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ETIOLOGICAL AND EPIDEMIOLOGICAL STUDIES ON THE RED LEAF DISEASE OF PINEAPPLE IN GHANA

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ABSTRACT

Possible cause(s) of red leaf disease (RLD) of pineapple (*Ananas comosus* L.) in Ghana were examined through field observations, isolation of suspected organisms from roots of diseased plants, growing plants in potted steam-sterilised and unsterilised natural field soils. Others included detection of the pineapple closterovirus (PCV) from symptomatic and healthy pineapple leaves using tissue blot immunoassay (TBIA). Effects of soil moisture, soil fertility, sucker condition and light intensity on the disease were studied in pots while some factor combinations were studied in mini plots in the field. Diseased plants from the field had reduced root systems and had *Neosartorya fischeri*. Nematodes of the genera *Aphelenchus*, *Pratylenchus* and *Helicotylenchus* were recovered from roots and infrequently from the rind of the underground stem. Pineapple plants grown outdoors in steam-sterilised field soil reddened just as those grown in unsterilised soil. The PCV was detected in only 53% of all typically symptomatic leaves examined. All non-symptomatic leaves also tested positive for PCV. Plants grown from symptomatic suckers in a plant house with diffuse light intensity (7,440 lm m^{-2}) and at 29 °C, recovered from RLD within 6 months. These plants, however, reddened when grown outdoors with intense light (39,751 lm m^{-2}) at 30 °C. Significantly ($P<0.05$) lower disease levels were recorded on mini-plots optimally fertilised with NPK and maintained at high moisture than on low fertility, low moisture plots. In a further mini plot trial, plants grown with reduced light had lower disease levels than those grown with full light. Overall, these results show that soil biotic factors have no primary etiological role in RLD. The viral nature of the disease is also doubtful. Leaf reddening in pineapple could be a physiological response to environmental stresses.

Key Words: *Ananas comosus*, nematodes, *Neosartorya fischeri,* mealybug wilt

RÉSUMÉ

Les possibles causes de la maladie des feuilles rouge (MFR) de l'anana (*Ananas comosus* L.) au Ghana étaient étudiées à travers des observations des champs, en isolation des organismes suspectés dans les racines des plantes infectées, en cultivant des plantes dans des pôts stérilisés par la vapeur et des champs naturels non stérilisés. D'autres méthodes incluaient la detection des closterovirus de l'anana (CVA) sur des feuilles d'anana présentant les symptômes et en bonne santé en utilisant des buvard immunoassay (BIA). Les effets de l'humidité du sol, la fertilité du sol, les conditions des rejetons et l'intensité de la lumière sur la maladie étaient étudiés dans des pôts alors que certains facteurs des combinaisons étaient étudiés dans des mini parcelles dans les champs. Les plantes infectées dans les champs avaient des systèmes des racines réduits et avaient *Neosartorya fischeri*. Les nématodes des genres *Aphelenchus, Pratylenchus* et le *Helicotylenchus* étaient récupérés des racines et de manière non regulière des écorces de la partie ensevelie dans le sol de la tige. Les ananas plantés en plain air dans des champs dont le sol était stérilisé à la vapeur rougis comme ceux la qui ont été planté dans un sol non stérilisé. Les CVA étaient detecté dans 53% des cas seulement sur les feuilles symptomatiques examinées. Toutes les feuilles non symptomatiques étaient aussi testées positives pour les CVA. Les plantes cultivées à partir des rejetons

symptomatiques dans une maison des plantes avec de la lumière diffuse (7440 lm m^{-2}) et à 29°C récupéra de la MFR dans 6 mois. Ces plantes devinrent rouge quand elles étaient plantées en plain air pour l'intensité lumineuse de 39751 lm m^{-2}) à 30°C. Des niveaux de maladies inférieurs étaient enregistrés sur des mini plots fertilisés de façon optimale avec le NPK et maintenus à l'état d'humidité élévée que sur des parcelles de faible fertilité et faible humidité. Dans d'autres essais sur des mini parcelles, ces résultats montrent que les facteurs biotiques n'ont pas de rôle étiologique dans pour MFR. La nature virale de la maladie est aussi à douter. Le rougissement de la feuille d'anana peurrait être une réponse au stress environnementaux.

