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CONTENTS

INFLUENCE OF ENVIRONMENTAL FACTOR (RAINFALL) ON COCOA BEAN QUALITY PARAMETERS.

Noel Y. Kuman

ABSTRACT

Cocoa bean quality attributes are influenced by environmental factors. Therefore, in this study the influence of one of the environmental factors (rainfall) on the bean quality attributes was evaluated. The result of this study indicated a weak positive and negative correlation between rainfall and each of the bean quality parameters. The average monthly rainfall does not have a direct and significant effect on shell content, bean size, fat content and recovery rate as indicated by the weak correlation coefficient (r). Simple Pearson correlation coefficient was used to determine association between each of the beans quality attributes. There was no significant relationship between each of the bean quality attributes except between recovery rate and fat content.

Key words: *Cocoa Bean, quality attributes, environmental factors.*

INTRODUCTION

The physical quality characteristics of cocoa are bean size, fat content, shell content and recovery rate. Bean size is easily measured and it is negatively correlated with shell content. Bean size is influenced by genotype (Kuman 2005). Beans developed during the main season, normally after rainy season tended to be larger in size weighing in excess of 1g (Wood and Lass 1985). Bean size can be influenced by mineral and water availability, which is more likely to be limiting in high density planting (Lachenaud 1995). Average bean weight is determined by environment, including within and between season variations (Toxopeus and Wessel 1970), but there is no evidence of interaction between season and genotype (Lockwood and Edward 1980). Bean size is not correlated with yield (Tan 1990; Lockwood and Pang 1995).

Shells (residues of mucilage and testa) of the beans are waste. Therefore, it is preferable that shell weight be as low as possible, but of adequate thickness to protect the beans from mould and insect invasion (Wood 1975). The difference in shell can be influenced by the genotype and methods of fermentation. Fermentation method and season appears to affect shell content (Wood and Lass 1985). The shell content of the beans varies depending on genotype and is negatively correlated with bean size (Vello *et al.* 1972). Within the clones, there is no relationship between per cent testa and bean size (Eskes *et al.* 1977).

The fat content varies between genotype and is probably influenced by environmental factors. It appears that there is no genetic correlation between fat content and yield or bean size (Lockwood and Pang 1995), but there is correlation between bean size and cocoa butter within the clones (Beek *et al.* 1977). Seasonal variation is shown to affect fat content. Fat content was suggested to be influenced by bean size (Egbe and Owolabi 1972).

Recovery rate is the ratio of dry fermented beans to wet unfermented beans expressed as a percentage. Recovery rate can be influenced by season, genotype or method of processing. Pod storage prior to fermentation increased the recovery rate (Kuman 1998). Season is another major factor affecting the recovery rate (Are and Atanda 1972). The recovery rate of the genotype was significantly influenced by environmental variation (Kuman 2005).

In previous studies, suggestions were made that season, in particular rainfall, may have influenced the development of some of the important cocoa quality parameters. There were also suggestions of correlation existing between different quality parameters.

Objective

The objective of this study is to statistically verify the influence of rainfall on the bean quality parameters and the association between each of the quality parameter.

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MATERIAL AND METHODS

Location

The quality data of 31 different genotypes were collected from a breeding trial conducted at the Papua New Guinea Cocoa and Coconut Research Institute. Urguhart *et al.* (1951) provided a comprehensive description of the environmental conditions of most of the cocoa growing areas including the site for this trial. The average rainfall of 134.9 mm with a maximum of (343.9 mm) and minimum (12.5 mm) was recorded during the 24 months (from 1999-2001) of the trial.

Experimental Design

The breeding trial was divided into three sub trials based on their size. The trees of each class were of the same age uniform in each replicate and were planted in split-plot design. Four replicates were used with 12 trees in a (3 m x 3 m) plot.

Data collection

The bean content from the 31 genotypes were harvested twice every month and processed before their shell content, bean count, fat content and recovery rate were assessed. Ripe pods of approximately 1 kg (equivalent to 6 pods) were harvested twice every month from each of the 31 tested genotypes, labeled, wrapped inside the shade cloth bags and fermented using a procedure described by Clapperton *et al.* 1994. At the end of 6 days of fermentation, the test samples were dried before their shell content; bean size and fat content were assessed.

Sample collection & preparation

Approximately 600g of dried beans from each genotype was dried inside the oven (Contherm, New Zealand) at 115 ^{0}C for 15 min to standardize moisture content to less than 7p% before being emptied into a mixing container and thoroughly mixed. Any foreign material and debris was removed before passing the samples into a funnel of a quartering device, which randomly divided the samples into four quarters. Approximately 150g of beans received from each quartering devise was used to analyse the bean size (bean count), fat content and shell content (shell coat). Recovery rate is determined by weighing beans before fermentation and after drying. Weight difference is expressed as percentage.

Sample Analysis

Fat content of the beans was extracted using standard fat extraction method (AOAC, 1970). Fat

Table 1. **Correlation between bean count, shell content, fat content and recovery**

Parameter	Bean count	Shell content	Fat content
Shell count	0.029		
	0.868		
	31	31	
Fat content	0.024	0.021	
	0.893	0.906	
	31	31	31
Recovery rate	-0.165	-0.124	0.484
	0.344	0.478	0.0043
	31	31	31

The analysis of variance to determine the influence of rainfall on bean quality parameters (Table 2-5) indicated a weak positive and negative correlations.

analysis for the hybrid clones was conducted by Cadbury Schweppes, Australia. The other quality attributes (bean size and shell content) were determined using the standard cocoa quality analysis procedure.

