whilst 1.1% did not harvest suckers or slips from mother plants within one square metre (1 m^2) perimeter of an infected mother plot.

The responses of farmers on the management of MWP on their main farms are shown in Table 11. The majority of farmers interviewed (58.4%) said they did not use any forms of control, and affected plants are left on the field till they completely die; 22.2% said they only removed infected plants with their hands whilst 19.4% of the applied pesticide. Concerning reasons why the majority of the farmers did not use pesticides, about 70.5% of them said that the chemicals were not effective after application. Furthermore, 23.8% said the pesticides were very expensive while 5.7% said they had no reason for not using any pesticides. When the farmers were asked how they keep track of the diseased spots on their planted fields, the majority (78.9%) of them indicated that they leave them alone, 17.2% said they tagged the individual diseased plants whilst 3.3% said they flagged diseased plots (area affected with the disease) (Table 11).

Table 11: Management of MWP disease by the respondent pineapple farmers

Variables	Frequency	Percentage (%)
Method of controlling mealybug associated with wilt on fallow plots		
No control	130	72.2
Chemical application	20	11.1
Removal of infected plants	30	16.7
Total	**180**	**100**
Means of managing MWP disease in your mother plots		
Spraying with insecticides	33	18.3
Physical destruction of infected mother plants	47	26.1
No Control	100	55.6
Total	**180**	**100**
Time of controlling of MWP disease on mother plots		
3 months interval	13	7.3
6 months interval	5	2.7
Any time the disease appeals	162	90.0
Total	**180**	**100**
Sucker harvesting from mother plots		
Harvesting from all mother plants provided the suckers look healthy	98	54.5
Not harvesting suckers from an infected mother plant	80	44.4
Not harvesting from within 1 m2 perimeter of an infected mother plant	2	1.1
Total	**180**	**100.0**
Method of keeping track of diseased areas on the field		
Tagging individual diseased plants	31	17.2
Indicating on the map of the plot	1	0.6
Flagging plots with diseased plants	6	3.3
leave it alone	142	78.9
Total	**180**	**100.0**
Control of disease on the field		
Chemical application	35	19.4
Removal of infected plants	40	22.2
No control	105	58.4
Total	**180**	**100**
If no control give reasons		
The high cost of insecticide	25	23.8
No effect after insecticide application	74	70.5
No reason	6	5.7
Total	**105**	**100.0**

Source: Field Survey, Nyarko (2018)

Management of pest by the respondent pineapple farmers

Table 12 shows the pest management methods employed by the respondents**.** The majority of farmers (75.0%) interviewed said they did not apply any control measure on their farms; 19.4% apply pesticide (insecticides) whilst 8.3% of the farmers employ handpicking and crashing as a means of controlling pests on their farms. Farmers who did not control pests on their farms gave reasons such as high cost of pesticides (51.9%) and ineffectiveness of pesticides (35.6%) whilst 12.6% gave no reasons (Table 12).

For the respondents who used pesticides, a greater percentage of them (71.4%) said they sprayed with chlorpyrifos (insecticides) to control the ants and mealybugs, 20.0% said they had no idea of the pesticide they use whilst 8.6% said that they used DDT as a means of controlling the ants and mealybugs (Table 12). In respect of the frequency of pesticide application, 42.9% and 34.3% of respondents applied the insecticides twice and three-times respectively in a growing season whilst 22.9% of them do the application once in a growing season. With the source of pesticides, most farmers (42.9%) indicated that they obtained their pesticides from agro-input shops; 28.6% from MoFA, whilst 14.3% obtained their pesticides from friends. In respect of the farmers' re-entry intervals, 37.1%, 28.6%, and 14.3% of them indicated 3, 5, and 7 days respectively whilst 20.0% said they did not observe any re-entry interval.

The majority of respondents (88.6%) said they used the same pesticide throughout the growing season whilst only 11.4% rotates their pesticides. 94.3% of the respondents said that treatments against the mealybugs were effective whilst 11.4% said they were not effective. The majority (94.3%) of the

respondents indicated that they applied the insecticide as a preventive measure in contrast to 5.7% who applied the chemical as a curative measure. In response to a question on the source of advice in the selection of pesticides, most farmers (77.1%) indicated agricultural extension agent (AEA), 14.3% indicated agro-chemical shops whilst 8.6% indicated other farmers.

Table 12: Management of MWP pest by the respondent pineapple farmers

Variables	Frequency	Percentage (%)
The management method of pest encountered		
Chemical application	35	19.4
Botanical application	0	0.0
Hand-picking and crashing	10	5.6
No control	135	75.0
Total	**180**	**100.0**
If no control give reasons		
The high cost of insecticide	70	51.9
No effect after insecticide application	48	35.6
No reason	17	12.5
Total	**135**	**100.0**
If chemical control, what kind of chemical		
Chlorpyrifos	25	71.4
DDT	3	8.6
No idea	7	20
Total	**35**	**100**
Number of times chemical is applied		
Once	8	22.9
Twice	15	42.9
3 times	12	34.2
Total	**35**	**100.0**
Source of pesticides		
Agro-chemical shop	17	48.6
MoFA	12	34.3
Other farmers/ Friends	6	17.1
Total	**35**	**100**
Re-entry interval observed for insecticide usage		
3 days re-entry interval	13	37.1
5 days re-entry interval	5	14.3
7 days re-entry interval	10	28.6
Don’t observe re-entry interval	**7**	20.0
Total	**35**	**100.0**
Alternate use of insecticides		
Yes	4	11.4

Table 12Cont'D

No	31	88.6
Total	**35**	**100.0**
Effectiveness of the pest control program		
Yes	33	94.3
No	2	5.7
Total	**35**	**100.0**
Effectiveness of the pest control program		
Source of advice if alternate use of chemicals		
AEA	3	8.6
Other farmers	27	77.1
Agro-input dealers	5	14.3
Total	**35**	**100.0**
Why alternate the use of chemicals		
For Protection	33	94.3
For curative	2	5.7
Total	**35**	**100.0**

Source: Field Survey, Nyarko (2018)

Effect of farmers' educational level and farming experience on their perception and management of MWP disease

From Table 13, it can be seen that the educational level of farmers had significant influence on the disease control method used by the farmers against the MWP disease ($\chi2 = 15.466$; df = 6; $P = 0.017$), how they manage MWP disease in their mother plots ($\chi2 = 11.579$; df = 4; $P < 0.021$), and how they managed pests on their farms ($\chi2 = 12.226$; df = 4; $P < 0.016$).

There was also a significant relationship between the educational levels of farmers and their awareness of the relationship between ants and mealybug population and the incidence of MWP ($\chi2 = 10.773$; df = 4; $P < 0.029$). It can, however, be seen from Table 13 that there was no significant effect of the educational level of farmers on the source of planting materials ($\chi2 = 1.692$; df = 2; $P= 0.429$) and on the knowledge of the causes of MWP disease ($\chi2 = 8.126$; df = 6; $P = 0.229$). Table 13 also revealed that the farming experience of the respondents had a significant influence on the source of planting materials ($\chi2$

= 36.027; df = 5; *P* = 0.000). However, their number of years in farming did not have any significant influence on their knowledge on the causes of disease ($\chi 2$ = 17.757; df = 15; *P* = 0.276), and how they control MWP disease ($\chi 2$ = 21.802; df = 15; P = 0.113). Table 13 further shows that the experience of respondents in farming did not influence on their knowledge on the relationship between ants and mealybug population and the incidence of MWP ($\chi 2$ = 3.612; df = 5; *P* = 0.607) (*P* < 0.05).

Table 13: Effect of farmers' educational level and farming experience on their perception and management of MWP disease

Variables	Pearson Chi-square	Df	*p* value
Educational level*Source of planting materials	1.692	2	0.429
Educational level*Control of mother plots against mealybug associated virus of pineapple	10.270	6	0.114
Educational level*Observation of MWP disease	1.079	2	0.583
Education level*Causes of the MWP disease	8.126	6	0.229
Education level*Estimated yield loss after infection	5.053	2	0.080
Education level*Relationship between ants and mealybug population and the incidence and severity of the mealybug wilt virus disease	10.773	4	0.029
Education level*By what means do you prevent MWP disease in your mother plots	11.579	4	0.021
Education level*Control these diseases	15.466	6	0.017
Education level*Management of pests on the farm	12.226	4	0.016
Experience*Source of planting materials	36.027	5	0.000
Experience*Control of mother plots against mealybug associated virus of pineapple	15.330	15	0.428
Experience* Observation of MWP disease	5.225	5	0.389
Experience*Causes of the MWP disease	17.757	15	0.276
Experience*Estimated yield loss after infection	3.475	5	0.627
Experience*Relationship between ants and mealybug population and the incidence and severity of the mealybug wilt virus disease	3.612	5	0.607
Experience*By what means do you prevent MWP disease in your mother plots	9.338	10	0.500
Experience*Control of these diseases	21.802	15	0.113
Experience*Management of pests on the farm	10.254	10	0.419
N of Valid cases	180		

Source: Field Survey, Nyarko (2018)

Field Survey

Disease symptoms observed on the pineapple crops

The crops displayed a wide range of disease symptoms during the field survey. The most commonly observed symptoms on all pineapple crops were definite and sudden change in leaf colour from red to pink. The leaf margins turn yellow and roll under which start at the leaf tips. Affected leaves become limp and droop, downward leaves curls (Figure 5). The other symptoms encountered were stunting, narrowing of leaves, leaf rolling, and yellowing.

Figure 5: pineapple plant attacked by mealybug wilt of pineapple showing symptoms in a surveyed field

Prevalence and severity of MWP disease in both pre- and post-induction stages in the three districts in the Central region

Table 14 shows the mean incidence of MWP disease recorded at the three districts during the pre-induction surveyed. It was observed that the disease was prevalent in all the districts. Analysis of variance showed significant difference in the incidence of MWP disease recorded at the various districts ($F_{2,48}$ =17.93; $P < 0.001$; mean =7.65; lsd = 2.24). The highest mean incidence was recorded at AAK (9.45± 1.10%), but it was not significantly different from that at KEEA district (8.90±0.58%) but significantly higher than that at Ekumfi district (4.60±0.58%). The ANOVA on mean incidence at the various communities across the three districts also revealed significant differences among them ($F_{9,48}$ = 4.77; P < 0.001; mean = 7.65; lsd = 3.57), with Asuansi in the AAK district having the highest score of 14.60±2.79, whilst Abor (2.80±0.66) in the Ekumfi district having the lowest (Appendix 1).

From the table 14, an ANOVA on the mean incidence of MWP disease recorded at the three districts during the post-induction stages showed significant difference among the three districts ($F_{2,48}$ = 34.53; $P < 0.001$; mean =5.23; lsd = 1.87). The highest mean incidence was recorded at AAK (7.00±0.80%), but it was not significantly different from that of the KEEA district (6.50±0.68%). However, significantly higher than that of Ekumfi district (2.20±0.46%). The ANOVA on the mean incidence during the post-induction survey also revealed significant differences among the communities across the districts ($F_{9,48}$ = 8.39; $P < 0.001$; lsd = 2.55) with Asuansi in the AAK district having the highest score of 11.0±1.18, whilst Abor in the Ekumfi district having the lowest of 0.80±0.37 (Appendix 1).

The mean severity score of MWP disease recorded at the three districts that were surveyed during the pre-induction stage are shown in Table 14. An ANOVA showed significant difference in the severity of MWP disease among the three districts ($F_{2,48}$ = 9394; P < 0.001; Mean = 1.12; lsd = 0.24). The highest mean severity score was recorded at AAK (1.29± 0.10) which was not significantly different from that of KEEA district (1.21±0.09), but significantly higher than that of Ekumfi district (0.86±0.06). It can be seen from the table 14 that ANOVA showed significant difference among the communities across the districts with respect to the mean severity of MWP diseases recorded during the pre-induction stage ($F_{9,48}$ = 3.05; P = 0.006; mean = 1.12; lsd = 0.41) with Asuansi in the AAK district having the highest score of 1.66±0.32, whilst Abor in the Ekumfi district having the lowest of 0.68±0.11(Appendix 2).