Mots Clés: *Ananas comosus*, nématodes, *Neosartorya fischeri*, mealbug wilt

INTRODUCTION

Production of pineapple is an important component of the non-traditional export crop programme in Ghana. In 1997, total fresh pineapple exported from Ghana was 27,600 MT, an increase of about 5,000% over the 1983 export of 500 MT (Anon., 1997). Much of this occurred in the Akwapim South, Ga and Gomoa districts as a result of expansion in the area planted to the crop.

According to Awuah (1998), the role of pineapple as a non-traditional export crop could be curtailed by a new disease which is present in most commercial plantings. The disease is most problematic on the exportable Smooth Cayenne cultivar though the non-exportable Sugar Loaf cultivar is also susceptible to a lesser extent. Symptoms of the disease have been described by Awuah (1998) and include reduction of the root system, stunting of plants and reddening of leaves, the most readily recognisable symptom. The name 'red leaf disease' (RLD) of pineapple was proposed for this disease (Awuah, 1998). Corbett and Pagden (1941), Singh and Shastry (1975) and Petty (1994) reported a pineapple disease similar in symptomology to the red leaf disease.

In Ghana, the cause of RLD has been conjectural. The dominant view is that the disease is the same as the virus - mediated mealybug wilt (MBW) present elsewhere (Ullman *et al.*, 1989; German *et al.*, 1992; Hu *et al.*, 1996) and transmitted by mealybugs, mainly *Dysmicoccus brevipes* Cockerell and *D. neobrevipes* Beardsley (Rohrbach *et al.*, 1988; Sether *et al.*, 1998; Sether and Hu, 2000). The virus involved in the MBW is a closterovirus now referred to as pineapple closterovirus (PCV) (Ullman *et al.* 1989). Two different types of the pineapple mealybug wilt-associated virus (PMWaV) have been reported. These are PMWaV-1 and PMWaV-2 (Melzer *et al.*, 2001).

Other views on the causes of RLD in Ghana include environmental stresses as either primary agents or pre-disposition factors (stress factor hypothesis; Awuah, 1998). Involvement of plant parasitic nematodes in the disease is also suspected (biotic factor hypothesis; Awuah, 1998). Thus, the cause as well as factors affecting RLD are not unequivocal and need to be determined to provide the basis for its effective management.

This paper describes experiments that aid in determining the cause of the RLD of pineapple in Ghana. We also examined the effects of stresses from soil moisture deficit, weed competition, low soil fertility and intense light on RLD expression.

MATERIALS AND METHODS

Possible aetiologic role of biotic agents. The pattern of spread of the disease was initially studied by monitoring the distribution of diseased plants on pineapple fields at the Pexin and Better and Best Farms at Ashalaga and Kojo Ashong, respectively, in the Ga District of the Greater Accra Region. Roots and underground stem tissues of severely diseased plants were also examined for evidence of fungal infection and infestation by insects. Stems, roots and root zone soil samples from such plants were further analysed in the laboratory at the Kwame Nkrumah University of Science and Technology (KNUST) for fungi and nematodes.

Fungi were isolated by plating roots and stem tissues on water agar plates and incubating plates on a laboratory bench (diffuse sunlight supplemented with fluorescent lighting; 25-30°C) for 1 week. Fungal colonies growing out of plated tissues were either examined directly with a

microscope or sub-cultured on chloramphenicol (500 ppm) potato-dextrose agar (CPDA) plates and examined after 5 - 7 days. Cultures were sent to the Commonwealth Agricultural Bureau International (CABI) Bioscience in the UK for confirmation of identification.