Data Analysis

Quality data were subjected to analysis of variance to determine the influence on bean quality by time of the year (monthly rainfall). The simple F-tests were not often possible for this analysis because of the complexity of the experimental design and the degree of imbalance due to missing of (SAS version 8, SAS Institute 1990 1996) values. F-tests with appropriate error terms were constructed by the general linear model (GLM) procedure . Simple Pearson correlation coefficient was used to determine association between each quality attributes.

RESULTS

Pearson correlation coefficient (Table 1) indicated there is no association between any of the quality parameters except between the recovery rate and the fat content.

DISCUSSION & CONCLUSION

The simple Pearson correlation coefficient (Table 1) indicated that each of the bean quality parameter is independent of each other except for a significant ($p< 0.004$) relationship between fat content and recovery rate. The recovery rate is the amount of dry yield obtained from wet beans after fermentation and drying. Fat makes up slightly more than 50% of the weight of unfermented dry beans (Lehrian *et al.* 1983), and it does not change very much during fermentation. Beans that are produced during wet season are normally large in size and have high fat content and would be expected to produce high recovery rate. However that has not always been the case as indi-

Table 2. **Rainfall effects on shell content of the bean.**

Dependent variable: Shell content		**Regression coefficient**	Std error	t-statistic	Prob (HO):coef = 0, 2 tailed)
Monthly rainfall (mls) (Year 1999)					
January	79	**0.0001**	0.0003	0.37	0.7078
February	60	**0.0002**	0.0003	0.80	0.4254
March	344	**0.0002**	0.0003	0.59	0.5571
April	179	**-0.0006**	0.0003	-2.58	0.0100
May	108	**-0.0009**	0.0002	-3.99-	<0.001
June	113	**-0.0002**	0.0002	-7.15	<0.0001
July	76	**-0.0018**	0.0002	-7.64	<0.0001
August	12	**-0.0015**	0.0002	-6.48	<0.001
September	117	**-0.0001**	0.0002	-0.55	0.5845
October	145	**0.0002**	0.0002	0.92	0.3572
November	179	**0.0007**	0.0002	3.42	0.0006
December	188	**0.0010**	0.0002	4.78	<0.0001
January Year (2000)	79	**0.0011**	0.0002	6.61	<0.0001
February	197	**0.0013**	0.0002	6.06	<0.0001
March	201	**0.0012**	0.0002	5.58	<0.0001
April	150	**0.0011**	0.0002	4.56	<0.0001
May	103	**-0.0003**	0.0003	-1.28	0.19999
June	96	**-0.0016**	0.0003	-6.04	<0.0001
July	91	**-0.0017**	0.0003	5.58	<0.0001
August	96	**-0.0019**	0.0003	4.56	<0.0001
September	130	**-0.0006**	0.0003	-1.28	0.0281
October	152	**0.0003**	0.0002	-6.04	0.2214
November	139	**0.0040**	0.0015	-0.28	0.783
December	205	**0.0016**	0.0002	-1.05	0.293

**All rainfall figures are rounded off to the nearest whole number with an average rainfall of 134 mls.*

Table 3. **Rainfall effects on fat content of the bean.**

Dependent variable: Fat content		Regression coefficient	Std error	t-statistic	Prob (HO):coef = 0, 2 tailed)
Monthly rainfall (mls) (Year 1999)					
January	79	**-0.0001**	0.0001	-0.25	0.8008
February	60	**0.00055**	0.0004	1.20	0.2298
March	344	**0.00056**	0.0004	1.30	0.1929
April	179	**0.00131**	0.0005	2.45	0.0144
May	108	**0.00187**	0.0006	3.27	0.0011
June	113	**0.00381**	0.0010	3.62	0.0003
July	76	**0.00097**	0.0011	0.85	0.3955
August	12	**0.0005**	0.0007	0.67	0.5057
September	117	**0.0006**	0.0004	-1.53	0.1269
October	145	**-0.0006**	0.0004	-1.0	0.1329
November	179	**-0.0009**	0.0004	-2.09	0.0372
December	188	**-0.0011**	0.0005	-2.22	0.0265
JanuaryYear (2000)	79	**-0.0017**	0.0006	-3.11	0.0019
February	197	**0.0001**	0.0005	0.29	0.7740
March	201	**0.0057**	0.0004	1.28	0.2002
April	150	**0.0004**	0.0005	0.67	0.5055
May	103	**0.0010**	0.0007	1.46	0.1442
June	96	**0.0024**	0.0007	3.29	0.0011
July	91	**0.0025**	0.0008	3.06	0.0023
August	96	**0.0001**	0.0014	0.11	0.9128
September	130	**-0.0019**	0.0006	-1.47	0.1429
October	152	**-0.0003**	0.0004	-0.67	0.4928
November	139	**-0.0005**	0.0004	-1.12	0.2620
December	205	**-0.0072**	0.0004	-1.60	0.1104

