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Research Application Summary

Prevalence of pineapple mealybug wilt disease in the Central region of Ghana

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Abstract

Mealybug wilt ofpineapple (MWP) disease is a major biotic constraint to the production ofpineapple in Ghana. The study assessed farmers' awareness of MWP disease 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. Incidence and severity scores of MWP disease were determined from 20 pineapple farms selected from each of the three districts. The field data were subjected to analysis of variance (ANOVA) and the means separated with the least significant difference (LSD) method at $P<0.05$. 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.

Keywords: Ampeloviruses, *Ananas comosus*, Ghana, pineapple, PMWaVs R

Resume

La maladie du flétrissement bactérien de l'ananas (MWP) est une contrainte biotique majeure pour la production d'ananas au Ghana. L'étude a évalué la conscientisation des agriculteurs à la maladie MWP et leurs pratiques de gestion de la maladie dans les districts de Komenda-Edina-Eguafo-Abirem (KEEA), Abura-Asebu-Kwamankese (AAK), et Ekumfi dans la région centrale du Ghana. L'incidence et les niveaux de sévérité de la maladie MWP ont été déterminés à partir de 20 plantations d'ananas sélectionnées dans chacun des trois districts. Les données récoltées sur le terrain ont été soumises à une analyse de la variance (ANOVA) et les moyennes ont été séparées par la méthode de la différence la moins significative (LSD) à avec un p<0,05. L'incidence de la maladie MWP était significativement différente entre les stades de croissance pré et post-induction et entre les trois districts (p<0,05), allant de 2,20±0,46% à 9,45±1,10%. L'état de fertilité des sols des exploitations était fondamentalement faible.

Mots clés : Ampélovirus, *Ananas comosus*, Ghana, ananas, PMWaVs R

Introduction

Pineapple (*Ananas comosus* L. Merrill), a Bromeliaceae, is the third most importance fresh fruit crop after citrus and banana worldwide (Usman *et al*., 2013). Pineapple contributes to over 20 % of the world production of tropical fruits and about 70% of the pineapple is consumed as fresh fruit in producing countries (Medina and Garcia, 2005). In Ghana, the pineapple sector is the most developed horticultural sector (Kuwomu *et al*., 2013). Pineapple production is a source of income for thousands of people ranging from farmers to market women and small-holder farmers. The crop provides raw material to feed industries, leading to establishment of cottage industries. For instance, a fruit juice processing factory is being established at Ekumfi Nanaben to process pineapple in response to the Government of Ghana's one district one factory policy. 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 levels. Overall, pineapple contributed more than USD 283,000,000 in foreign exchange to the economy of Ghana between 1990 and 2013.

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

Mealybug wilt of pineapple (MWP) disease is among the major diseases of pineapple in the world causing up to 100% yield losses (Hu and Sether, 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-I (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*., 2002; 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 brevipe* Cockerell), and also by man through planting of infected planting materials (Jahn *et al*., 2003). The MWP disease symptoms are shown by tip dieback, descending curling, reddening, and wilting oft e leaves which can prompt a complete breakdown of the plant (Sether and Hu, 2002). This study assessed the prevalence of the pineapple mealybug wilt disease and how the soil influence the disease spread in the Central region of Ghana.

Materials and Methods

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 of Ghana which included Komenda-Edina-Eguafo-Abirem (KEEA), Abura-Asebu-Kwamankese (AAK) and Ekumfi districts. The disease assessment was done at both pre-induction and post-induction stages of the 2018/2019 planting season. The disease assessment was carried out in 12 communities which covered the three districts. Five farms were selected per community, and in each farm four MWP-affected plots were purposively selected and 250 plants from each were scored for incidence and severity. Thus, a total of 1000 plants were assessed per farm. The incidence and severity of the disease was computed using the following formulae:

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

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

Soil samples were collected from all the farms surveyed in order to find the relationship between the soil fertility status (N, P, K, organic carbon contents, pH, bulk density, moisture content and texture) in each farm and disease incidence and severity. Surface samples (0 -15 cm) were collected from different spots of each farm in a zigzag pattern using 5 cm diameter coring cylinder auger and 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. Total N concentration in the soil was determined using the micro Kjeldahl method. Available phosphorus was determined using the method of Bray and Kurtz (2000). 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 glass electrode of a pH meter in the soil suspension after the soil had been shaken for 15 min using a mechanical shaker according to the method described by Rowell (2012).