Nematodes were extracted from roots and stem tissue by homogenising 5-g samples in 100 ml tap water in a blender at low speed for 10 seconds. The macerated tissue was extracted for 24 h using a modification of the method of Whitehead and Hemming (1965). Extracted nematodes were heat relaxed and fixed in a solution containing 10 ml 40% formalin, 1 ml glacial acetic acid and 89 ml distilled water before examination. Nematodes were extracted from soil as above using 100 ml soil samples.

In an additional experiment to determine whether or not soil biotic factors are involved in the disease, several soil samples were obtained from root zones of severely diseased plants at the Pexin Farm, bulked and thoroughly mixed together. Half of the soil sample (40 kg approx.) was oven sterilised (100 °C for 3 h on each of two occassions) and distributed into four clay pots (22 cm-rim diameter; 19 cm deep) at a rate of 10 kg pot^{-1}. Pots were planted 1 week later with non-symptomatic suckers of the susceptible pineapple cultivar, Smooth Cayenne (one sucker per pot). Suckers planted in non-sterilised natural field soil were used for comparison. Plants were grown outdoors for 5 months and observed for RLD symptom development.

Detection of the pineapple closterovirus (PCV) in pineapple leaves was done with tissue blot immunoassay (TBIA) (Hu *et al.*, 1997). The surfaces of freshly cut pineapple leaf bases were firmly pressed onto 0.45 µm nitro ME nitrocellulose membrane (supplied by Dr. John Hu of the University of Hawaii). Blotted membranes were sent to Dr. John Hu for processing. In all, 227 leaves from nine farms/ plantings at eight locations (Table 1) were blotted. Of these, 197 and 30 were from symptomatic and non-symptomatic plants, respectively.

Effect of soil moisture, soil sterilisation, soil fertility, sucker condition and light intensity. Plant house pot experiments and a field mini-plot trial were used to study the effect of the above factors on RLD severity. Suckers of Smooth Cayenne pineapple were used in these and subsequent experiments.

For the plant house pot experiment, several soil samples were obtained from root zones of severely diseased plants at the Pexin Farm. The soil samples were bulked, thoroughly mixed and half of it distributed into plastic buckets (26 cm rim diameter; 26 cm deep) at a rate of 14 kg per bucket. Each bucket was provided with three drainage holes at the base. The rest of the soil was steam sterilised and distributed into identical

TABLE 1. Detection of pineapple closterovirus (PCV) in symptomatic and non symptomatic pineapple leaves using tissue blot immunoassay[a]

Location sample	Detection of PCV in plants samples (%)	
	Samples with RLD symptoms	Samples without RLD symptoms
Atwia (Sugar Loaf)	0	-
Akraman (Queen Farms)	44.4	-
Pokuase (NARP research plot)	0	30/30
Kojo Ashong (Better & Best Farm)	67.6	-
Obom (Integral Farm)	100	-
Ashalaga (Pexin Farm)	0	-
Pokrom	58.3	-
KNUST	38.9	-
KNUST (Sugar Loaf)	83.3	-

[a]Smooth Cayenne pineapple was tested except where indicated
- = no test performed

plastic buckets. Symptomatic and non-symptomatic pineapple suckers obtained from the Pexin Farm and the Bechem area (disease - free area), respectively, were planted in potted soil (one sucker per pot) and kept in a plant house (temperature 26 - 32 °C) and with diffuse lighting (approximately 7,740 lm m^{-2}). The various treatment combinations thus studied using a completely randomised design were as follows (i) soil treatment (sterilised and unsterilised), (ii) pineapple sucker condition (non-symptomatic and symptomatic), (iii) soil fertility (high and low), and (iv) soil moisture (high and low).

A high soil moisture regime was ensured by covering the soil surface with a piece of black polyethylene sheet cover. Low moisture soils were left uncovered. Two equal split applications of 56 kg NPK (15-15-15) fertiliser applied per pot 1 and 4 months after planting ensured high soil fertility. Low fertility plants did not receive fertilisers. Each plant received 500 ml water when the uncovered soil appeared dry and crusty.