The mean severity score of MWP disease recorded at the three districts during the post-induction growth stage surveyed are shown in Table 14. An ANOVA showed significant difference in the severity of MWP disease recorded at the various districts ($F_{2,48}$ = 9.09; P < 0.001; Mean = 0.90; lsd = 0.23). The highest mean severity score was recorded at AAK (1.10±0.10) which was not significantly different from that of KEEA district (0.94±0.07), but significantly higher than that of Ekumfi district (0.67±0.08) (P < 0.05). The ANOVA also revealed significant difference among the communities across the districts with respect to the mean severity score of MWP disease ($F_{9,48}$ = 3.05; P = 0.006; mean = 0.90; lsd = 0.41). The highest mean severity index of MWP disease with Asuansi in the AAK district having the highest score of 1.47±0.18, whilst Abor (0.35±0.15) in the Ekumfi district having the lowest (Appendix 2).

Table 14: Mean prevalence and severity score of viral disease in both pre and post-induction stages in the three districts in the Central region

Districts	Prevalence (%)		Severity (%)	
	pre-induction stage	post-induction stage	Pre-induction stage	post-induction stage
KEEA	8.90±0.58[b]	6.50±0.68[b]	1.21±0.09[b]	0.94±0.07[b]
AAK	9.45± 1.10[b]	7.00±0.80[b]	1.29± 0.10[b]	1.10±0.10[b]
Ekumfi	4.60±0.58[a]	2.20±0.46[a]	0.86±0.06[a]	0.67±0.08[a]
Mean	7.65	5.23	1.12	0.90
LSD ($p \leq 0.05$)	2.24	1.87	0.24	0.23
P	<.001	<.001	<.001	<.001

Source: Field Data, Nyarko (2019)

Means in the same column bearing the same letters are not significantly different from each other ($P < 0.05$) *Mean± Standard error; KEEA: Komenda-Edina-Eguafo-Abirem; AAK: Abura-Asebu-Kwamankese

Comparison of mean incidence and severity scores of MWP disease in both pre- and post-induction stages

From table 15, an independent sample t-test analysis revealed that the mean prevalence of virus disease in the pre-induction stage (7.65%) was significantly higher ($t = 3.41$; $p = 0.001$) than that of the post-induction stage (5.23%). The mean severity score during the pre-induction stage (1.12) was also significantly higher ($t = 2.84$; $p = 0.003$) than in the post-induction stage (0.90) as shown in Table 15.

Table 15: Comparison of mean incidence and severity scores of MWP disease in both pre- and post-induction stages

Growth stage	Mean prevalence (%)	Mean severity scores
Pre-induction	7.65	1.116
Post-induction	5.23	0.904
t-test	3.41	2.84
p-value	< 0.001	0.003

Source: Field Data, Nyarko (2019)

Relationship between the incidence and severity scores of MWP both in pre- and post- induction stages.

Figure 6 shows a significant and positive correlation between mean incidence and severity score of MWP disease across the three districts during the pre-induction stage survey ($r = 0.9053$; $p < 0.001$). Figure 7 also revealed a significant positive correlation between mean incidence and severity score of MWP disease across the three districts during the post-induction stage survey ($r = 0.9164$; $p < 0.001$).

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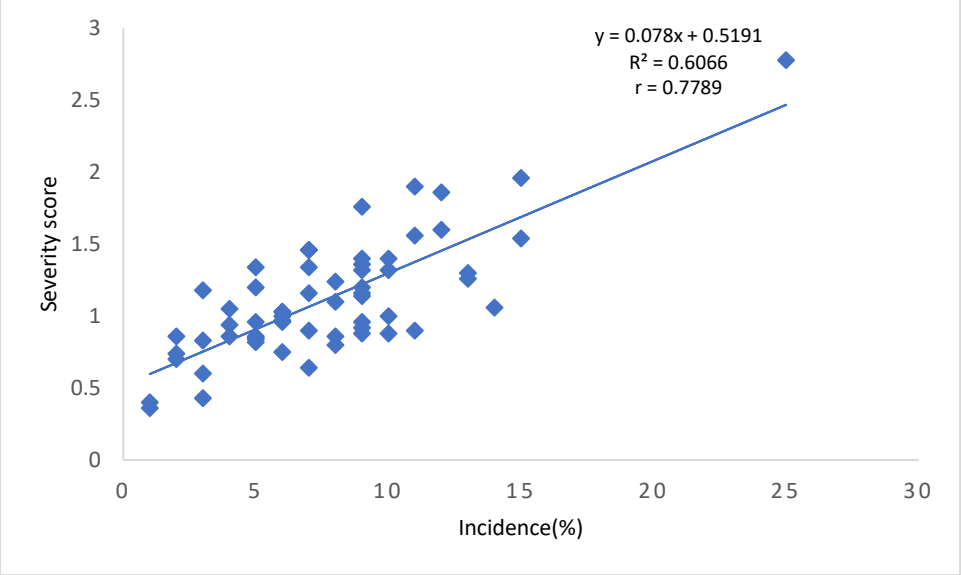

Figure 6: Relationship between the incidence and severity of mealybug wilt of pineapple (MWP) in pre-induction stages within three districts in Central region

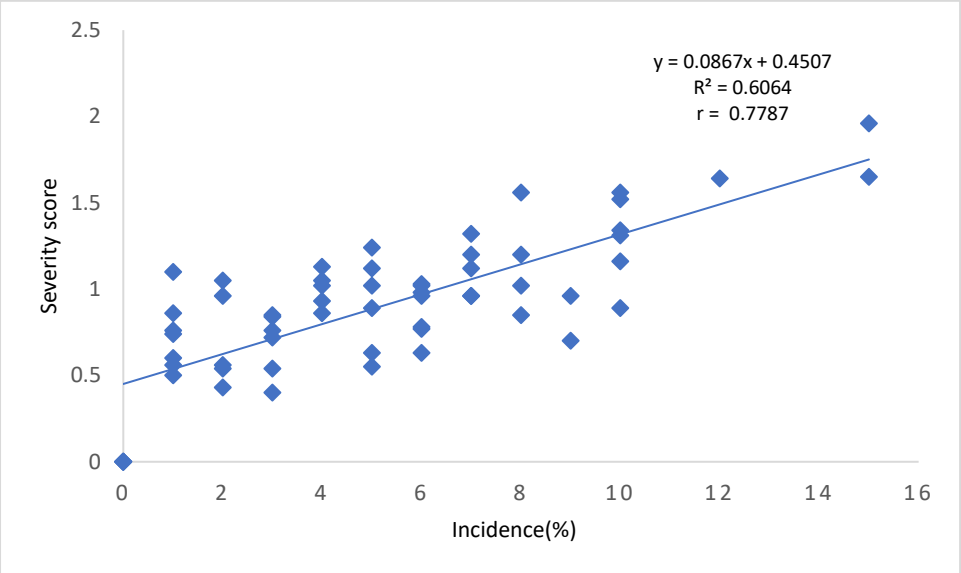

Figure 7: Relationship between the incidence and severity of MWP in post-induction stages among the various communities within the districts.

Influence of ant and mealybug populations on the extent of disease infections within the three districts.

The means of ant populations on pineapple crops in the various districts during the field survey are shown in Table 16. The ANOVA did not show any significant difference (F2,48 = 1.14; *P* = 0.327; mean = 10.90) in the mean ant populations among the districts surveyed. However, AAK had the highest population of 11.70±0.82 whereas Ekumfi had the lowest (9.95±0.75). There was no significant difference (F9,48 = 0.93; *P* = 0.506; mean = 10.90) in the ant populations among the communities across the districts (Appendix 3).

The means population of mealybugs on pineapple crops surveyed from the three districts during the field survey are shown in Table 16. The ANOVA did not show any significant difference (F2,48 = 3.22; *P* = 0.050; mean = 12.68) it means population of mealybugs among the districts surveyed. However, AAK had the highest population of 14.00±1.27, whereas Ekumfi had the lowest (10.70±0.91). With respect to the population of mealybugs, ANOVA also revealed significant difference (F9,48 = 1.74; *P* < 0.11;1 mean = 10.90) among the communities in the districts (Appendix 3).

Table 16 shows that the mean severity scores of MWP disease at the three districts that were surveyed varied significantly among them (F2,48 = 6.00; *P* < 0.005; mean =2.34; LSD = 0.46). The highest mean severity score was recorded at AAK (2.69±0.17) which was not significantly different from that of the KEEA district (0.94±0.07), but significantly higher (*P* < 0.05) than that of Ekumfi district (1.96±0.14). The mean MWP severity scores at the various communities across the three districts also differed significantly (F9,48 = 2.33; *P* < 0.029; mean = 2.34; LSD = 0.84) among them (Appendix 3).

Table 16: Mean ants and mealybugs population on the extent of disease infections with the three districts

Districts	Mean ant population (%)	Mean mealybug population (%)	Mean severity
KEEA	11.05±0.89	13.35 ± 0.87	2.36±0.17[ab]
AAK	11.70±0.82	14.00±1.27	2.69±0.17[b]
Ekumfi	9.95±0.75	10.70±0.91	1.96±0.14[a]
Mean	10.90	12.68	2.34
P	0.322	0.064	0.005
l.s.d.	-	-	0.460

Source: Field Data, Nyarko (2019)

Means in the same column bearing the same letters are not significantly different from each other ($P < 0.05$). KEEA: Komenda-Edina-Eguafo-Abirem; AAK: Abura-Asebu-Kwamankese

Influence of soil fertility on the incidence and severity of MWP diseases in three districts.

The soil fertility levels of the pineapple farms surveyed from the three districts in the Central region are shown in Table 17. An ANOVA did not show any significant difference ($p > 0.05$) among the various districts in terms of pH, organic matter, nitrogen, phosphorus, potassium, organic carbon, CEC, C_N_ ratio, but their moisture contents differed significantly among them ($P<0.05$). KEEA had the highest moisture content of 10.51%, followed by Ekumfi (6.6%) whilst AAK had the lowest (5.65%).

The mean soil fertility parameters recorded from the pineapple farms in the various communities across the three districts are shown in Appendix 4.

With respect to pH, moisture content, organic matter, nitrogen, phosphorus, organic carbon, potassium, and CEC in the various communities, ANOVA showed significant differences ($p > 0.05$) among the communities. However, the C_N_ ratio recorded for the communities varied significantly among the communities (Appendix 4).

Table 17: Soil fertility levels of the pineapple farms surveyed from three districts.

District	pH	%MC	%OM	%N	Exchangeable K (cmolkg^{-1})	Available P (µgg^{-1})	%OC	C_N_ratio	CEC (cmol_kg)
KEEA	5.376	10.51^{b}	2.51	0.1297	0.238	13.0	1.45	11.57	6.04
AAK	5.317	5.65^{a}	3.06	0.1381	0.287	6.4	1.77	13.58	6.94
Ekumfi	5.615	6.60^{a}	2.97	0.1494	0.348	7.3	1.72	11.82	7.44
means	5.436	7.58	2.85	0.1391	0.291	8.9	1.65	12.32	6.80
p-value	0.309	0.007	0.513	0.569	0.115	0.309	0.513	0.570	0.133
lsd	-	3.150	-	-	-	-	-	-	-

Source: Field Data, Nyarko (2019)

Means in the same column bearing the same letters are not significantly different from each other ($P < 0.05$); KEEA: Komenda-Edina-Eguafo-Abirem; AAK: Abura-Asebu-Kwamankese

MC: moisture content; OM: Organic matter; N: Nitrogen; K: Potassium; P: Phosphorous; OC: Organic carbon

Relationships between soil fertility status and MWP disease incidence and severities

Table 18. Shows the Pearson's correlation coefficients calculated to ascertain the relationships between soil fertility status and incidence and severity of MWP disease. Results revealed no significant correlations between soil fertility levels (pH, moisture content, organic matter, nitrogen, phosphorus, organic carbon, potassium, and CEC) and MWP disease incidence ($P > 0.05$) and severity ($P>0.05$).