** All rainfall figures are rounded off to the nearest whole number.*

Table 4. **Rainfall effects on recovery rate of the bean.**

Dependent variable: Recovery rate		**Regression coefficient**	Std error	t-statistic	Prob (HO):coef = 0, 2 tailed)
Monthly rainfall (mls) (Year 1999)					
January	79	**0.0001**	0.0008	0.12	0.9070
February	60	**-0.0017**	0.0007	-2.43	0.0153
March	344	**0.0022**	0.0006	3.30	0.0010
April	179	**0.0025**	0.0006	3.94	<0.0001
May	108	**0.0035**	0.0006	5.79	<0.0001
June	113	**0.0036**	0.0006	5.84	<0.0001
July	76	**0.0029**	0.0006	4.63	0.0543
August	12	**0.0012**	00006	1.93	0.6024
September	117	**-0.0002**	0.000	-0.53	0.0254
October	145	**-0.0013**	0.0001	-2.24	<0.0001
November	179	**-0.0022**	0.0006	-3.83	<0.0001
December	188	**-0.0035**	0.0005	-6.29	<0.0001
January Year (2000)	79	**-0.0030**	0.0005	-5.60	<0.0001
February	197	**-0.0024**	0.0005	-4.23	<0.0001
March	201	**0.0001**	0.0006	0.25	0.8044
April	150	**-0.0004**	0.0006	-0.76	0.4471
May	103	**0.0028**	0.0006	4.59	<0.0001
June	96	**0.0023**	0.0007	3.23	0.0013
July	91	**0.0032**	0.0007	4.81	<0.0001
August	96	**0.0032**	0.0007	4.78	<0.0001
September	130	**-0.0054**	0.0007	-0.70	0.4848
October	152	**-0.0008**	0.0005	-1.68	0.0931
November	139	**-0.0002**	0.0007	-0.34	0.7346
December	205	**-0.0021**	0.0006	-3.69	0.0002

** All rainfall figures are rounded off to the nearest whole number.*

Table 5. **Rainfall effects on bean size of the bean.**

Dependent variable: Bean count		**Regression coefficient**	Std error	t-statistic	Prob (HO):coef = 0, 2 tailed)
Monthly rainfall (mls) (Year 1999)					
January	79	**-0.0028**	0.0018	-1.59	0.1128
February	60	**-0.0009**	0.0016	-0.58	0.5654
March	344	**-0.0004**	0.0015	-0.28	0.7812
April	179	**0.0005**	0.0014	0.35	0.7284
May	108	**-0.0018**	0.0013	-0.58	0.5571
June	113	**-0.0013**	0.0014	-0.95	0.3417
July	76	**-0.0026**	0.0014	-1.77	0.0761
August	12	**0.0002**	0.0017	0.14	0.8888
September	117	**0.0011**	0.0011	0.85	0.3934
October	145	**0.0017**	0.0012	1.37	0.1698
November	179	**0.0006**	0.0012	0.48	0.6323
December	188	**-0.0008**	0.0012	-0.66	0.5092
January Year (2000)	79	**-0.0001**	0.0012	-0.01	0.9954
February	197	**0.0015**	0.0012	1.18	0.2379
March	201	**0.0020**	0.0013	1.48	0.1401
April	150	**0.0006**	0.0015	0.42	0.6770
May	103	**-0.0015**	0.0014	-1.05	0.2934
June	96	**-0.0011**	0.0014	-0.68	0.4969
July	91	**-0.0018**	0.0015	-1.20	0.2294
August	96	**-0.0021**	0.0022	-0.92	0.3589
September	130	**-0.0009**	0.0018	-0.54	0.5904
October	152	**0.0023**	0.0012	1.87	0.0619
November	139	**0.0017**	0.0013	1.27	0.2058
December	205	**0.0002**	0.0013	0.17	0.8665

* *All rainfall figures are rounded off to the nearest whole number.*

cated by the results of this study. The recovery rate can be influenced by the amount and structure of bean endocarp especially the pulp, which varies between different genotypes. Beans with large pulp volume to bean ratio produced during wet season would normally have a lower recovery rates than beans produced in dry seasons (Wood and Lass 1985). Recovery rate can also be influenced by genotype, pod ripeness and season. Kuman (1998) reported a variation in recovery rates between 30% to 40% for different genotypes and an increase in recovery rate for cocoa pods that are stored prior to fermentation.

There is a weak positive and negative correlation between rainfall and each of the quality parameters (Tables 2-5). The result indicated that the average monthly rainfall alone does not have a significant influence on each of the bean quality parameters; however the rainfall in combination with other environmental factors can affect the bean quality parameters. Genotype by month interactions influenced bean quality of the genotypes and this interaction contributed 12% of the total sum of squares (SS). In the analysis to study the main effects separately, monthly environmental factors contributed 3% to the total SS (Kuman 2005).

Studies of Toxopeus and Wessel (1970) indicated that there was a positive correlation between bean size and fat content and a negative correlation between bean size and shell content. The correlation between bean size and fat content is often positive and significant but there is no genetic correlation (Ekes and Lanaud 2000). In this study, no such correlation was shown to exist between any of the quality parameters except between fat content and recovery rate. The differences between the result of this study and that of Toxopeus and Wessel and Ekes and Lanaud could be explained in terms of genotypic response to genotype x environment (GXE) interactions. Different cultivars response differently to environmental variation at different stages of their growth and development and these interactions could affect the final bean quality, hence influencing the relationship between each of the quality parameters. The shell content, bean count, recovery rate and fat content contributed 8%, 12%, 12% and 28% respectively to the total sum of square (SS). The SS indicated the different responses of genotypes to environmental factors (Kuman 2005).

CONCLUSION

Rainfall alone does not have a significant influence on the development of each of the bean quality parameters, but can in combination with other environmental factors. Beans produce during wet seasons would be expected to yield high recovery rate since they are large size and contain high fat content. However this has not always been the case as the recovery rate can be influenced by genotype, season and pod storage. Recovery can be improved by pod storage prior to fermentation or through selective breeding of genotypes that only produce high recovery rate. The bean quality parameters are influenced by combinations of environmental factors including any relationship between each of the quality parameters.