At approximately 6 months in the plant house, plants were rated for RLD severity and subsequently transferred outdoors and grown for an additional 8 months. They were assessed for the disease at 1, 4, 5, 6, 7, and 8 months during these periods. Ambient plant house and outdoor light intensities and temperatures were recorded.

Combinations of soil moisture and fertility, and competition from weeds were tested in a mini-plot experiment at the Department of Crop Science, KNUST on a field with no history of pineapple culture. Soil from the field was analysed for pH, total nitrogen, available phosphorous and exchangeable potassium at the soil science section, Department of Crop Science KNUST following standard procedure (Anon., 1979). The land was clean-weeded and sub-divided into blocks approximately 2 m apart. Four ridges each 3 m long and 1m apart, equivalent to mini-plots were constructed on each block. Symptomatic pineapple suckers were obtained from severely diseased plants at the Pexin Farm and planted, without pesticide treatment, on mini-plots. They were spaced at 40 cm.

Treatments studied were (i) high soil moisture with high soil fertility, (ii) low moisture with high fertility, (iii) low soil moisture with low soil fertility; no weed competition, and (iv) low soil moisture with low soil fertility; partial weed competition. The experiment was factorial in a randomised complete block design (RCBD) with four replications.

High soil moisture regime was ensured by completely covering the appropriate plot with high density black polyethylene sheet (held down along the sides and ends with soil). Low moisture plots were not covered. High soil fertility was achieved with NPK (15-15-15) fertiliser at a rate of 56 g per plant in two equal split applications, 3 and 6 months after planting. Low fertility plots received no fertiliser. Partial weed competition was imposed by allowing weeds to co-exist with the pineapple plants and hoeing them only when they completely outgrew the pineapple plants. Where weed competition was not desired, the plot was clean weeded throughout the trial. Plants were rated for RLD symptoms after 6 months and subsequently at monthly intervals until the 14th month.

Soil samples were obtained from each plot (four/plot) on 15 August, 1998 three days after a heavy rain. Samples from plots receiving similar treatments were bulked and composite samples analysed for pH, total nitrogen, available phosphorus and exchangeable potassium at the Soil Science Section, Department of Crop Science, KNUST as before (Anon., 1979).

Effect of light and soil fertility. This was determined in a field mini-plot trial at the Department of Crop Science, KNUST. The site was clean weeded and prepared into four blocks each measuring 8.8 m x 3.4 m (2 m between blocks). Each block was further sub-divided into four ridges (representing four mini-plots), each ridge measuring 3.4 m x 0.4 m. Symptomless Smooth Cayenne pineapple suckers were obtained from the Crops Research Institute's trial plots at Pokuase, Accra. They were planted in late November 1999 at a spacing of 0.4 m giving eight plants per plot.

The factors studied were light intensity and soil fertility at two levels each, giving a 2 x 2 factorial arrangement in a randomised complete block design. Low light intensity was imposed by shading the plants, 5 weeks after planting, with 1 m high palm frond shed (6 large fronds, each with approximately 136 leaflets) erected over the

appropriate plot. The sides of the sheds were also partially covered with palm fronds to ensure optimal shading. Palm fronds were arranged uniformly to allow approximately equal amount of light into each plot. Sheds were repaired when damaged by replacing unusable palm fronds. High light intensity plots were left unshaded. High soil fertility was established with 20 g NPK (15-15-15) fertiliser applied in two split applications to each plant (first application at 3 months and second application at 6 months). Low fertility plants received 10 g of the fertiliser in two split applications. From 24 November, 2000 (i.e.12 months after planting) and subsequently at approximately monthly intervals up to 23 March 2001, plants were scored for red leaf disease severity.