Table 18: Correlations between soil fertility and incidence and severity of MWPD,

Variable	Incidence	Severity
%N	0.1612	0.1685
%P	-0.0612	-0.1976
%K	-0.1028	-0.1214
%OM	-0.0292	0.0684
%OC	-0.0292	0.0684
CEC_(cmol_kg)	0.0390	0.0817
Soil pH	0.0944	0.0708
C_N_ratio	-0.1548	-0.0484

Source: Field Data, Nyarko (2019)

MC: moisture content; OM: Organic matter; N: Nitrogen; K: Potassium; P: Phosphorous; OC: Organic carbon

Detection and Characterisation of Pineapple Mealybug Wilt Associated Virus Species in Diseased Pineapple Samples

Detection of the viral species responsible for MWP disease

Five different viral species namely PMWaV-1, PMWaV-2, PMWaV-3, PMWaV-4, and PMWaV-5 were detected by qRT-PCR from the plant samples during the study. Twenty-three (23) samples were positive at least to one of the five species of PMWaVs identified, except sample 9 which shows negative to all the five PMWaVs species (Table 19; Figure 8). PMWaV-5 had the highest infection rate across the districts with a relative abundance of 91.7% (Figure 8) this was followed by PMWaV-2, PMWaV-4, PMWaV-1, and PMWaV-3 with relative abundances of 62.5%, 45.8%, 33.3%, and 8.3% respectively (Figure 8). For distribution of the five viral species in the districts, all the five viral species were found in AAK, whilst only 4 (PMWaV-1, PMWaV-2, PMWaV-3, and PMWaV-4) were found in Ekumfi and KEEA districts, implying that PMWaV-3 was only found in AAK.

Table 19: Pineapple mealybug wilt associated virus (PMWaV) species detected on 24 diseased pineapple plant samples from three districts in the Central Region

District	Sample	Virus species				
		PMWaV-1 (Ct)	PMWaV-2 (Ct)	PMWaV-3 (Ct)	PMWaV-4 (Ct)	PMWaV-5 (Ct)
AAK	17	+	+	-	+	+
AAK	18	+	+	-	+	+
AAK	19	+	+	-	+	+
AAK	20	+	+	+	-	+
AAK	21	+	+	-	+	+
AAK	22	+	+	+	+	+
AAK	23	-	+	-	+	+
AAK	24	-	+	-	+	+
Ekumfi	1	-	+	-	-	+
Ekumfi	2	+	-	-	-	+
Ekumfi	3	-	+	-	-	+
Ekumfi	4	-	-	-	-	+
Ekumfi	5	-	-	-	+	+
Ekumfi	6	-	-	-	-	+
Ekumfi	7	-	-	-	-	+
Ekumfi	8	-	+	-	-	-
KEEA	9	-	-	-	-	-
KEEA	10	-	+	-	-	+
KEEA	11	-	+	-	-	+
KEEA	12	-	-	-	-	+
KEEA	13	-	+	-	-	+
KEEA	14	-	-	-	+	+
KEEA	15	+	+	-	+	+
KEEA	16	-	-	-	+	+

Source: Field Data, Nyarko (2019)

Present (+) and Absent (-); KEEA: Komenda-Edina-Eguafo-Abirem; AAK: Abura-Asebu-Kwamankese

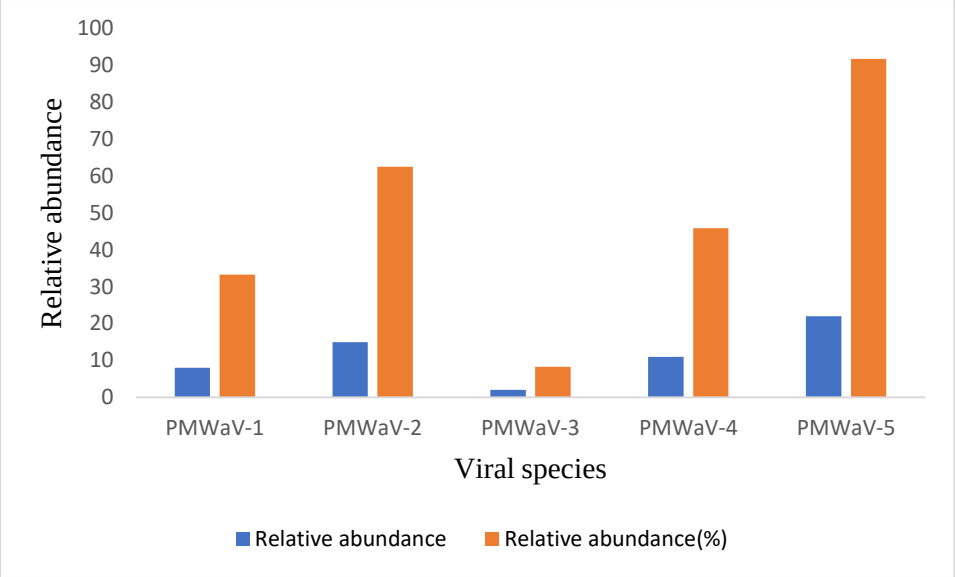

Figure 8: Relative abundance of pineapple mealybug wilt associated virus (PMWaV) species detected from 24 pineapple samples from three districts in the Central Region.

Performance of the PMWaVs primers on samples across the growing area

Figure 9 shows qPCR amplification of the Closterovirus with the PMWaVs primers of cDNA fragment size that varies from 400 – 610 bp from all the MWPdisease-affected samples (lanes 1-12 and 13- 24) but no band for negative control (NTC).

The amplicon of PMWaV-1 obtained from pineapple samples using PMWaV-1 primer pairs of band size 590 bp. The primer detected the virus from only two out of eight samples at KEEA districts, and at AAK, almost all the samples tested positive indicating the presence of PMWaV-1 except two samples (sample 23 and 24) that was absent in terms of PMWaV-1. However, none of the eight samples at Ekumfi showed the presence of PMWaV-1.

With PMWaV-2 amplicon obtained from pineapple samples using PMWaV-2 primer pairs of band size 610 bp, the primer detected the virus from only two out of eight samples from Ekumfi, four out of eight samples from

KEEA, whilst at AAK, all the samples tested indicated the presence of PMWaV-2. PMWaV-3 was detected in only two out of eight samples from AAK. The virus, however, was not detected in any of the samples from KEEA and Ekumfi districts.

The amplicon of PMWaV-4 of band size 590-600 bp was obtained from pineapple samples using PMWaV-4 primer pairs. The primer detected the virus from only one out of eight samples in Ekumfi, four out of eight samples at KEEA districts, whilst at AAK, seven out of eight samples tested indicated the presence of PMWaV-5.

The amplicon of PMWaV-5 of band size 500 – 600 bp was obtained from pineapple samples using PMWaV-5 primer pairs (Figure 9). The primer detected the virus from six out of eight samples from Ekumfi, and all the samples from AAK and KEEA indicated the presence of PMWaV-5.

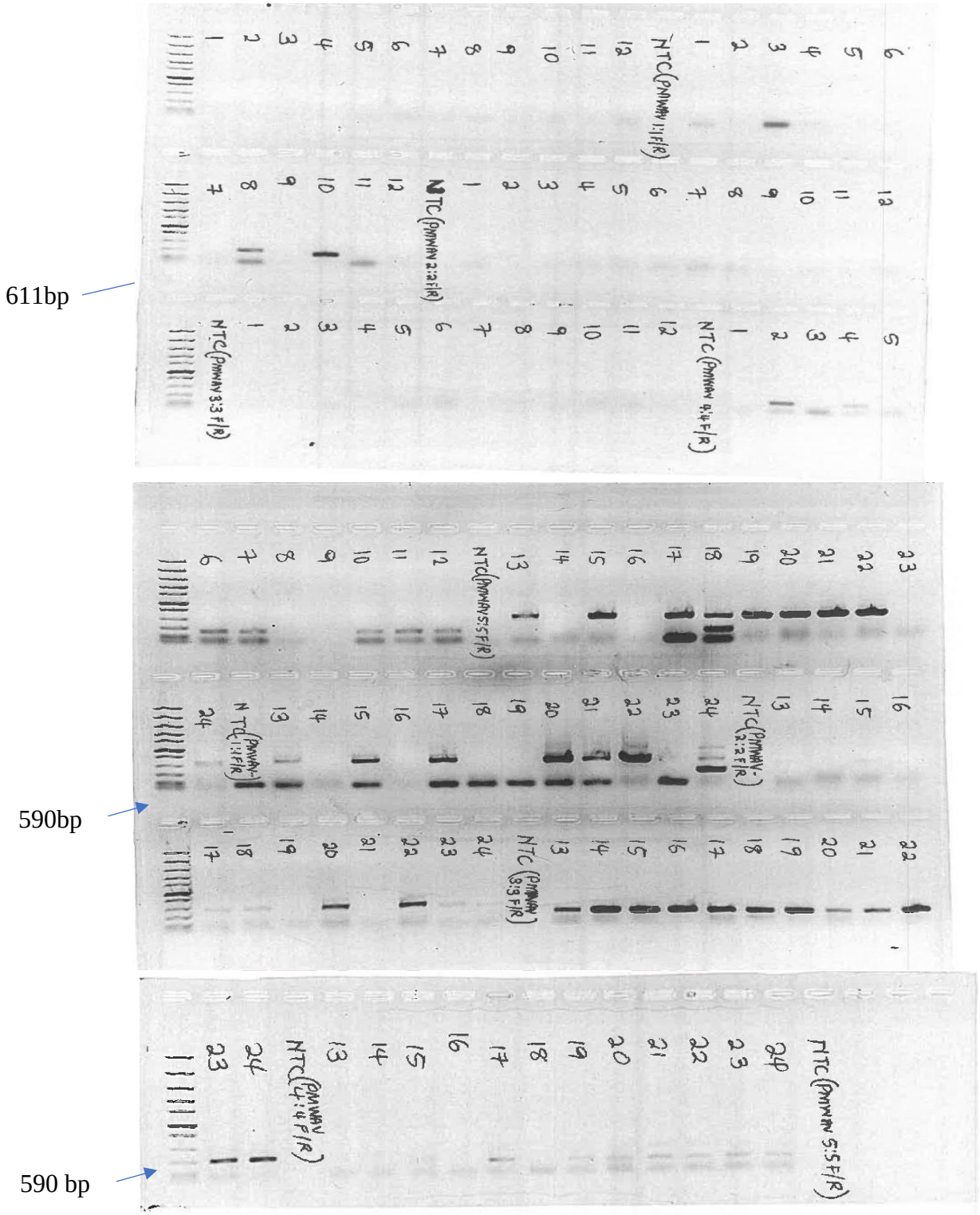

Figure 9: The amplicon of PMWaVs obtained from PMW pineapple sample using PMWaV-1, -2 -3 -4 and -5 primer pairs; The amplification was done in two part; Samples 1 to 12 and samples 13 to 24 with band sizes of 500 – 610 bp; Samples 1-8 were obtained from Ekumfi; Samples 9-16 were obtained from KEEA and samples 17-24 were obtainted from AAK.