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TESTING THE EFFICIENCY OF MINI-FERMENTATION BOX TO FERMENT SMALL QUANTITIES OF COCOA BEANS.

Noel Y. Kuman and Neil W. Hollywood

ABSTRACT

A mini- fermentation box was developed that successfully fermented small quantities of wet beans as low as 200 kg. The minibox fermented beans produce bean quality and flavor characteristics similar to beans fermented in conventional fermentation box. The minibox was released by the Cocoa Board of Papua New Guinea and now enables majority of the smallholder farmers to ferment and sell dried beans, and receive maximum benefits, unlike selling wet beans in the past for a lower price.

***Keywords**: Minibox fermentation, bean quality attributes.*

INTRODUCTION

Cocoa scientifically known as *Theobroma Cocoa* L is a major cash crop for the majority of the population living in the lowlands of Papua New Guinea (PNG). Cocoa is grown in 14 provinces of PNG. Out of these provinces, East New Britain (ENB) and Bouganville are the major producers. A survey on wet bean marketing in PNG revealed that 92 % of the farmers relied on cocoa as their main source of income (Gimbol 1989). Another similar study by Yabro and Nobel (1989) indicated that between 80 -100 percent of the smallholders in ENB, East Sepik and Oro province depended on cocoa as their main crop for cash income. Cocoa production trend show that smallholders are the major producer, unlike in the early 1970's when cocoa production was dominated by the plantation sector. From the mid 1980s to the current period, smallholders' production has increased to well over 20,000 tonnes of cocoa, which represents two thirds of the total PNG's cocoa production.

The principal aim of farmers is to maximize net returns for their crop. However, under the current arrangement, most of the farmers are selling wet beans because PNG Cocoa Board regulation restricts the smallholder farmers from fermenting their wet beans to sell them as dry beans. The board issues fermentry licenses only to farmers who can produce in excess of 2 tonnes of cocoa per annum. This restriction is imposed to ensure that beans are properly fermented to maintain consistent in quality especially flavour. Under fermented or unfermented beans do not produce flavor precursor required for flavour development. Similarly, over fermented bean resulting mainly from fermenting small quantities of beans, produce purifying odour that leads to producing off flavours detrimental to bean quality. Unfortunately, most of the smallholder farmers can not produce the required quantities of cocoa to meet the cocoa board requirements and therefore are not eligible for the fermentry licenses.

Analysis of economic factors reveal that farmers are getting half the price when selling wet beans as compared to fermented dried beans. A post devaluation analysis using 1996 average price, shows that a smallholder farmer selling wet bean would have generated K1,011 per tonne dry bean equivalent, while the average price of a tonne dry bean was K1,441.00. A farmer selling dried beans would have earned K430.00 (42.5 %) more than wet beans (Omuru 1997). This implies that although the smallholders are major producers, most of them have not actually received the maximum benefit. This is one of the major problems encountered by the cocoa growers in Papua New Guinea that has not been addressed. With the introduction of the mini-fermentation box, farmers are now able to ferment and sell their beans as dried beans thus receiving the maximum benefits.

The problem was addressed through the Australian International Development Assistance Bureau (AIDAB) funded Cocoa Quality Improvement Project (CQIP) undertaken by the Papua New Guinea Cocoa and Coconut Research Institute (PNGCCRI) from 1990-1996. In this project, one of the studies conducted was designing of a mini fermentation box that can ferment small quantities of beans. Different size mini-fermentation boxes were designed and tested to select a model that could successfully ferment small quantities of beans equivalent to what most of the

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small holder farmers can produce. The smallholder farmers can use the mini fermentation box to ferment their wet beans and sell them as dry beans for a better price subject to the Cocoa Boards approval and granting of licenses.

Post harvest process

In a conventional fermentation box (120 x 90 x 90 cm), the temperature range attained is between 24-30 ^{0}C at the start of the fermentation and this rises to 50-51 °C by the third and forth day, and remains around this temperature until the end of the fermentation. The increase in temperature is the result of microbial activities and can be affected by the batch size of the fermentation. Lower maximum temperatures between 40-41 °C can be attained in small scale fermentation. Maximum temperature of 48-50°C is required to cause the death of the beans resulting in the disintegration of the beans internal structure allowing endogenous enzymes to come into contact with their substrates. This causes the loss of cell membrane and integrity of cellular compartments. The loss of hydrophilic compartment results in the intra and intercellular mixing of water-soluble components (Lehrian and Patterson 1983), which allows series of biochemical reactions to take place to produce the chocolate flavour precursor, which later develops into chocolate and other ancillary flavours during roasting. A minibox fermentation would require a temperature regime similar to commercial fermentation to successfully ferment the beans. Incorrect processing conditions would lead to development of off-flavours. Quantities of bean less than 200 kg when fermented become over fermented, producing purifying odours. Chemical testing of these beans produce high pH values and strong off flavor indicated by low titratable acidity (TA) values (Kuman 1977).

The degree of fermentation is assessed by the cut test (Wood and Lass 1975). The higher percentage of brown bean indicated that the beans are properly fermented. Flavour test can also be used to assess the difference in flavour produced by different processing methods. Similar flavours can be produced when beans are fermented under similar processing conditions.

OBJECTIVE

To test whether the mini fermentation box can successfully ferment small quantities of wet cocoa beans

MATERIAL & METHOD

Experimental Design

Different sizes of mini fermentation box of different dimensions were constructed and tested to ferment small quantities of cocoa beans. The efficiency of each of the mini fermentation boxes was judged by measuring the degree of fermented beans. Thirty five replicates of fermentation were conducted testing the efficiency of 8 different box sizes before an ideal mini fermentation box (60 x 60 x 60 cm) length, width, and height was selected that can successfully ferment small quantities of cocoa beans between 200-250 kg . Same treatments were applied to all fermentations conducted except the quantities of bean used in each of the fermentation boxes (Kuman 1997).