Data collection and analysis. Where the effect of light was studied, light intensity was periodically taken in the morning (0800 hr), afternoon (1400 hr) and in the evening (1700 hr) with a General Electric Triple Range light meter (model 217, GE Lighting, Nela Park, Cleveland, USA). In the mini-plot shading trial, light intensity was taken just above the plants but in the plant house pot experiment, ambient plant house and outdoor light intensities were recorded. Temperatures were similarly taken with a mercury thermometer. In all experiments, plants were assessed for RLD using a scale of 1-12 (1 = 0% disease, 2 = 0-3%, 3 = 3-6%, 4 = 6-12%, 5 = 12-25%, 6 = 25-50%, 7 = 50-75%, 8 = 75-87%, 9 = 87-94%, 10 = 94-97%, 11 = 97-100% and 12 = 100%) after Horsfall and Barratt (1945). Disease ratings were converted into percentages (Redman *et al.*, 1962) and percentages acrsine transformed to normalise the variance before analysis with MSTAT C.

RESULTS

Soil analysis showed the field to be acidic and deficient in nitrogen, available phosphorous and exchangeable potassium. In a typical RLD epidemic, diseased plants generally occurred in groups which are randomly distributed throughout the affected field. Mealybugs (*Dysmicoccus brevipes* Cockerell) and ants (*Pheidole megacephala* F. and *Crematogaster africana* Mayr) are sometimes associated with diseased plants. Roots of such plants were reduced and in severely diseased plants, several of the roots were at various stages of decay. Association of termites with the underground stem tissue increased disease severity. Internal browning of the underground stem was, however, absent except in cases of termite colonisation. *Neosartorya fischeri* (Wehmer) Mallock & Cain, a common soil fungus was frequently isolated from decaying roots. No vascular wilt fungus nor any of the well known fungal root pathogens was isolated from either the roots or the internal tissue of the underground stem.

Plant parasitic nematodes of the genera *Aphelenchus*, *Pratylenchus* and *Helicotylenchus* were isolated mainly from the roots (five out of eight samples). The nematodes occurred infrequently in the rind of the underground stem (two out of eight samples) and were completely absent from the pith. When detected in roots and the rind, their number per 5g sample was low, ranging from 1-3 (average 2) per root sample and 1-8 (average 5) in the rind of underground stem. The above nematodes also occurred in root zone soil samples.

The PCV was detected with TBIA in 127 out of 239 (53%) typically symptomatic pineapple leaves examined (Table 1). All 30 symptomless leaves examined were also positive for PCV.

Pineapple plants grown outdoors in steam sterilised soil manifested typical leaf reddening just as those grown in natural field soil after 5 months. The root systems and vigour of the two categories of plants were also similar.

Plants grown in pots in the plant house from symptomatic suckers completely recovered from RLD after 6 months regardless of soil sterilisation, fertility and moisture status. The light intensity in the plant house ranged from 2,153-48,438 lm m^{-2} and the temperature range was 24-38 °C. Plants from healthy suckers were also disease-free. When the same plants were taken outdoors and grown for an additional period of 8 months, they became variously diseased. The light intensities outdoors ranged from 4,037-100,000 lm mm^{-2}, while outdoor temperatures ranged from 24-38 °C.

Probability values from ANOVA of RLD levels as affected by soil sterilisation, sucker condition, soil fertility and soil moisture when potted plants were grown outdoors are presented in Table 2.

The effects of soil treatment (sterilisation) and sucker condition were generally not significant ($P>0.05$) but soil fertility had significant effects at most disease assessment periods.

Soil analysis showed the field to be acidic and deficient in nitrogen, available phosphorus and exchageable potassium. Significantly lower ($P<0.05$) RLD levels were recorded at all assessment periods on the high fertility than on the low fertility plots (Fig. 1). Disease levels associated with the high fertility/high moisture treatment, though lower, generally were not

TABLE 2. Probability values for the effect of soil treatment (sterilised and unsterilised), sucker conditions (symptomatic and non-symptomatic), soil fertility (high and low) and plastic mulching (with and without) on RLD severity at various periods in potted, outdoor-grown pineapple plants[a]