Mixed Viral Infections of pineapple samples by Pineapple mealybug wilt associated virus species

Mixed viral infections by two or more of the five viruses identified (PMWaV-1**,** PMWaV-2, PMWaV-3, PMWaV-4, and PMWaV-5) were detected in the pineapple samples from all the three districts (Table 20). Double infections were detected in 4 out of 8 samples from Ekumfi where there was co-infection by PMWaV-5 and either of PMWaV-1 (sample 2)**,** PMWaV-2 (samples 2 and 3) and PMWaV-4 (sample 5). Six out of 8 samples from KEEA showed mixed infections; where there was co-infection by PMWaV-5 and either PMWaV-2 or PMWaV-4 (in double infections); or co-infection between PMWaV-5 and PMWaV-1**,** PMWaV-2 and PMWaV-41 (quadruple infections). Mixed infections were detected in all the 8 samples from AAK, where there was co-infection by PMWaV-5 and the other four viral species in triple (2 samples), Quintuple (5 samples), and quadruple infections (1 sample).

Table 20: Mixed infections of pineapple mealybug wilt associated virus species (PMWaVs) in pineapple crop samples from three districts in the Central region

	Number of mixed infections (%)				
District	Double	Triple	Quadruple	Quintuple	Total (%)
AAK	-	2	5	1	8 (100)
Ekumfi	4	-	-	-	4 (50)
KEEA	5	-	1	-	6 (75)

Source: Field Data, Nyarko (2019)

Sequence analysis

Heat shock protein 70 (HSP70) gene was sequenced to estimate the genetic variability of PMWaV-1, PMWaV-2, and PMWaV-3 isolates. This gene encodes a protein that is involved in post-transcriptional gene silencing, host range specificity, and symptom expression. After editing, the final sequences analysed were the partial sequence gene of 419nt of PMWaV-1, 590nt of PMWaV-2, and 486nt of PMWaV-3.

The field isolates analysed shared nucleotide identities ranging from 95.2 to 99.7% for PMWaV-1, from 98.9 to 100% for PMWaV-2 and 98.3% for PMWaV-3. The deduced amino acid sequences of the sequenced isolates also ranged from 86.5 to 99.2% for the PMWaV-1, from 97.1 to 100% for PMWaV-2 and 95.3% for the PMWaV-3 (Table 21), indicating narrow variability (close identities) within each viral species.

The heat shock protein 70 gene sequences of the sequenced isolates shared 95.2 to 100% nucleotide identities for PMWaV-1, 98.2 to 100%, for PMWaV-2 and 97.5 to 99.3% for PMWaV-3 with a published isolate from Genbank. Deduced amino acid identities with that of published isolates from GenBank also ranged from 86.5 to 100% for PMWaV-1, from 95.5 to 100% and 93.3 to 98.0% for PMWaV-3 (Table 21).

Table 21: Nucleotides (nt) and amino acid (aa) sequence identities of Pineapple mealybugs wilt associated virus field isolates and selected published isolates retrieved from GenBank.

Sequences	Sequence identities (%)	
	Nucleotide	Amino acid
(a) PMWaV-1 (HSP70)		
Between sequenced isolates	95.2 - 99.7	86.5 – 99.2
Between sequenced isolates and published isolates	95.2 – 100	86.5 – 100
(b) PMWaV-2 (HSP70)		
Between sequenced isolates	98.9 – 100	97.1 – 100
Between sequenced isolates and published isolates	98.2 – 100	95.5 – 100
(c) PMWaV-3 (HSP70)		
Between sequenced isolates	98.3	95.3
Between sequenced isolates and published isolates	97.5 - 99.3	93.3 – 98.0

Source: Field Data, Nyarko (2019)

Phylogenetic analyses

The maximum likelihood tree for the partial HSP70 gene nucleotide sequence data revealed that the 38 sequence of PMWAV-1, -2 and -3 isolates from PMW disease pineapple plant formed three main genetic groups corresponding to three clades supported by bootstrap values of 100%.

PMWAV-1 isolates collected from the Central regions form clade 1 containing the majority of the sequenced isolates (19 isolates) of which seven field isolates clustered with the twelve published isolates from different countries. Clade 2 consists of PMWAV-3 isolates, which contain two field isolates (AAK301, AAK302) that also clustered with three published isolates

from GenBank. The third clade contains a phylogenetic analysis of HSP70 gene nucleotide sequences of PMWaV-2 isolates of which all the five filed isolates (AAK202, AAK203, AAK204, AAK205) cluster with eight published sequence isolates from different countries (Figure 10).

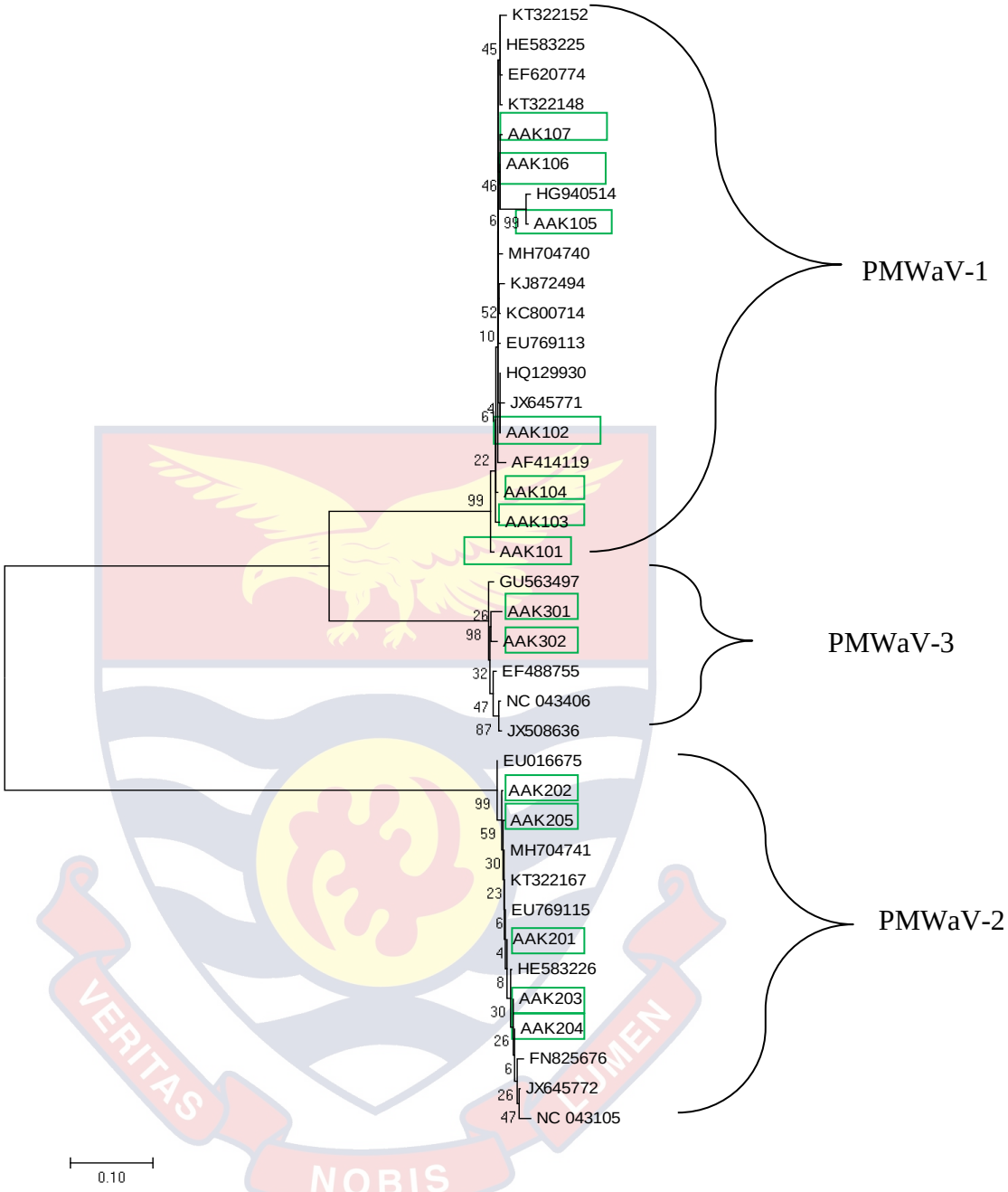

Figure 10: Maximum-likelihood phylogenetic tree of nucleotide sequences of HSP70 gene of PMWaV-3 isolates (n=38) sampled from the Central region of Ghana. The sequence isolates are in green boxes whilst the rest are accession names of isolates from the GenBank. The scale bar signifies a genetic distance of 0.10 nucleotide substitutions per site.

The maximum likelihood tree for the amino acid sequences for HSP70 gene for PMWaV-1, PMWaV-2 and PMWaV-3 had sample topology as their nucleotide sequence (Figure 11).

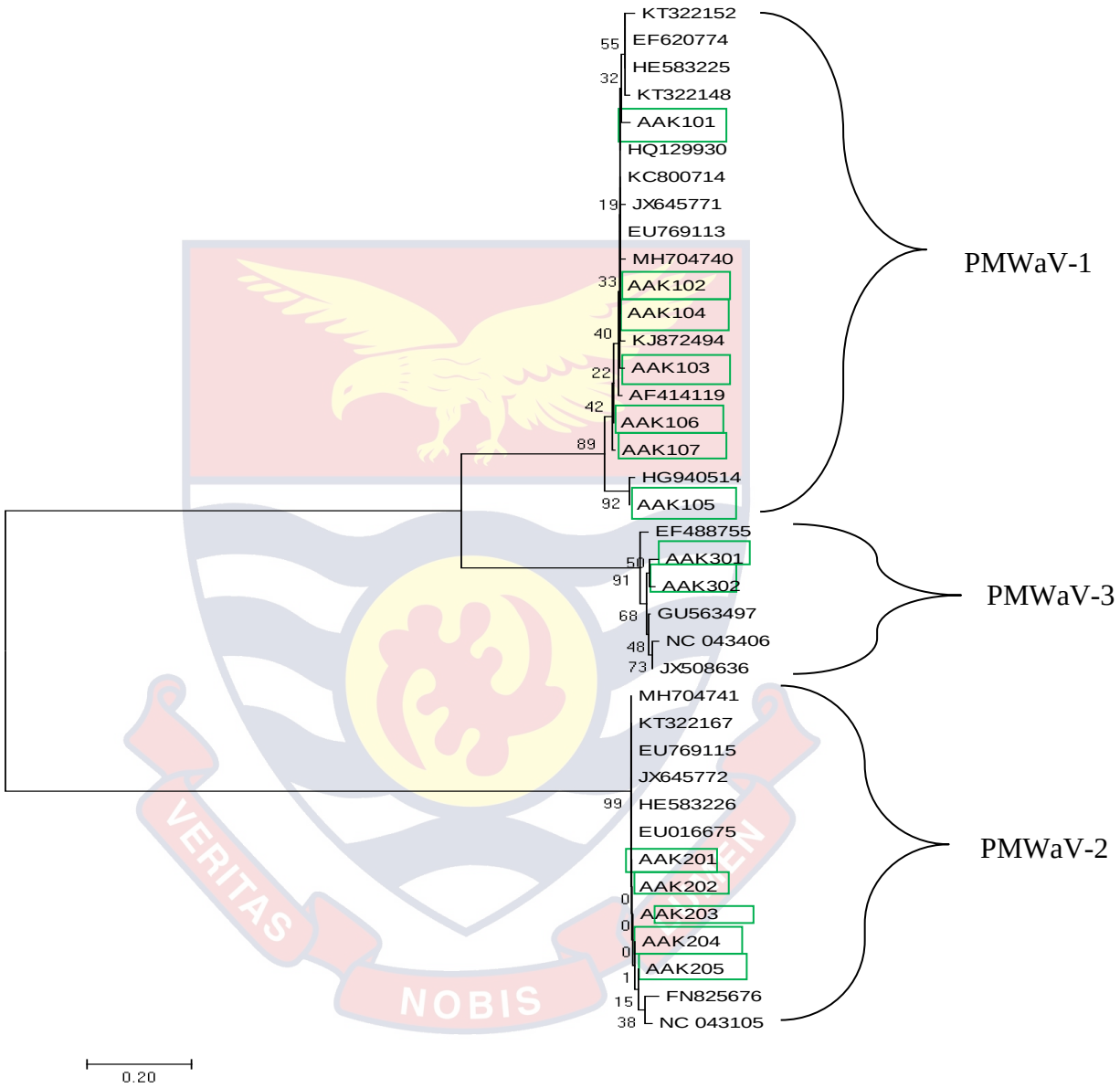

Figure 11: Maximum likelihood phylogenetic tree (abridged) of hsp70 amino acid sequences of PMWaV-2 isolates (n= 38) sampled in the Central region of Ghana. The sequence isolates are in green boxes whilst the rest are accession names of publish isolates from the GenBank. The scale bar signifies a genetic distance of 0.20 nucleotide substitutions per site.