Fermentation process

Cocoa pods of mixed cultivar were harvested from field and transported to the fermentry before the pods were removed. The wet beans collected were thoroughly mixed before they were weighed to collect 200 kg of wet beans and fermented in the mini fermentation box. The remaining batch (5-600 kg) was fermented in the large conventional box. The fermenting beans were covered with hessian bags or alternatively banana leaves to control and maintain the temperature and moisture. The fermenting beans were turned daily for 5 days before the cured beans were dried in a solar dryer. For comparison purpose, dried beans processed by conventional methods were also sampled from the commercial fermentry together with minibox fermented bean for quality assessment. Twenty fermentation replicates were carried out to generate samples for quality assessment.

Flavours assessment

Organoleptic evaluation was conducted by trained taste panel following the procedure described by (BCCA 1996; Sukha 2001). The flavour intensity was estimated using 0-10 scale, with 0 being weak and 10 being strong. Individual flavor attribute scores from the flavor profiling were used to determine mean. Variance components were investigated using analysis of variance (ANOVA) using Minitab Release 14 (Minitab Inc.) to determine the significance of treatments effect and interactions.

Cut test

The cut test procedure, significance of cut test and its use as a tool to measure the fermentation index is discussed by Shamsuddina and Dimick

(1986) and Dand (1993).

RESULTS AND DISCUSSION

The results shown in Tables 1 & 2 are average results of 120 batches of samples collected from each treatment and analysed. As for the flavour result, it represents the average result of 60 samples assessed (Table 3).

and partly brown beans with 20 percent of purple beans. Purple colour indicates unfermented beans, which when present in lower number (percent) is acceptable (Dand 1993). Chemicals assay of the samples (Table 2) indicated a similar range of pH and TA for beans fermented from the two fermentation boxes. This may indicate a similar conditions in both fermentation processes (minibox & conventional) with a succession of microbial activities (Ostovar and Kenney 1973, Carr *et al.* 1979), which are prerequisite for successful fermentation, though the fermentation process can be influence by heterogeneities in aeration, temperature and other fermentation conditions.

Table 1. **Average cut test results for minibox and conventional fermentation box.**

Fermentation Method	Brown	Partly brown	Purple	Insect	Germination	Slaty	Criollo	Mouldy
Minibox	44 ± 21	32 ± 22	20 ± 32	-	-	-	3	1
Conventional	39 ± 25	30 ± 20	27 ± 26	-	-	-	3	1

Commercial fermentation box (range of brown bean = brown + partly brown): 61-77 %
Minibox (range of brown beans): 54-71 %. (NS) P>0.05

No significant variation in the degree of fermentation between the samples generated from the two fermentation boxes

Table 2. **Average chemical assay results of beans generated by minibox and conventional fermentation box.**

Method of fermentation	PH	Titratable Acidity (TA)
Minibox	4.9	0.21
Range	4.4 - 5.3	0.06 - 0.29
Conventional fermentation box	4.6	0.26
Range	4.4 - 4.9	0.21- 0.31

Table 3. **Average flavor assessment results of beans produce from minibox and conventional fermentation box.**

Sample	Choco-late	Acidity	Bitterness	Astringency
Minibox	7.5	5.8	1.6	1.3
Conventional fermentation box	7.5	5.3	1.6	1.4

(NS) P>0.05
No significant variation was shown for samples generated from two fermentation boxes

DISCUSSION

The cut test results (Table 1) indicated that beans produce by the two processes were properly fermented, as indicated by the percentage of brown and partly brown beans. Both fermentation processes produced on average 70 percent of brown

The success of a fermentation process is also measured by the temperature range of the fermentation cycle. The maximum temperature

range for a successful fermentation is between 47-51 °C. The maximum temperature range achieved in minibox fermentation was between 47-51 °C, similar to temperature range attained in conventional fermentation. Other minibox designs failed to successfully ferment small quantities of beans because they can not maintained the fermentation temperature; as a result fermented beans got dried and turned black producing putrefying odour (Kuman 1997).

The organoleptic assessment result (Table 3) indicated that minibox fermented beans produce similar flavour profile as compared to beans produced by conventional fermentation method. Individual flavor attribute scores of the two fermentation process were compared to determine the significance of treatment effects and interactions. The flavor results indicated that there was no significant ($p<0.05$) difference between flavour profiles of beans produced by the two fermentation processes (Kuman 1997).

CONCLUSION

An mini-fermentation box (60x 60 x 60 cm) can successfully ferment small quantities of wet beans between 200-250 kg, which is equivalent to the average quantities of beans produce by most of the smallholder famers who own between 0.5 to 2 hectares of cocoa. Minibox fermented beans produced quality and flavour characteristic similar to those beans produced by the conventional fermentation process. The minibox was approved for use by the PNG Cocoa Board and is now widely used by smallholder cocoa farmers. The farmers are benefiting from this technology by fermenting their beans and selling them as dried beans for a better price.

However, one of the major problems observed in the acaptive trial were farmers producing over fermented and putrefied beans when using minibox. This problem was widespread during the off flush period when farmers were not able to collect sufficient beans to fill up the minibox to the required depth of 60 cm, equivalent to 200 kg of wet beans. To address this problem, new trials were initiated to ferment small quantities of beans between 10 to 100 kg using eskies.