Source	Probability values against duration outdoors (months)					
	1	4	5	6	7	8
Soil treatment (A)	0.041		0.163		0.2241	
Sucker type (B)	0.086	0.000	0.000			0.1411
Fertilisation (C)		0.000	0.000	0.000	0.000	0.0001
Mulching (D)	0.005					
A x B		0.136	0.234		0.0001	
A x C	0.259			0.116		
A x D	0.041	0.207	0.250	0.213		0.0257
B x C		0.167	0.038	0.036	0.000	0.0001
B x D	0.086			0.216		0.0006
C x D				0.039	0.000	0.0001
A x B x C			0.311		0.035	0.1085
A x B x D			0.051			0.0115
A x C x D	0..259	0.159	0.058		0.064	0.2804
B x C x D	0.023	0.234			0.202	0.0001
A x B x C x D					0.011	

[a]Spaces indicate very high probability values; 1-8 represents duration in months

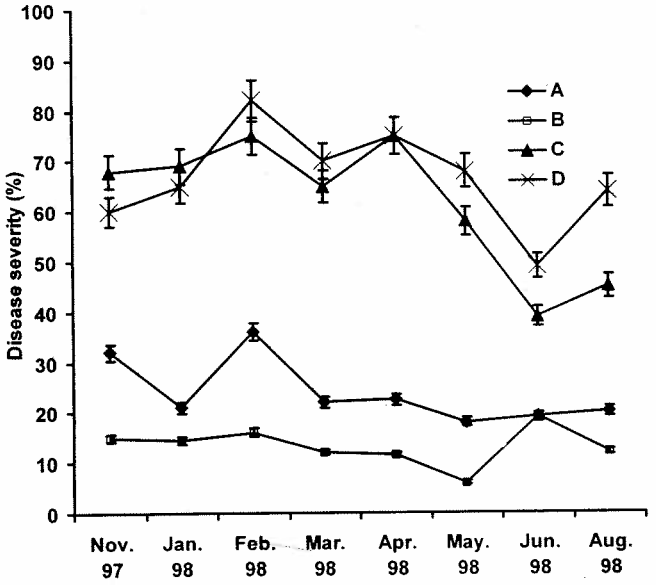

Figure 1. Effect of NPK fertilisation, plastic mulching and weed control on red leaf disease at KNUST. A = NPK without mulch; B = NPK with mulch; C = No NPK, no mulch, full weeding; D = No NPK, no mulch, partial weeding. At each period vertical bars represent standard errors of the means.

significantly (P>0.05) different from levels obtained with the high fertility/low moisture treatment (Fig. 1). Plants receiving the high fertility/high moisture treatments, however, grew more vigorously. Under the low fertility regimes, clean weeded and partially weeded plots had similar disease levels.

The moisture content associated with the high fertility/high moisture treatment three days after a heavy downpour was 10.1% (Table 3). This is contrasted with the low values of 3.4 - 4.4% associated with the low moisture treatments.

Soil fertility, light intensity and their interactions significantly (P<0.05) affected RLD severity at almost all assessment periods (Table 4). Shaded plants generally had significantly (P<0.05) lower RLD levels (ranging from 0.07 to 14.8% than unshaded plants (RLD levels ranged from 12.79 to 91.43 (Table 5). The light intensity associated with shaded plots averaged 18,848 (morning), 23,358 (afternoon) and 6,792 lm m^{-2} (evening) giving an overall average light intensity of 16,333 lm m^{-2}. The corresponding temperatures were 27.5, 34.7, and 29.9 °C (overall average = 30.7

TABLE 3. Characteristics of soils from mini-plots under four management regimes[a]

Management regime	Soil characteristics				
	Moisture (%)	Total N (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})	pH (H_2O)
High moisture, high fertility	10.1	0.22	285	185	4.1
Low moisture, high fertility	4.4	0.22	145	235	4.4
Low moisture, low fertility	3.4	0.15	26.1	145	4.2
Low moisture,low fertility, weed competition	3.7	0.15	24	165	4.4