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 5% probability level. Data on soil fertility status (and soil pH, moisture content, organic matter, nitrogen, phosphorus, organic carbon, potassium and CEC) were subjected to ANOVA and the mean separated by least significance difference (LSD) method at 5% level of probability. Pearson's correlation coefficients were calculated in order 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).

Results

Disease symptoms observed on the pineapple crops. The crops displayed a wide range of disease symptoms during the field surveyed. The most commonly observed symptoms on all pineapple crops were definite and sudden change in leaf colour from red to pink and the leaf margins turn yellow and roll under at the leaf tips. Affected leaves change colour, become limp and droop downward with pronounced leaf rolling (Figure 1). The other symptoms encountered were stunting, narrowing, leaf rolling and yellowing.

Figure 1. Mealybug wilt of pineapple (MWP) disease symptoms

Prevalence and severity of MWP disease in both pre and post-induction stages. Table I 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 incidences of MWP amongest the three districts ($F2, 48 = 17.93$; $P < 0.001$; mean =7.65; lsd = 2.24). The highest mean incidence was recorded at AAK (9.451 1.10%), but it was not significantly different from that at KEEA district (8.9010.58%) but significantly higher than that at Ekumfi district (4.60±0.58%). An ANOVA on the mean incidence of MWP disease recorded at the three districts during the post-induction stages also showed significant difference among the three districts. The highest mean incidence was recorded at AAK (7.0010.80%), but it was not significantly different from that of the KEEA district (6.50±0.68%). but significantly higher than that at Ekumfi district (2.2010.46%).

The mean severity score of MWP disease recorded in the three districts during the pre-induction stage are shown in Table 1. An ANOVA showed significant difference in the severity of MWP disease among the three districts. The highest mean severity score was recorded at AAK (1.291 0.10) which was not significantly different from that of KEEA district (1.2110.09) but significantly higher than that of Ekumfi district (0.8610.06). The mean severity score of MWP disease recorded at the three districts during the post-induction growth stage s (Table 1) indicate that 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 (0.6710.08) (P < 0.05).

Table 1. 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±0.58b	6.50±0.68b	1.21±0.09b	0.94±0.07b
AAK	9.45± 1.10b	7.00±0.80b	1.29± 0.10b	1.10±0.10b
Ekumfi	4.60±0.58a	2.20±0.46a	0.86±0.06a	0.67±0.08a
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

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. 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 rainy season (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 3.

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 3. 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%).

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.

Table 4 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 2. Comparison of mean incidence and severity scores of Mealybug wilt of pineapple (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

Table 3. 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	10.51b	2.51	0.1297	0.238	13.0	1.45	11.57	6.04
AAK	5.317	5.65a	3.06	0.1381	0.287	6.4	1.77	13.58	6.94
Ekumfi	5.615	6.60a	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	-	-	-	-	-	-	-

Discusion

Incidence and Severity of the MWP Disease in the Selected Districts. Symptoms of the MWP diseases were found in all the farms assessed in the three districts at both pre- and post-flowering induction growth stages. Mean disease incidence ranged from 4.6% to 8.9% in the pre-induction survey, and from 2.2% to 6.5% in the post-induction survey in the three districts (Table 1). This indicates very high prevalence of MWP disease in the Central region and at all growth stages as reported by Rohrbach and Mau (2005), Sether *et al*. (2008) and Sindhu and Joy (2012). The high incidence and severity observed in this study pose serious threat to the pineapple industry in the Central region and in Ghana as a whole. Judging from the fact that most pineapple farmers do not treat their mother plots and pineapple fields (Table 1), they will inadvertently spread the MWP disease in the region and possibly to the other major growing districts and regions in Ghana. This is expected because it has been reported (Sindhu and Joy, 2012) that if less than 3% of plants show wilt symptoms, pull out and crush those affected; if more than 3% wilt is observed in a field, as well as destroying the individual plants, also implement a mealybug control spray program and if more than 10% of plants in a field exhibit MWP disease symptoms early, treat as above and do not use planting material from this field, even if control of wilt appears effective. The high prevalence and severity of MWP disease observed in the study could also be attributed to poor farming practices adopted by the pineapple farmers. This is expected because poor agronomic practices are possible causes of viral disease epidemics in tropical and sub-tropical countries (Bartholomew *et al*., 2003; Kuwornu et at., 2013). It was realized during the household survey that many farmers practice continuous cropping, and also intercrop pineapple with plantain, maize, cassava and even citrus which are known to be alternate hosts for the vector mealybug and the PMWaVs (Bartholomew et at., 2003). The farmers also do not practice land fallowing, and do not control the disease in both the mother plots and the field. Continuous cropping could bring about the build-up of PMWaVs and its mealybug vector (Jahn et at., 2003). The practice of monocropping over large land areas could also serve as a source that favours the production of vector populations and establishment of viruses.