\Genetic diversity within HSP70 genes in PMWaV-1, 2 and 3

Analysis of genetic diversity within the HSP70 genes of PMWaV-1, 2, and 3 isolates showed that the genes were variable with a high number of mutations, a high number of polymorphic sites, and very high haplotype diversity but low nucleotide diversity (Table 22).

Table 22: Genetic variability within HSP70 DNA sequences of PMWaV-1, 2 and 3 isolates

Dataset	Number of sequences	Number of polymorphic sites (S)	Total number of mutations (Eta)	Nucleotide diversity (π) [a]	Haplotype diversity (h) [a]
PMWaV-1	19	64	69	0.0172 ± 0.0032	1.000 ± 0.0029
PMWaV-2	13	23	23	0.0074 ± 0.0016	0.9870±0.0035
PMWaV-3	6	27	28	0.0199 ± 0.0024	1.000 ± 0.0076

Source: Field Data, Nyarko (2019); [a] Mean ± standard deviation

Analyses of genetic distance and the natural selection within HSP70 genes of the PMWaV-1, PMWaV-2 and PMWaV-3 isolates

The overall mean genetic distances within and between the nucleotide sequence datasets for HSP70 genes for PMWaV-1, PMWaV-2, and PMWaV-3 were determined using the Maximum Likelihood model. The mean genetic

distance within the PMWaV-1 isolates was 0.018±0.002, PMWaV-2 was 0.007±0.002 and PMWaV-3 isolates was 0.020±0.004 (Table 23).

Using the Maximum Likelihood method via the HyPhy package, 17 detected codon positions in the HSP70 gene of PMWaV-1, 7 for PMWaV-2 and 4 codon positions in the HSP70 gene for PMWaV-3, were under significant positive selection ($P < 0.05$) (Table 23) This provided evidence of heterogeneous selection pressures among codon sites in HSP70 genes for PMWaV-1, PMWaV-2 and PMWaV-3 dataset. There was also a comparison of the overall selection intensity in the HSP70 genes. The results showed that the selection intensity was (mean pairwise dN / dS this gene was 0.2587 for PMWaV-1, 0.2696 for PMWaV-2 and 0.1545 for PMWaV-3) (Table 23). Thus, overall, the values of the dN/dS were low, i.e. dN/dS < 1, implying that the genes of PMWaV-1, PMWaV-2, and PMWaV-3 were under negative selection.

Table 23: Mean pairwise genetic distance and the selective pressures within HSP70 genes of the PMWaV-1, 2 and 3 isolates

Specie	MeanGenetic distance[a]	d_N	d_S	dN/dS	Total number of codons	Codon positions under positive selection[b]
PMWaV-1	0.018±0.002	0.0652	0.2521	0.2587	189	17
PMWaV-2	0.007±0.002	0.0199	0.0738	0.2696	196	7
PMWaV-3	0.020±0.004	0.0214	0.1385	0.1545	160	4

Source: Field Data, Nyarko (2019)

[a] Mean ± standard error. Standard error was estimated by a bootstrap procedure of 1000 replicates. The overall genetic distance within and between HSP70

genes of PMWaV-1, 2 and 3 nucleotide sequences datasets were estimated using the Maximum likelihood model.

[b]Codons that have undergone positive selection (P < 0.05), rejecting hypothesis of neutral evolution. Maximum Likelihood analysis of natural selection codon-by-codon method was via HyPhy package implemented in MEGA7.

Neutrality tests

The results for the various neutrality tests are summarised in Table 24 and aside Tajima's D, test and Fu and Li's F* test for PMWav-1 that was significant in terms of neutrality deviation ($P < 0.05$), the rest of the tests (Fu and Li's D* and Fu and Li's F* tests) detected had no significant neutrality deviation ($P < 0.05$) for the PMWaV-1, PMWaV-2, and PMWaV-3 populations.

Table 24: Neutrality test for HSP70 of PMWaV-1, 2 and 3

Species	Tajima's D	*P*-value	Fu and Li's D*	*P*-value	Fu and Li's F*	*P*-value
PMWaV-1	-2.07401	< 0.05[a]	-2.26722	> 0.05[b]	-2.57250	< 0.05 [a]
PMWaV-2	-1.79390	<0.05[a]	-2.14661	>0.05 [b]	-2.34611	>0.05 [b]
PMWaV-3	-0.87754	<0.5[a]	-0.70049	>0.05 [b]	-0.81754	>0.05 [b]

Source: Field Data, Nyarko (2019)

[a] $P < 0.05$, significant at $P < 0.05$ [b] $P > 0.05$ not significant

CHAPTER FIVE

DISCUSSION

Farmers' Awareness and Knowledge of MWP Disease and their Agronomic Practices

The study revealed that the majority of the respondents were males. This result is consistent with the findings of Sarpong et al. (2017) and others which indicated that the majority of pineapple farmers from the Central and Eastern regions of Ghana are males. The dominance of males in pineapple production is expected because pineapple production is labour intensive which may be too tedious and time-consuming for females who have to combine farming activities with their domestic duties (Sarpong *et al.*, 2017; Apatanku *et al.*, 2016; Bawura, 2013). Also, according to Duncan (1997), access and control of land are influenced by customary law and that limits the role of women land acquisition.

The age of the majority of farmers ranging between 29 years and 38 years which agrees with the findings of Sarpong et al. (2017) which revealed that the majority of pineapple farmers in Ghana are in the age range of 20 to 50 years, indicating that they are in their youthful ages. The youthfulness of the farmers could enable them to adopt good agronomic practices such as early weeding, spraying of pesticides among others. These help in reducing the spread of pests and diseases in their pineapple farms. Also, youthful farmers have the physical strength to do labour-intensive work and are likely to adopt improved technologies (Nwosu, 2011).

Basic knowledge about disease management is one of the main tools in the reduction of the prevalence of diseases (Sarpong *et al.*, 2017). It was observed in this current study that most pineapple farmers had some level of

education (primary and secondary education) which suggests that they might have had basic knowledge about disease management and thus accounting for the low level of incidence of the MWP disease. And also, aside from them having a formal education, their experience in farming (number of years in farming) might have contributed to their knowledge of the disease (Anon, 2005; Sarpong *et al.*, 2017). Also, from the study, about 32.2% of the respondents were illiterate, they might have probably had their knowledge about the disease through experience. Apantaku et al. (2016) also argued that the experience of farmers in farming counts more than formal education to increase productivity. However, it has been reported that formal education as well as experience in farming are means through which farmers get information (Nagaraju *et al.*, 2002).

Although some farmers produce pineapple on a large scale in the study areas, the majority of the respondents were small scale farmers with average total land size less than three hectares. This confirms the report from MoFA (2013) that the majority of farmers in Ghana are mostly smallholders with farming lands size less than five hectares.

Results from the study indicate that the majority (86.1%) of respondents produce the local variety which is the Sugarloaf whilst the remaining farmers cultivate either Smooth cayenne or MD2varieties. Sugarloaf variety is reported to be highly susceptible to PMWaVs (Trienekens *et al.*, 2004). The preference of farmers for Sugarloaf could perhaps be because it does not require intensive care such as ploughing and harrowing, use of plastic mulch, and regular application of fertilizer. Also, local markets' preference for sugarloaf is due to its sweetness or the high Brix level.

It was observed that the majority of the respondents got their source of planting materials from their farms whilst others rely on other farmers for planting materials. This result agrees with the finding of Sarpong et al. (2017) that more than two-thirds of pineapple farmers in Ghana get their planting materials from farms of their own and neighbours. However, when farmers chose to acquire planting materials from their source, they are unable to produce it in large quantities or enough to cover their farm areas. This forces them to fall on friends for planting materials and this could lead to a source of infection and the spread of these diseases (Anon, 2005, 2006). Pesticide Initiative Programme (PIP) (2004), however, suggested that farmers can produce their planting materials or rely on friends for their propagules, provided they are trained on the symptoms of the MWP disease and they can carefully select healthy plantlets from whole planting materials.

The results of the study also revealed that the source of finance for the majority of the farmers came from their savings and this suggests a lack of external financial support to expand their production. The results further revealed that the farmers adopt various agronomic practices in the production of pineapples (Table 8) that could affect the incidence of MWP and other diseases in their farms. The majority (93.3%) of the respondents affirmed keeping fallow plots, and this finding agrees with that of Sarpong et al. (2017). This practice of keeping fallow plots by farmers could result in a reduction of the incidence of MWP disease in their pineapple farms. Allowing fallow periods aid in breaking disease and pest cycles and also that ensures lands for cultivation regain their fertility (PIP, 2004). Contrary to the recommendation by Paulle and Duarte (2011) on the fertilization of pineapple crops to ensure good yields, the

majority of the respondents in this study (see Table 8) do not apply any form of fertilizer in the production of pineapple. The farmers explained that they are not able to afford the high price of fertilizers. This is true for smallholder farming families in Ghana who are resource-poor and are not able to afford some farming inputs such as fertilizers and pesticides (Donkoh and Abgoka, 1995; Paulle and Duarte, 2011; Leon and Kellon, 2012).

The results also revealed that the majority (67.8%) of the respondent's practice monocropping with only 22.2% practicing mixed cropping. Although monocropping offers the farmers insurance against crop failure since there is less competition between pineapple and other crops, it exposes the crops to a high incidence of pests and diseases due to the continuous cultivation of the same crop. Moreover, monocropping is characterised by dense populations with genetic homogeneity and as a result, once a disease becomes established, it can rapidly spread to epidemic proportions (Arya, 2002). Mixed cropping on the other hand is known to reduce the incidence of plant diseases through diversification of production and it is mainly recommended for smallholder farmers to prevent the risk of disease spread (Iwuchukwu *et al.*, 2017; Sumbali and Mehrotra, 2009). Some of the farmers also intercrop with banana, plantain, and citrus, which are known to be alternative hosts for the vector mealybug and the virus. This confirms the works of Williams and Willink (1992), and Mau et al. (2007) which report that the mealybug having a wide range of hosts and being polyphagous, doubles the incidence of the virus.

Farmers Awareness, Knowledge and Percentage Loss of MWP disease

The disease was well known to farmers in all the three major pineapple production areas in the region. Almost all the farmers had experienced the MWP

disease in their farms and were even able to give a vivid description of the symptoms of the disease and were also able to differentiate between MWP disease and other wilt conditions. Some common MWP disease symptoms described by the farmers include slight reddening of the leaves about halfway up the plant, definite or sudden change in leaf colour from red to pink and the leaf margins turn yellow and roll under. Others include the leaf tip dieback and affected leaves become limp and droop, the entire plant completely withers and all leaves pulled off from the heart. These symptoms are consistent with MWP disease symptoms reported by Broadley et al., (1993) and PIP, (2004).