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GROWTH PERFORMANCE OF BROILER CHICKENS ON DIETS FORMULATED FROM LOCAL INGREDIENTS.

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ABSTRACT

An experiment was conducted to study growth performance of broiler chickens fed five treatment diets formulated using sorghum, copra meal (CM), palm kernel meal (PKM), sweet potato wagi besta (SPWB) variety and sweet potato SI.172 (SPIL) variety as energy sources and to correlate this performance with estimates of metabolisable energy content of the diets. There were five chickens per replicate and four replicates per treatment and feeding period was five weeks after three weeks of brooding. Replicates were randomly assigned to 20 rooms located in an open shed at the National Agricultural Research Institute, Lae, Papua New Guinea. A rapid bioassay method was used to estimate metabolisable energy content (in mJ/kg) of the diets to be 13.64, 13.53, 13.44, 13.55 and 13.54 for Sorghum, PKM, CM, SPWB and SPIL diets respectively. Body weight gains of chickens on the five diets were not significantly different indicating that the local ingredients could be used to feed chickens. However feed intake and feed conversion ratios varied between diets. Feed intake was found to be inversely related to energy content of the diets generally.

Key words: *Papua New Guinea, chicken, feed, local ingredient, sweet potato, copra meal, palm kernel meal, metabolisable energy.*

INTRODUCTION

Village chicken production for meat, eggs and feathers is a widespread agricultural activity throughout Papua New Guinea (PNG) and the South Pacific Island countries (SPICs). According to Bangunan *et al.* (1996) there were between 550,000-570,000 rural households in PNG in 1996 out of which 155,000 raise poultry. Until the mid 1970's indigenous breeds of chickens formed the backbone of chicken production and currently they far outnumber the genetically improved genotypes used for commercial chicken production in PNG. Recently however, the trend in some rural and peri-urban areas of PNG has been towards small scale, intensive production of chickens for commercial purposes (Low and Low 2000). The economic value of this activity has been estimated at about 67 million Australian dollars annually (ACIAR 2008).

These small scale commercial broiler producers use genetically improved strains and commercial feeds. Generally feed costs account for over 70% of running costs in chicken production and this is probably the most important constraint to production of chickens in PNG and the SPICs (Aregheore 2001). Cost of commercial feeds in PNG are high because some of the ingredients used in their preparation are imported. According to Kumar (1996), some 50,000 tonnes of feed grains costing over eight million kina are imported each year for commercial livestock production in PNG. Other important feedstuffs imported into PNG include soybean meal, fish meal and blood meal. It is therefore thought that the use of locally available and novel feed ingredients to formulate and manufacture chicken feeds could contribute to lowering the cost of chicken feeds (Preston 1995). This intervention in PNG and the SPICs could potentially decrease product prices with positive effects on human nutrition, sustainability of the chicken industry and farmer incomes and livelihoods. In this respect, protein and energy content of feeds are the two most important considerations in formulating diets for chicken because they are related to cost and efficiency of chicken production.

PNG has high energy staples such as sweet potato, cassava, banana and agro-industrial by-products such as palm kernel and copra meal that can be used as base for feed formulation to substitute for imported grains. However, to effectively use these ingredients for feeding chickens, information is required on not only the nutrient composition of the feed ingredients and their utilization but also actual performance of chickens on such diets. Such information is generally neither well developed nor available in PNG and other SPICs (Aregheore 2001).

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The main objective of this study was therefore to measure the growth response of broiler chickens which were fed five diets formulated from local ingredients and to correlate this performance with estimates of energy content of the diets.

MATERIALS AND METHODS

Two experiments were conducted. In the first experiment, growth performance of broiler chickens which were fed five experimental diets was measured. The second experiment was necessitated by lack of equipment for direct measurement of energy content of the treatment diets so a rapid bioassay method was used. Both experiments were conducted at the Animal Research Unit of the National Agricultural Research Institute (NARI) at Labu situated at latitude 06° 40' South and longitude 146° 54' East.

In the first experiment 208 one-day old chickens of the commercial Ross hybrid broiler breed were bought from a local commercial supplier and reared in a brooder for 20 days using commercial broiler starter feed. One hundred of these chickens were selected (50 males and 50 females) on the basis of uniformity of body weight at the end of the brooding period to be used in the experiment. Five experimental diets for feeding broiler chickens were formulated using sorghum, copra meal, palm kernel meal, sweet potato wagi besta (SPWB) variety and sweet potato SI.172 (SPiL) variety as energy sources. The two sweet potato varieties were chipped, oven dried and ground to meal form before mixing. Other ingredients were available in dried meal form. These ingredients were mixed with a protein concentrate prepared by a local feed mill. The composition of the diets and protein concentrate are shown in Table 1.

All five diets were used as treatments labeled as Sorghum, PKM, CM, SPWB and SPIL (see Table 1). Each treatment was replicated four (4) times with two of the replicates assigned to female chickens and the other two assigned to male chickens. A completely randomized experimental design was used whereby the treatments were randomly assigned to 20 rooms with deep litter floor located in an open, well ventilated poultry shed at NARI.

There was a one-week adaptation period followed by 4 weeks feeding and measurement period. During the trial, chickens were fed ad libitum. The amount of feed given each day and the residual feed in each feed container was measured at the end of each day to obtain a value of the total feed intake for each treatment. The total intake was divided by the number of chickens to obtain the average feed intake (FI) for each treatment. Other response variables measured were average body weight gain per bird (BWG) and feed conversion ratio (FCR). BWG was measured as the difference between average body weight at the start and end of the feeding period and FCR was calculated as FI divided by BWG. Analysis of variance (ANOVA) was carried out on each of the response variables to study the effect of diets on these variables.