Table 4. 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

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

The use of resistant varieties could be a greater contributor in disease control (Thresh, 1983; Bartholomew *et al*., 2003). The study also revealed that farmers cultivate Sugar loaf variety of pineapple which is kn wn 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 improve varieties probably because of lack of financial means, or the resistant varieties may not be available due to the lack of effective multiplication and distribution of planting materials. All the above factors favour the spread of MWP disease and hence its high prevalence in the Central region.

The findings show that AAK and KEEA are hotspots of MWP disease in the Central region compared to Ektimfi had the lowest incidence and severity scores (Table 1). 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 agronomy practices, pineapple cultivar and viral species/ strain. Pineapple growers and plant pathologists should therefore take this observation into consideration in planning for effective management strategies against MWP disease in the Central region.

From the result, regardless of the district, MWP disease was observed in both pre and post induction stages of growth of the plants, the incidence and severity during the pre-induction stages were higher than that at the post-induction stage of the pineapple (Table 1). The reasons for the difference in incidence and severities recorded in three districts mighty be an interplay of agronomic practices by the farmers, climatic and geographical factor, viral species or strain and also mixed disease infection, as earlier suggested by Jahn *et al* (2003), Kuwornu *et al*. (2013) and Iwuchukwu *et al*. (2017). There were reports that suggested that seasonal changes could also affect vectors, hosts and pathogens and these influence the quantities of vectors and replication of the pathogen (virion) which could determine the rate at which hosts plant become infected (Nakasone and Paull, 1998; Williams *et al*., 2017)

The high temperatures experienced during pre-induction stage could possibly increase the susceptibility of host plants to virus infection since it causes stress on the plant and the plant is unable to fight the disease infection and that accelerate the fitness of vinises to cause infection. The pre-induction stage is the vegetative stage of the plant and where the pathogen (virion) have enough food and water and that enable them to replicate and spread fast to all part of the plants. However, during the post-induction stage, which is the reproductive stage of the plant, some of the plants are able to recover from the disease infection since they are able to get adequate nutrients and water which make them produce more resistant substance which help in reducing the infection rate.

Influence of soil fertility status on the disease prevalence. The study has revealed that the soils surveyed had low inherent fertility status, in their total N concentrations and available P and exchangeable K concentrations Organic matter, and Organic carbon were all generally low (Table 3). The critical limits of N, P and Kcommended by Industrial Research (CSIR) are 0.13%, 20 μg^{-1}, 0.47 c mol kg^{-1} or 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 of 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 3). 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 might mean that 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 4). However, there were no significant correlations between soil N, P and K and incidence and severity of MWP disease (see Table 4). This suggests that the incidence and severity of the viral diseases among the pineapple farms surveyed were independent of the levels of soil N, P and K. This could be due to the general inherent low soil fertility status of the pineapple farms (Paulle and Duarte, 2011)

Conclusion

Mean incidences and severity of MWP disease varied among the three districts surveyed, with AAK having the highest values whilst Ekumfi had the lowest. The mean incidence of MWP disease ranged from 2.2% to 9.45% whereas the mean severity score ranged from 0.67 to 1.29. The levels of incidence and severity scores were higher during the pre-flower induction stage than at the post flower induction. 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 of MWP disease.

We recommend that the study should be repeated in the other leading pineapple growing regions namely Eastern, Greater Accra and Volta regions of Ghana. This is necessary because Knowledge on prevalence of MWP disease and genetic structure of associated PMWaVs would help in designing a comprehensive disease management strategy for effective management of MWP disease in Ghana.

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