Among the farmers who were aware of the MWP disease, the majority of them did not know the exact cause; others attributed the symptoms to climatic factors such as high temperature, low rainfall, and soil factors such as inadequate soil nutrients to the plant or the used excessive fertilizer. This was similar to the finding by Sarpong et al. (2017) where pineapple farmers from parts of Central and Eastern regions of Ghana were able to describe the symptoms of MWP disease but did not know the causes and epidemiology of the viral disease. The farmers were not aware of mealybugs is the vector that transmits the PMWaVs but rather associated the disease with climatic and soil factors (Sarpong *et al.*, 2017; PIP, 2004; Broadley *et al.*, 1993).

Most farmers indicated that they experienced the MWP symptoms during the flower induction growth stage. This finding suggests that the plants were infected by the virus earlier at the vegetative stage because it can take from five months to a year for the symptoms to appear after the actual feeding by the viruliferous mealybugs. An infected plant could go through the full growth cycle without showing any MWP disease symptoms which could lead to loss of

fruit quality (Anon, 2005; Joy and Sindhu, 2012; Sarpong *et al.*, 2017). The results of the present study contradict the report of Sarpong et al. (2017) where the majority of the pineapple farmers in Ghana indicated that occurrence and severity of the MWP were higher during the pre-flowering growth stage than the post-flowering growth stage. However, PIP (2011) reported that MWP could be severe at all the growth stages of pineapples.

Most farmers (83.3%) were aware of the relationship between ants and mealybug populations and that of incidence and severity of the MWP disease (Table 9). The farmers further confirmed that the higher the ants/mealybug populations, the higher the incidence and severity of the disease. This suggests that the farmers were able to confirm the symbiotic relationship between the ants and the mealybug and the disease spread of which they may or may not know directly. It has been reported by several scientists that PMWaVs, the causal agent of MWP disease is transmitted by two species of mealybug, *Dysmicoccus brevipes*, and *D. Neobrevipes*, (Sether *et al.*, 1998; 2005), which are in a symbiotic relationship with ants such as the ants offering protection to mealybugs against their natural enemies. This discourages the parasitoid from attaching the mealybugs, in return of honeydew rich in amino acids and sugars secreted by mealybugs. (Jahn and Beardsley, 2000; Rohrbach and Johnson 2003; Jahn *et al.*, 2003).

Several strategies have been recommended for the management of MWP disease in pineapple farms. These include controlling mealybug associated with wilt on fallow plots, controlling the disease on mother plots, and controlling the disease on the field including not harvesting suckers from infected mother plant/ plot (Rohrbach and Mau, 2002; Kuwornu and Mustapha, 2013; Iwuchukwu *et*

al., 2017). Report from Sarpong et al. (2017) also indicates that most pineapple farmers in Ghana control ant and mealybug vectors from the mother plots with insecticide, keep fallow plots, and do not harvest suckers from infected mother plots. On the contrary, however, in this present study, most farmers did not manage the MWP disease on fallow plots, mother plots, and the field.

The inability of most farmers to control MWP disease in their mother plot contributed to the high prevalence of the disease in the study areas since mother plots are mainly used as sources of planting materials. This confirms the reports by Joy and Sindhu, (2012) and Anon, (2005) that suckers or slips used as planting material should not be harvested from the mother plant within about 1.2 square metres of an infected plant. It has also been recommended that, if less than 3% of plants show wilt symptoms, those affected should be pulled out and destroyed. However, if more than 3% wilt is observed in a field, apart from destroying the individual plants, a mealybug control spray programme should be implemented. On the other hand, if more than 10% of plants in a field exhibit MWP disease symptom early, planting material from this field should not be used, even if control of wilt appears effective (Anon, 2005; PIP, 2011; Joy and Sindhu, 2012). Most farmers also harvest from all mother plants provided the suckers look healthy (Table 11), and this could be due to their inadequate knowledge of the epidemiology of MWP disease. The result also revealed that the majority of the farmers do not use pesticides to control ants and mealybug and for that matter, MWP disease and attributed this to high cost and ineffectiveness of the insecticides and other unknown reasons (Table 11). It was observed that about 22.2% of the respondents manage MWP disease on the pineapple fields by removal or rogueing of diseased plants. According to Joy

and Sindhu (2012), removal of the infected plants (rogueing) from the rest of the field is one major way of controlling MWP disease within an infected field and also reducing the population of the vectors (mealybug and ants).

The effects of MWP disease on pineapple production over the years have been known to portray serious tip dieback, descending curling, reddening, and wilting of the leaves which cause total death (Sether and Hu, 2002). Yield losses due to MWP disease could reach 100% as a result of transmission occurring either at the induction stage or at the fruiting stage (Sether and Hu, 2002; Jahn *et al.*, 2003). Similarly, yield loses due to MWP diseases reported by the farmers ranged from less than 10% to 30%, and this was discouraging them from continuous cultivation of pineapple as reported by Sarpong et al. (2017) who observed yield losses ranging between 1% and 60% due to MWP disease.

The study also revealed that the farmers' educational levels and experience in farming had a significant influence on their awareness and management of the MWP pests and disease. These findings are consistent with that of Sarpong et al. (2017) and Iwuchukwu et al. (2017) who reported that both formal education and experience in farming could serve as a means through which farmers get informed. This affirms the reason why the majority of farmers were aware of the incidence of viral diseases and pests' damages in their farms. However, farmers' educational levels and experience did not have a significant influence on the cause of MWP disease. Thus, irrespective of the farmers' educational levels and their experience in pineapple farming, the majority of them did not know the cause of the disease.

Incidence and Severity of the MWP Disease in the Selected Districts

The observation MWP in all the farms at both pre- and post-flowering induction growth stages surveys in the three districts, the study is an indication of the high prevalence of MWP in the Central region. This was also reported by Rohrbach and Mau (2002), Hughes et al. (2002), Sether *et al.* (2009), and Joy and Sindhu (2012). The high prevalence of MWP could be due to farmers not treating their mother plots and pineapple fields as well as poor farming practices adopted by the pineapple farmers as noted by Bartholomew et al. (2003), Kuwornu and Mustapha (2013). The high prevalence and severity of MWP could also be due to the practicing continuous cropping, do not allow land to fallow, and do not control the disease in both the mother plots and the field (Donkoh and Abgoka, 1995). Bartholomew et al. (2003) and Jahn et al. (2003) have reported that intercrop pineapple with plantain, maize, cassava, and even citrus which are known to be alternate hosts for the vector mealybug bring about the build-up of PMWaVs and its mealybug vector.

The study also revealed that farmers cultivate Sugarloaf variety of pineapples which is known to be susceptible to MWP disease (Sarpong *et al.*, 2017) compared to varieties such as MD2 which is resistant to MWP disease (d'Eeckenbrugge and Leal, 2003; Jahn *et al.*, 2003). Famers do not use resistant or improved varieties due to financial constraints or the resistant varieties could not be available due to the lack of effective multiplication and distribution of planting materials. Furthermore, the study revealed that the farmers were not aware of the benefits they will gain from planting these improve or resistance varieties (personal communication with some of the farmers), and all these

practices favour the spread of MWP disease, and hence its high prevalence in the Central region.

The result of the study has also shown that AAK and KEEA are hotspots of MWP disease in the Central region compared to Ekumfi with the lowest incidence and severity scores (Table 14). The differences in incidence and severity of MWP disease among the three districts could be attributed to an interplay of different climatic and soil factors, farmers' agronomic practices, pineapple cultivar, and viral species/ strain.

From the result, it suggests that irrespective of the district, the incidence, and severities of MWP disease amongst the three districts in both pre and post-induction stages of growth of the plants occurred the dry and rainy periods respectively and also incidence and severity during the pre-induction stages were higher than that of the post-induction stage of the pineapple (Table 14). The reasons for the difference in incidence and severities recorded in these districts mighty be an interplay of agronomic practices by the farmers, climatic and geographical factor, viral species or strain and also mixed disease infection which also supports the reports by Jahn et al. (2003), Kuwornu and Mustapha (2013) and Iwuchukwu et al. (2017) Donkoh and Abgoka (1995). Some reports suggested that seasonal changes could affect vectors, hosts, and pathogens. These influence the quantities of vectors reproduced, the replication of the pathogen (virion) which could determine the rate at which hosts plant is affected, since, these might influence parasite transmission thus altering the behaviour of hosts and the biology of vectors or parasite infectious stages in the environment (Nakasone and Paull, 1998; Williams *et al.*, 2017).

Influence of Mealybugs and ants Population and the MWP Disease

The study revealed that there was a positive correlation on the mealybugs, ant's population, and the extent of disease infection which means that there is a symbiotic relationship between ant and mealybug's population which influence the spread of the MWP disease within the districts (Table 16). This agrees with the report by Rohrbach et al. (1988), Rohrbach and Johnson, (2003) and Jahn et al. (2003) that there is a mutualistic relationship between ants and mealybugs, with the ants playing a key role in dispersing mealybugs from the alternate host or older pineapples to newer plantings of pineapple. The ants offer protection to mealybugs against their natural enemies discouraging the parasitoid from infecting the mealybugs, which in return gets honeydew rich in amino acids and sugars secreted by mealybugs.

However, the difference in ant and mealybugs' population and severities recorded within these districts mighty be an interplay, climatic or geographical factor and planting materials. The variation of the climatic condition within the districts may favour the parthenogenetic status of the female mealybugs which confirms the report by Jahn and Beardsley (2000). Temperature influences directly the plant host genotype or on the virus replication thus high temperature favours ant and mealybugs population which leads to producing large quantities of eggs and viruses able to replicate well within the plant as a report by Sether et al. (2009).

The districts with low ant mealybug population also show a reduction in the severity of the disease during the rain period and these may be due to rains with wind washing the eggs and dislodging other growth stages of the mealybugs and the ants thereby reducing their population. This is confirmed

reports by Jahn and Beardsley (2000) that rainfall affects the mealybug and ant's population since it disorients them especially when the rains are associated with wind since they wash the mealybug and the ants away. However, some farms surveyed had favourable amount of rainfall which influenced increased ant's mealybug's population which led to an increase of MWP severity (see Table 1).

Other reasons for the low or increased population of the vectors and the severity level of the disease could probably be attributed to more vegetative growth. This makes mealybugs or ants stay or prefer those farms and or crops providing them with a constant supply of food. And that affirms the report by Jahn and Beardsley (2000), and Rohrbach and Johnson (2003). Sether et al., (2009) observed that mealybugs possibly fed on the pineapple crops on the farms with more vegetative growth and those with thick leaves. Jahn and Beardsley (2000) also revealed that the pest *Dysmococcus species* were related to MWP disease as a vector for conveying pineapple mealybug wilt associated viruses (PMWaVs) and that the pink pineapple mealybugs commonly feed on the roots, leaves, stems, fruit, and crowns of pineapple. On the other hand, gray pineapple mealybugs infest only the aerial roots, stems, fruit, and crowns of pineapple. And that mealybugs initially show up on roots and make it hard to handle in its beginning times. The roots stop developing and result in a breakdown of the tissue. Mealybugs are also found on the basal parts of the plant, mostly in the leaf axils and on the forming fruits and they feed on plant sap in the phloem of their host plants. As population builds mealybugs turn out to be increasingly destructive since the bugs suck the sap from leaves bringing about wilting manifestations.

Influence of Soil Fertility Status on the Disease Prevalence

The study has revealed that the soils surveyed had low inherent fertility status, in that their total N concentrations and available P and exchangeable K concentrations Organic matter, and Organic carbon were all generally low (see Table 17). The critical limits of N, P, and K recommended by the Council for Scientific and Industrial Research (CSIR) are 0.13%, 20 µg g-1, 0.47 c mol kg-1 for N, P, and K, respectively (Yeboah *et al.*, 2012). The low nutrient content of the soil can be related to continuous cropping in the soils or weathering parent material (Evans *et al.*, 2002; Paulle and Duarte 2011).