In the second experiment a rapid bioassay method combined with the proximate values of nutrients in the diets and faeces of chickens was used to obtain estimates of the metabolisable energy content of each diet. During the bioassay

Table 1. **Percentage of ingredients and proximate composition of experimental diets for broiler chickens.**

	Treatment diets				
Ingredient	Sorghum	PKM	CM	SPWB	SPIL
Sorghum	57.5	43.1	43.1	43.1	43.1
Palm kernel meal (PKM)		25			
Copra meal (CM)			25		
Sweet potato waghi besta (SPWB)				25	
Sweet potato (SIL) (SPIL)					25
Protein concentrate	42.5	31.9	31.9	31.9	31.9
Total	100	100	100	100	100
Proximate nutrient composition (dry matter basis)					
Crude protein	22.9	18.4	23.5	18.9	18.4
Fat	6.8	8.5	6.9	5.3	4.9
Crude fibre	2.0	4.0	2.8	1.7	2.2
Ash	6.7	5.9	6.4	5.0	5.1

trial each of the same five diets used in the feeding trial were fed to groups of four chickens which were three weeks old and of the same breed as those used in the feeding trial described above. There were six replicates per diet, three replicates being assigned to female chickens and the rest three to male chickens. Each replicate was located in a cage in an open shed at NARI. The first three days of the bioassay period were regarded as adaptation period during which birds were fed test diets but no measurements were taken. From the fourth day (inclusive) to the seventh day total faeces output from each cage was collected. Each faeces sample was dried in an oven and subjected to proximate analyses. Each of the five diets were similarly subjected to proximate analyses. Since poultry pass out faeces and urine together metabolisable energy content of the diet was measured as the difference between the energy contents of each diet and its corresponding faeces using the following formula:

EME = [(GE in diet x FI) – (GE in faeces x Total faecal output)]/FI.

Where: EME is estimated metabolisable energy (mJ/kg DM) in test diet; GE is total gross energy of test diet (mJ/kg DM) and FI is total feed intake (g) during the bioassay period.

The GE content of proteins, fats, crude fibre and nitrogen free extract were taken from the literature (McDonald et al., 1995) to be 24.5 MJ/Kg, 39.0 MJ/Kg, 15.6 MJ/Kg and 17.7 MJ/Kg respectively.

RESULTS

A summary of the results of ANOVA of the response variables measured during the feeding trial are shown in Table 2. The EME content of each diet are shown in Table 3. Results of ANOVA showed that diet had highly significant effect (P<0.01) on FI and FCR but no significant effect on BWG. These results showed that, even though the EME content of the diets varied, chickens ate enough of each diet to obtain similar average body weights by the end of the feeding period. Sex of chicken also had highly significant effect on BWG and FI (P<0.01) but not on FCR. Mean FI and FCR for the five experimental diets are shown in Table 3. PKM had the highest FI and FCR of all diets and these values were significantly higher than the FI and FCR of all other diets except CM. However, chickens on CM diet had similar FI and FCR to those on Sorghum, SPWB and SIL diets.

DISCUSSION

At the end of the experiment chickens on all experimental diets attained statistically similar body weights even though EME content of the diets were different (Table 3). In this experiment the

Table 2. **Summary of ANOVA of response variables measured on broiler chickens fed different experimental diets.**

Parameter	Response variable measured		
	Body Weight Gain (mean = 2511g)	Feed Intake (mean = 4482g)	Feed Conversion Ratio (mean = 1.78)
Diet	ns	hs	hs
Sex	hs	hs	ns

Note: ns: non-significant effect (P<0.05); hs: highly significant effect (P<0.01).

Table 3. **Estimated metabolisable energy content of diets and growth performance of broiler chickens.**

Diet	Estimated Metabolisable Energy (MJ/Kg)	Feed Intake (g)	Feed Conversion Ratio	Body Weight Gain (g)
1. Sorghum	13.64	4310^{a}	1.63^{a}	2642^{a}
2. PKM	13.53	5483^{b}	2.12^{b}	2585^{a}
3. CM	13.44	4735^{ab}	1.9^{ab}	2495^{a}
4. SPWB	13.55	3862^{a}	1.59^{a}	2432^{a}
5. SIL	13.54	4019^{a}	1.67^{ab}	2403^{a}

Note: Values within columns which have different superscripts are significantly different (P<0.05) from each other.

diets were provided ad libitum and it appears that the chickens ate enough of each diet to meet their nutrient requirements for growth and maintenance. Dietary energy level is the main factor influencing feed intake as birds will, under normal circumstances, eat to satisfy their energy requirements. Chickens fed on low energy diets tend to eat more of that diet than if they were fed a higher energy diet (Olomu and Offiong, 1980; Olomu, 1995). This feeding behaviour of chickens was demonstrated in this study. The PKM and CM diets had the lowest EME compared to all other diets and chickens on these diets had the highest feed intakes and poorest FCR as well. The FI and FCR values calculated for chickens on CM PKM diets was not statistically different.