[a] Soil samples were taken 3 days after a heavy rain. pH was determined for a 1:1 (wt:vol) soil:distilled water suspension

TABLE 4. Probability values for the effects of fertiliser (A) light (B) and their interaction on RLD levels at four disease assessment periods

Source	24/11/00	22/12/00	23/1/01	22/3/01
Block				
A	0.007	0.004	0.000	0.000
B	0.000	0.000	0.000	0.236
AB	0.041	0.043	0.150	0.312

TABLE 5. Effect of combination of light and soil fertilisation on RLD levels at four disease assessment periods

Treatment	RLD severity levels (%)[a]			
	24/11/00	22/12/00	23/1/01	22/3/01
No Fert, with reduced light	0.07	0.29	0.07	7.5
No Fert, with full light	12.79	23.02	31.81	52.2
Fert, with reduced light	1.46	4.20	5.15	14.8
Fert, with full light	54.54	68.55	91.43	74.8
LSD (0.05)	13.77	13.54	14.84	19.79
CV (%)	53.0	41	35.0	39.4

[a]Data was arcsine transformed before analysis and back transformed as reported here

°C). In unshaded plots, the light intensities were 41,398 (morning), 69,966 (afternoon) and 11,173 lm m^{-2} (evening) giving an overall average of 40,849 lm m^{-2}. The corresponding temperatures were 28.7, 36.0 and 31.5 °C (overall average = 32°C).

DISCUSSION

The cause of RLD of pineapple in Ghana has not been fully established. Because symptoms of RLD resemble those of the MBW reported elsewhere (Ullman *et al.*, 1989; German *et al.*, 1992; Hu *et al.*, 1996), it has been reasoned that the two diseases are the same with a common viral etiology. Awuah (1998), however, ruled out involvement of a virus and mealybugs in the disease because of two main reasons. The first reason is that field development of RLD is spotty instead of diseased plants originating from the edges of fields along the paths of the migrating mealybugs as reported for the virus-mediated MBW (Illingworth, 1931; Beardsley *et al.*, 1982; German *et al.*, 1992). The second reason is that only few ants and mealybugs, if any at all, were associated with diseased plants in contrast with the MBW where mealybug and ant numbers are strongly correlated with wilt development (Rohrbach *et al.*, 1988). Petty (1994) also noted that ants and mealybugs are generally not found on pineapple plants with symptoms similar to those of the RLD.

Based on preliminary evidence, RLD was thought to have a complex etiology with nematodes constituting one major group of primary agents and stress factors, mainly moisture stress and low soil fertility, either being primary agents or just pre-disposition factors (Awuah, 1998). In the present study, pineapple plants grown outdoors in pots using sterilised and unsterilised soils from RLD endemic fields manifested similar levels of leaf reddening despite absence of plant parasitic nematodes, wilt/root rot fungi and other soil biotic agents in the sterilised soil. When the internal tissues of the stems of diseased plants from the field were examined, they were generally free from decay, indicating absence of vascular wilt fungi and further suggesting non-involvement of wilt fungi in RLD development. The most frequently isolated fungus from the roots was *N. fischeri*, a common soil fungus which is generally non- pathogenic to plants. These findings make a revision to Awuah's (1998) hypothesis necessary to exclude the involvement of soil-borne biotic factors as primary agents of red leaf disease.

The cause of the MBW which occurs in the United States and assumed to be the same as the RLD in Ghana is thought to be a PCV (Rohrbach *et al.*, 1988). In the present study, the PCV was detected in several diseased and symptomless, apparently healthy pineapple leaves. The assay, however, failed to detect the PCV in up to 48% of typically symptomatic leaves examined. Presence of the PCV in some of the Ghanaian pineapple leaf samples does not, however, indicate a causative association. For example, Sether *et al.* (1998) successfully transmitted the PCV with mealybugs to healthy pineapple plants in glass house pot studies. The authors, however, did not indicate whether virus-positive plants also became diseased and developed typical MBW symptoms but their conclusion that the study was useful in assessing the role of the PCV in MBW (which has symptoms similar to those of RLD in Ghana) suggests that the viral nature of and the involvement of mealybugs in even MBW is equivocal.