Furthermore, the soils surveyed were found to be slightly acidic with soil pH of 5.38, 5.32, and 5.62 in the KEEA, AAK, and Ekumfi districts respectively (Table 17). This low soil pH could be as a result of continuous cropping and also leaching of soil basic cations that were reported by Ficciagroindia, (2007). This means the soil pH plays an important role in the overall health status of plants since it is one of the deciding factors affecting plant nutrient uptake and movement and many soil attributes and reactions. However, pineapple crops grow well in slightly acidic soils and that explains the slight positive correlation between soil pH and incidence and severity of MWP disease (see Table 18). However, there were no significant correlations between soil N, P, and K and incidence and severity of MWP indicating that the degrees of incidence of MWP among the pineapple farms surveyed were not dependent on the levels of soil N, P, and K. According to Paulle and Duarte (2011), it could be due to the general inherent low soil fertility status of the pineapple farms.

Molecular Detection of PMWaVs in Plant Samples in Major Pineapple Growing Areas in the Central Region.

Over the years, symptoms alone have not been effective in the detection of the plant viral disease (Agrios 2005). The detection of PMWaVs by molecular means has however been shown to be reliable and efficient (Gambley *et al.*, 2008; Sether *et al.*, 2009). To confirm the presence of the virus as illustrated by phenotypic detection, viral RNA was detected by quantitative real-time polymerase chain reaction (qRT-PCR) using viral RNA by specific primers varied from districts. The study qRT-PCR assays detected five closteroviruses (PMWaV-1- PMWaV-2 - PMWaV-3 -PMWaV-4 -PMWaV-5) from the diseased pineapple samples collected from the three districts, which are leading pineapple producing centres of Ghana. This is the first time all the five viral species have been identified in one country in Africa. Similarly, all five virus species have been identified from Hawaii (Dey et al., 2018) and Australia (Gambley *et al.*, 2008). Three out of the five viral species namely PMWaV-1, PMWaV-2, and PMWaV-3, have been identified in Taiwan (Shen *et al.*, 2009), Mexico (Ochoa-Martinez *et al.*, 2013), China (Yu *et al.*, 2015), Cuba (Hernandez et al., 2012), etc. These countries are major pineapple growing countries in the world, suggesting that these viruses are prevalent in all major pineapple growing countries worldwide as reported by Sether and Hu, (2002) and Dey et al. (2018). The presence of all the five viruses in Ghana is a clear indication that MWP poses a serious threat to the pineapple industry in Ghana. The study also detected multiple viral infections in all the three districts, with all five viral species detected in a sample in AAK. The mixed infections could result in the recombination of viral species and lead to a variety of intrahost

virus-virus interactions. Many of these virus-virus interactions may result in the generation of variants showing novel genetic features, and thus changing the genetic structure of the viral population (Syller, 2012).

AAK district had significantly higher PMWaV infection than that of KEEA and this could allude to the report by Dey et al., (2018) that the presence of large numbers of viruliferous mealybugs feeding, and were always present in areas with MWP symptoms. It was not surprising that AAK districts recorded a higher PMWaVs infection due to the high mealybug population. It also indicates that PMWaVs are acquired and transmitted in the field by their mealybug vector (Dey *et al.,* 2018). The least PMWaVs infection recorded on the farms at Ekumfi could also be as a result of the small number of samples tested or the absence of exposure to viruliferous mealybugs under field conditions. It can also allude to farmers practicing proper agronomy practices such as good sanitation, proper pest management which reduces the abundance of the viruliferous mealybugs on their farm as reported by Sether and Hu, (2002).

Genetic Confirmation of PMWaVs

Genetic variability of PMWaV-1, PMWaV-2, and PMWaV-3 populations infecting pineapple crops in the Central region of Ghana was analysed using the sequences encoding HSP70 homologous genes of the viral genome. The results revealed that PMWaV-1, PMWaV-2, and PMWaV-3 isolates clustered into three main genetic groups (evolutionary, divergent, and lineages) corresponding to three clades supported by bootstrap values more than 98%, irrespective of the geographical origin. This result confirms the Variability of PMWaV-1, PMWaV-2, and PMWaV-3 isolates infecting pineapple crops in Ghana.

The nucleotide diversity of the HSP70h gene for PMWaV-1, PMWaV-2, and PMWaV-3 also revealed that PMWaV-3 had the higher nucleotide diversity than that of PMWaV-1 and PMWaV-2 isolates, with PMWaV-2 having the least diversity in HSP70h gene. This could be due to the greater number of mutations and recombination in the genes of PMWaV-3 and PMWaV-1 than the genes of PMWaV-2 (Melzer *et al.,* 2001). According to reports by Roossinck (1997), mutation and recombination are the initial sources of variation in populations. RNA viruses use all known genetic variation processes to guarantee their survival, mutation, and recombination are the main cause of errors that occur during the replication of RNA viruses. This results in a high degree of variability (Domingo and Holland 1997; Elena *et al.*, 2014) and these may account for the high sequence variants or haplotypes observed (Wimp and Whitham, 2001; Sacristan and García-Arenal 2008; Elena *et al.,* 2014).

Despite the high number of mutations and the consequent high number of haplotypes recorded for the PMWaV-1, 2 and 3 HSP70 genes, the genetic diversity was low (0.0172 ± 0.0032 for the PMWaV-1 isolates, 0.0199 ± 0.0024 for PMWaV-3 and 0.0074 ± 0.0016 for the HSP70 gene of PMWaV-2), suggesting genetic homogeneity. This is in line with the observation made by Sacristan and García-Arenal (2008) and Elena et al. (2014), which indicated that populations of plant viruses are not extremely variable despite high genetic variation potential and high mutation rates are not necessarily adaptive as a portion of the mutations are deleterious. It has also been reported that analysed populations of plant viruses are genetically stable, and this is so regardless of the many haplotypes that may occur in the population (Elena *et al.*, 2014). For instance, research by Garcia-Arenal et al. (2001) of which out of twenty-two of

29 virus species that was worked on genetic diversities of below 0.10 who recorded. The high rate of mutation in RNA viruses could not be due to an evolutionary strategy but to the need for replication of their chemically unstable RNA genome (Roossinck and Ali, 1997; Roossinck and Garcia-Arenaal, 2015). However, High mutation rates for RNA viruses have been revealed by Garcia-Arenal et al. (2001) to represent an evolutionary strategy.

An indication of population substructuring was the important neutrality deviation observed from the neutrality trials. All the tests for neutrality showed negative values (see Table 24), indicating that all PMWaV-1, 2, and 3 populations were in active evolution.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

From the study, it can be concluded that MWP is prevalent and severe in the pineapple farms in the Central Region of Ghana. The majority of the farmers were aware of the MWP in their pineapple fields, and the disease causes serious tip dieback, downward curling, reddening, wilting of the leaves, and death of the plant. The majority of the farmers had low educational levels, who adopt some poor agronomic practices by relying on their farms for planting materials, and do not control disease in mother plots, fallow plots and on main fields, and practiced monocropping. They mainly cultivate Sugarloaf variety of pineapple, and many of them loss between 1% and 30% of their yield per hectare due to MWP disease attack.

Again, the outcome of the field survey suggests that there were significant differences in the mean incidences and mean severity scores of MWP disease among the three districts, with AAK having the highest values whilst Ekumfi had the lowest and also levels of incidence and severity scores were higher during the pre-flower induction stage than at the post flower induction. There was a relationship between the ants, mealybugs' population, and the incidence of PMW disease, however, there were no significant differences in the mean ant and mealybug populations among the three districts surveyed. The Soil fertility status of the farms surveyed at the three districts were inherently low and acidic and did not correlate significantly with the levels of mean incidence and severity scores of MWP disease.

Finally, it was revealed that five different closterovirus species namely PMWaV-1, PMWaV-2, PMWaV-3, PMWaV-4, and PMWaV-5 were detected from the plant samples during the study using qRT-PCR assays with five PMWaVs specific primers. Mixed viral infections by 2 or more of the five viral species were detected in the pineapple samples from all three districts. Phylogenetic analysis of both nucleotide and amino acid sequences of the heat shock gene (HSP70) confirmed the presence of PMWaV-1, PMWaV-2, and PMWaV-3 in the pineapple samples from the three districts of the Central region of Ghana. This is the first report of PMWaVs in Ghana and the whole of Africa.

Recommendations

1. Intensive education on the causes and management of MWP disease should be carried out in the pineapple growing areas in the Central region to save the pineapple industry.
2. Farmers should be encouraged to adopt integrated pest and disease management strategy. Farmers should be educated to manage MWP disease in the mother plots, fallow plots and field. They should be educated not to use planting materials from mother plots with the incidence of MWP disease.
3. Farmers should be educated to adopt good agronomic practices and good farm sanitation to prevent and minimise disease incidence and spread.
4. The study should be repeated at the other major pineapple growing regions namely Eastern, Greater Accra, and Volta regions of Ghana. Knowledge of the status of the prevalence of MWP disease and the

genetic structure of associated PMWaVs will lead to a comprehensive disease management strategy for MWP disease in Ghana.

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APPENDICE

APPENDIX ONE

SURVEY QUESTIONAIRE

DEPARTMENT OF CROP SCIENCE, SCHOOL OF AGRICULTURE, UNIVERSITY OF CAPE COAST

This questionnaire is designed to elicit information from Mealybug wilt of pineapple (MWP) disease on pineapple farms in some selected districts in the Central and Eastern region of Ghana. This questionnaire is strictly for research purposes and any information given will be treated with all confidentiality.

District code……

Name of community……

Serial number of respondents……

Demographic and Farm Characteristics of Respondents

1. Sex a) Male [ ] b) Female [ ]
2. Age……
3. Occupation……
4. Level of education reached: a) None [ ] b) Non-formal [ ] c) Some basic education [ ] e) Basic education [ ] f) Some secondary education [ ] g) Secondary education [ ] h) MSLC [ ] i) Tertiary [ ]
5. How long have you been cultivating pineapple? a) < 1 year [ ] b) 1-5 year [ ] c) above 5 years [ ]
6. Size of land under cultivation with: a) MD2……. b) Smooth Cayenne…… c) Queen Victoria……. d)Sugar loaf…….

7. What land tenure system do you practice? a) Self owned [ ] b) Rent [ ]
   c) Share cropping [ ] d) Others……

8. What is your source of labour? a) Hired [] b) Family labour [ ]
   c) Nnoboa [ ]

9. How do you finance your work? a) Self [ ] b) Bank [ ] c) Susu operator [ ] d) Family members [ ] e) Others……

Agronomic Practices

10. What method of land preparation do you employ?
    a) Zero tillage [ ] b) Slash and burn [ ] c) Tractor plough [ ] d) Others.

11. Source planting materials a) Own source [ ] b) Other farms [ ]

12. Do you keep fallow plots a) Yes [ ] b) No [ ]

13. If you answered yes to question 12 how do you control the mealybug associated wilt on your fallow plots ……

14. Duration of your fallow a)12 months [ ] b)18 months [ ]
    c) 24 months [ ] d) Beyond 24 months, specify

15. Do you use fertilizers on your farm? a. Yes [ ] b. No [ ]

16. If yes, which type? A) Chemical fertilizer [ ] b) Organic-manure [ ]
    3. Both [ ]

17. Why this type of fertilizer? A) Cheaper [ ] b). More efficient [ ] c) Easy to apply [ ] d) Other.....

18. What crops receive chemical fertilizers and why? .....

19. Which types of chemical fertilizers do you use? A) NPK [ ] b) Urea [ ] c) Ammonia [ ] d) Others, specify.....

20. What method of fertilizer application do you use? a) Broadcasting [ ] b)Spraying [ ]c) Drilling [ ] d) Others specify……

21. Estimate quantity of chemical fertilizer usage per acre on your farm.....

22. How many times do you apply fertilizer before you harvest your maize? a) Once [ ] b) Twice [ ] c) Other, specify.....

23. If multiple applications, state the order……

24. When (what stage) do you apply your fertilizer after planting? a) 1. 3 weeks [ ] b) 2. 6 weeks [ ] c). Other, specify.....

24. What farming practices do you use? a) Monocropping [] b) Mixed cropping [ ] c) Others……...

25. If mixed cropping, what kinds of crop do you intercrop?

a) Banana [ ] b) Okra [ ] c) Others……

26. Do you practice crop rotation? a) Yes [ ] b) No [ ]

27. What time do you plant your crop? a) Major season [ ] b) Minor season [ ]

28. What variety do you cultivate? a) MD2 [ ] b) sugar loaf [ ]

c) smooth cayenne [ ]

30. Control of mother plots against mealybug associated virus of pineapple

a) 3 months interval [ ]

b) 6 months interval [ ] c) 9 months interval [ ] d) Other, specify……

31. Do you treat the soil that has been planted in the earlier seasons before planting new suckers?

a) Yes [ ] b) No [ ]

32. Do you do spot soil treatment or whole plot treatment if you are replanting on a land that has been planted before with an incidence of mealybug wilt. A) Spot treatment [ ] b) Whole plot treatment [ ]

Farmers' awareness of viral diseases

33. Have you observed the MWP disease on your farm before? a) Yes [ ]
    b) No [ ]
34. If yes, describe the disease? ……
35. What causes the MWP disease?.....
36. At What growth stage do you first encounter the disease? a) Juvenile stage [ ] b) Induction stage [ ] c) Fruiting stage [ ]
37. Which season does the disease occur? a) Dry season [ ] b)Wet season [ ]
    b) Both seasons [ ]
38. At which season is the disease very severe? a) Dry season [ ] b) Wet season[ ] b)Both seasons [ ]
39. What is the estimated yield loss after infection? a) <10 [ ]
    b) 11-20% [ ] c) 21-30% [ ] d) 31-40% [ ] e) 41-50% [ ]
    f) above 50% [ ]
40. In your experience/opinion is there a relationship between ants and mealybug population and the incidence and severity of the mealybug wilt virus disease a) Yes [ ] b) No [ ]
41. If you answered yes to question 40 above, what is the relationship between the ants/mealybug populations and the mealybug associated wilt virus disease a) the higher the ants/mealybug the higher incidence and severity of the disease b) Other, Specify ……
42. The disease is restricted to certain portions of the field a) Yes [] b) No []
43. If you answered yes to question 42, then which spots/areas is the wilt restricted to during attack ……

44. Plants that are attacked by the wilt disease are able to recover to bear exportable fruits.

    a) Yes [ ] b) No [ ]

Disease management

45. By what means do you prevent MWP disease in your mother plots a) Spraying with insecticides [ ] b) Physical destruction of infected mother plants [ ] c)Not planting at the same spot for at least two seasons [ ] d) Other, specify……..

46. How do you control these diseases? a) Chemical application [ ]

    b) Removal of infected plants [ ] c) Botanicals [ ] d) No control [ ]

    e) Others…….....

47. If chemical, how often do you apply? Please specify……

48. If chemical is used, mention the kind of chemical (s)? ……

49. What are the sources of your pesticides? Specify……

50. Who advises you on the choice of chemical? a) AEA[ ] b) Agro-input dealers [ ] c) Other farmers [ ] d) Others……

51. Do you alternate the use of chemicals? a) Yes [ ] b) No [ ]

52. If you alternate, who advices you? Specify……

53. Why do you alternate?.....

54. How long do you wait after spraying before harvesting? Please specify….

55. Is the control measure effective? a) Yes [ ] b) No [ ]

56. What other diseases do you encounter on your pineapple farm?.....

57. What major pests do you encounter on your pineapple farm?.....

58. What type of damage do these pests cause to the plants? Please describe…

59. What is the estimated yield loss after infestation? a) <10% [ ] b) 11-30% [ ] c) 31-50% [ ] c) 51-80% [ ] d) above 80%[ ]
60. How do you manage the pests you encounter on your pineapple farm? a) Chemical application [ ] b) botanical application [ ] c) Hand picking and crashing[ ] d) No control [ ] e)Others ……
61. If chemical, how often do you apply? Please specify……
62. If chemical is used, mention the kind of chemical (s)?.....
63. Is the pest management programme effective? a) Yes [ ] b) No [ ]
64. Do you alternate the use of pesticides? a) Yes [ ] b) No [ ]
65. If chemical is used, mention the kind of chemical (s)? ……..
66. How many times do you apply the chemical? ……..
67. Do you alternate the chemical? a) Yes [ ] b) No [ ]
68. Do you control alternate crops? a) Yes[ ] b) No [ ]
69. Re-entry interval……
70. Pre-harvest interval……
71. Pesticide disposal method. a) Buried [ ] b) incineration [ ] c) throw it around [ ]
72. Mode of mixing pesticide. a Hand[ ] b) stick[ ] c)swirling/ shaking of sprayer[ ]
73. Application equipment used a) knapsack [ ] b) motorizes sprayer[ ] c)bucket and broom[ ]
74. Place of pesticides storage after acquisition. A) Bedroom[ ] b)kitchen[ ] c)bathroom[ ] d)storeroom[ ]

APPENDIX TWO

Table 14. Mean prevalence and severity score of viral disease in both pre and post-induction stages among the various communities within the districts

Communites	Prevalence (%)		Severity (%)	
	pre-induction stage	post-induction stage	Pre-induction stage	post-induction stage
Abrenu-Akyinim	8.00±0.71^{bcd}	4.00±0.45^{b}	0.89± 0.12^{abc}	0.68±0.1^{2abc}
Ankwanda	11.00±1.30^{d}	9.60±1.50^{de}	1.61± 0.20^{ef}	1.10±0.19^{de}
Atta-Badzi	9.20±1.28^{cd}	7.20±1.16^{cd}	1.23± 0.11^{cde}	1.04±0.06^{cd}
Essaman	7.40±0.81^{bc}	5.20±0.74^{bc}	1.09±0.08^{bcd}	0.93±0.06b^{cd}
Amoasima	9.20±1.32^{cd}	6.60±1.03^{c}	1.10±0.08^{bcd}	1.15±0.10^{de}
Asebu-Ekrofui	5.20±1.02^{ab}	3.20±1.07^{ab}	1.07± 0.10^{abcd}	0.79±0.21^{bcd}
Asuasi	14.60±2.79^{e}	11.0±1.18^{e}	1.66±0.32^{f}	1.47±0.18^{e}
Ayeldu	8.80±1.07^{cd}	7.20±0.86^{cd}	1.31± 0.12^{def}	0.99±0.16^{bcd}
Abor	2.80±0.66^{a}	0.80±0.37^{a}	0.68±0.11a	0.35±0.15^{a}
Asofa	7.60±0.87^{bcd}	5.20± 0.74^{bc}	1.06±0.10^{abcd}	0.99±0.09^{bcd}
Atwiaa	3.00±0.45^{a}	1.40±0.25^{a}	0.81±0.08^{ab}	0.52±0.04^{ab}
Ekumfi	5.00±1.14^{ab}	1.40±0.51^{a}	0.88±0.15^{abc}	0.77±0.20^{bcd}
Mean	7.65	5.23	1.12	0.90
LSD ($p \leq 0.05$)	3.57	2.55	0.41	0.41
P-value	<.001	<.001	<.001	<.001

Means in the same column bearing the same letters are not significantly different from each other ($P < 0.05$) *Mean± Standard error; KEEA: Komenda-Edina-Eguafo-Abirem; AAK: Abura-Asebu-Kwamankese.

APPENDIX THREE

Table 19. Ant and mealybug population and the extent of disease infections with the twelve communities in the three districts

Communities	Mean ant population (%)	Mean mealybug population (%)	Mean Severity
Abrenu-Akyinim	10.40±2.11^{ab}	12.20±2.45^{ab}	2.50±0.29^{abc}
Ankwanda	10.40±1.63^{ab}	13.20±2.03^{ab}	1.94 ±0.15^{ab}
Atta-Badzi	13.00±2.10^{b}	15.00±1.55^{bc}	2.89 ±0.43^{cd}
Essaman	10.40±1.50^{ab}	13.00±0.71^{ab}	2.09±0.35^{abc}
Amoasima	10.00±1.67^{ab}	11.20±2.08^{ab}	2.34±0.22^{abc}
Asebu-Ekrofui	10.20±1.77^{ab}	11.60±2.29^{ab}	2.20±0.17^{abc}
Asuasi	14.60±1.63^{b}	20.00±2.63^{c}	3.58 ±0.39^{d}
Ayeldu	12.00±0.95^{ab}	13.20± 1.20^{ab}	2.62 ±0.20^{bc}
Abor	10.80±1.39^{ab}	11.40±2.16^{ab}	2.10±0.25^{abc}
Asofa	10.60±1.17^{ab}	10.40±1.54^{ab}	2.13 ±0.17^{abc}
Atwiaa	10.20±1.59^{ab}	12.00±2.07^{ab}	1.68 ±0.28^{a}
Nanaben	8.20±1.93^{a}	9.00±1.73^{a}	1.94 ±0.31^{ab}
Mean	10.90	12.68	2.34
P	0.485	0.048	0.004
l.s.d.	4.705	5.539	0.839

(Source: Field Survey, 2018); Means in the same column bearing the same letters are not significantly different from each other ($P < 0.05$).

APPENDIX THREE

Table 21. Various soil fertility levels in the soils of the communities where MWP disease was surveyed in the three districts.

District	Community	pH	%MC	%OM	%N	Exchangeable K (cmolkg^{-1})	Available P (µgg^{-1})	%OC	C_N_ratio	CEC_cmol_kg
KEEA	Abrenu-Akyinim	4.918	0.96	1.94	0.1060	38.6	0.177	1.12	10.62	4.82
KEEA	Ankwanda	5.464	18.60	2.00	0.0995	3.2	0.129	1.16	11.90	6.16
KEEA	Atta-Badzi	6.132	10.83	4.46	0.2385	6.8	0.529	2.59	10.99	8.99
KEEA	Essaman	4.990	11.65	1.63	0.0748	3.5	0.118	0.95	12.76	4.18
AAK	Amoasima	5.356	5.41	4.94	0.1478	14.4	0.366	2.86	21.06	6.69
AAK	Asebu-Ekrofui	4.692	7.19	2.27	0.1065	3.7	0.210	1.31	12.96	5.37
AAK	Asuasi	5.882	4.42	2.82	0.1924	3.5	0.246	1.64	8.64	9.13
AAK	Ayeldu	5.338	5.56	2.20	0.1058	3.8	0.326	1.28	11.63	6.58
Ekumfi	Abor	6.160	7.28	3.52	0.1663	2.9	0.415	2.04	11.39	9.20
Ekumfi	Asofa	4.772	3.58	3.32	0.1707	9.7	0.351	1.92	12.99	6.58
Ekumfi	Atwiaa	6.242	7.13	2.45	0.1204	13.5	0.335	1.42	12.99	5.90
Ekumfi	Nanaben	5.286	8.40	2.60	0.1401	3.1	0.289	1.51	10.68	8.06
means		5.436	7.58	2.85	0.139	8.9	0.291	1.65	12.32	6.80
p		<.001	<.001	0.008	<.001	0.001	<.001	0.008	0.369	<.001
lsd		0.515	4.246	1.768	0.052	15.42	0.1679	1.026	8.106	2.136

(Source: Field Survey, 2018); Means in the same column bearing the same letters are not significantly different from each other ($P < 0.05$).