Adjei-Boateng (1998) reported that both neem extract and dimethoate applied monthly to pineapple plants in the field in Ghana suppressed mealybugs on pineapple 5–8 months after planting. However, leaf wilting due to mealybug infestation, which should have decreased following successful control of the mealybugs rather increased during the period so that at 7 and 8 months, wilt levels associated with the neem extract and dimethoate treatments were not significantly different from those associated with the water control treatment which failed to control the mealybugs. This contradicts the assumption that mealybugs transmit the PCV believed to cause RLD in Ghana. Thus, the role of the mealybugs in the development of RLD in Ghana is, perhaps, unduly emphasised.

When suckers from severely diseased pineapple plants were grown in a plant house with diffuse sunlight for six months, none of the resulting plants developed RLD. When these healthy plants were transferred outdoors and grown in intense sunlight/heat, all of them manifested various levels of leaf reddening. Significantly less RLD levels

were also associated with plants grown outdoors on shaded mini-plots than on unshaded plots and several plants grown on shaded plots were free from RLD. Thus, leaf reddening (the diagnostic symptom of RLD), could partly be a physiological response of the Smooth Cayenne pineapple cultivar to bright sunlight with its accompanying heat. Petty (1994) made a similar observation on a wilt disease of pineapple in South Africa (with symptoms similar to those of the RLD in Ghana) and reported that pineapple plants growing in a glasshouse where diffuse sunlight conditions prevail are generally free of the disease. Reddening in plants results from synthesis of pigments mainly anthocyanin which, according to Fitter and Hay (1987), is a common response to stresses such as drought and high temperatures.

It is evident from the present study that other environmental factors apart from light, are important to RLD expression. Low soil fertility and to some extent, low soil moisture levels appear to be important. Red leaf disease was significantly reduced in the field with a combination of high soil fertility and plastic mulching to conserve soil moisture. Plastic mulch also prevents leaching of nutrient (Quin, 1973) which benefits pineapple plant growth by improving vigour and enhancing leaf greening. Pineapple plants grown under high fertility regime with plastic mulch in the present study were vigorous and green in colour. Norman (1986) made a similar observation. Singh and Sastry (1975) also reported that fertilisation, especially with nitrogen, reduced wilt incidence in pineapple. The symptoms of 'wilt' reported by Singh and Sastry (1975) are similar to those of the RLD reported in the current study. On farms in Ghana where plastic mulch is used, RLD is either absent or reduced. The positive effect of proper soil fertility on RLD levels observed in the present study accords with observations by Petty (1994) that on soils well fertilised with nitrogen fertilisers and on virgin lands, pineapple plants become resistant to wilt. Corbett and Pagden (1941) also noted in Malaysia the involvement of soil nutrients in pineapple wilt incidence/severity and recommended that new pineapple plantings be established in peat soil instead of in lateritic soil. They found that the disease was absent in virgin peat soil which prompted their conclusion that soil/good agronomic practices and pineapple cultivar are factors affecting disease severity. To conclusively prove the stress factor hypothesis, virus-negative and positive planting materials should be grown under various light and soil fertility regimes and the growth habits of plants compared. Sether *et al.* (2001) have described a method for producing virus-free planting materials which should be useful in such a study.

In conclusion, no evidence has been obtained in the present study indicating that RLD of pineapple in Ghana is caused by either soil biotic factors (nematodes and fungi) or by the PCV. Results of plant house and field experiments when examined together, rather suggest that intense sunlight, with its accompanying heat, is the most important factor affecting the incidence and severity of RLD of pineapple in Ghana. The results further show that when stresses resulting mainly from reduced soil fertility and to some extent low soil moisture are minimised, RLD levels are also reduced. A combination of black plastic mulch and optimal soil fertilisation, therefore, appears to be effective in reducing RLD levels and should be used to manage the disease.

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