Another important finding from this study was that the performance of chickens on SPWB, SIL and CM were not significantly different to those on the Sorghum diet. Sorghum is a grain that is commonly used in some standard and commercial poultry diets as energy source so performance of chickens on the Sorghum diet might give an indication of performance on commercial diets which were not used in this study. Studies elsewhere have also shown that performance of chickens on sweet potato diets were comparable to those of some grain crops (Yoshida and Morimoto 1958; Fetuga and Oluyemi 1976). However Gerpacio *et al.* (1978) reported that the poorer performance observed for chickens on sweet potato diets could be attributed to unidentified chemical factors in sweet potatoes which inhibit digestive and metabolic processes in chickens. Among the two sweet potato varieties SPWB had slightly higher EME than SIL and this reflected in the slightly better performance of chickens on SPWB compared to SIL in terms of FI and FCR. Of all the treatment diets only PKM had significantly poorer values of FI and FCR compared to the Sorghum diet. The significantly poorer FI of PKM may be due to its lower EME (see Table 3) so the chickens had to eat more of this diet to satisfy their requirements for energy. Also, in this study PKM had the highest crude fibre content (Table 1) which could contribute to poor performance of chickens. Sundu *et al.* (2006) also reported that, although PKM has no anti-nutritional factors its high fibre content leads to low digestibility and high feed intake in chickens.

CONCLUSION

At the end of this experiment broiler chickens on the five diets attained similar body weights. This indicates that the local ingredients used in the various diets in this study could be suitable sources of energy for feeding chickens. Also feed intake was found to be generally inversely related to estimates of metabolisable energy content of the various diets. However differences were found in feed intake and feed conversion ratios among the diets reflecting the chickens innate ability to satisfy its energy requirements when feeding. These differences may indicate differences in the utilization of the diets by chickens and this could have implications on cost of the diets. Further studies are therefore needed to fully assess the nutritional value of these diets and their cost structure and also to compare these diets to commonly used commercial diets in order to arrive at a clearer understanding of the use of the various local ingredients for feeding broiler chickens.

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GROWTH OF CULTURED TILAPIA (*OREOCHROMIS NILOTICUS*) ON DIETS FORMULATED FROM EARTHWORM AND OTHER LOCALLY AVAILABLE INGREDIENTS.

Gebo Suzi[1], Gariba Danbaro[1,2] and William Nano[1]

ABSTRACT

A growth trial was carried out to study the effect of experimental diets containing earthworm meal and fish meal as protein sources on the body weight and length of Tilapia (Oreochromis niloticus) fingerlings. The protein concentrate portions of the five diets contained 0% 25%, 50%, 75% and 100% earthworm meal and the remaining part was made from fishmeal. The basal portion of the diets was made from copra meal, cassava, millrun and sago. All diets were formulated to contain 35% crude protein but metabolisable energy estimates ranged from 12.7 to 13.8mJ/kg. Feeding period was seven weeks and live body weight and length were measured weekly. The results showed highly significant effects of diets on body weight and length of Tilapia. The highest and lowest body weight and body length were obtained for the diet made from concentrate of 100% fishmeal and 75% earthworm meal respectively. Body weight and length of Tilapia fed on diets containing 25% earthworm meal concentrate were similar to those of Tilapia on 100% fishmeal concentrate diet. Generally growth performance of Tilapia decreased as the proportion of earthworm meal in the diets increased. The results suggest that, there is potential for using earthworms to replace up to 25% of fishmeal in the protein concentrate of farm made Tilapia diets.

Keywords: *Feeding trial, tilapia, diet, body weight, body length, fishmeal, earthworm meal, growth.*

INTRODUCTION

In Papua New Guinea, the history of freshwater fish farming dates back to the 1960s with the introduction of the trout and carp species (Smith 2007). Currently more and more farmers are venturing into small scale Tilapia (*Oreochromis niloticus)* culture in ponds. Tilapia is a hardy and prolific species that is a suitable for pond culture because they are fast growing and tolerant to poor water conditions. They also eat a wide variety of feedstuffs and can produce very good quality flesh (Fitzsimmons 2001). However Tilapia farmers, as is the case with the whole fish farming industry of Papua New Guinea, are faced with the problem of not being able to meet the optimum nutrient requirements of fish and also the high costs of farm-made fish diets (Maclean 1978). One way of minimizing these problems is by using locally available ingredients to reduce costs and using scientific procedures to properly formulate diets to meet the nutrient requirements of fish. This study was undertaken to evaluate earthworm meal as a potential source of protein to replace the high cost fish meal in Tilapia diets. The specific objectives of the study were to formulate diets which meet the nutrient requirements of Tilapia using earthworm and fishmeal as a source of protein and copra meal and cassava as a source of energy and to assess the growth performance in terms of average body weight and length of Tilapia fed on the formulated diets.

MATERIALS AND METHODS

The feeding trial was conducted under an open shed at the Papua New Guinea University of Technology Agriculture Farm. Five experimental diets called 0EW, 100EW, 75EW, 50EW and 25EW relative to the proportion of earthworm meal in the protein concentrate, were formulated using the feed formulation software of Thomson (2006). The ingredients used as well as the overall crude protein and metabolisable energy contents of the diets are shown in Table 1. Each diet was made by mixing a protein concentrate with a basal portion. The protein concentrate was made from various proportions of earthworm meal and fishmeal (from tuna) while the basal portion was made from calculated proportions of copra meal, cassava, millrun and sago. Millrun and sago were used solely to enhance the floating and binding qualities of the diets respectively. Wheat mill run consists of wheat bran, wheat shorts, wheat germ, wheat flour and the offal from the tail of the

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mill. Ground run of the mill screenings are normally added to mill run.

All diets contained a fixed level of 35% crude protein but metabolisable energy estimates ranged from 12.78mJ/kg to about 13.83mJ/kg. Earthworms were dug from the ground at a local farm. They were killed in water at 60°C, oven dried at 50 – 60°C for three to four days and then milled to a meal form. All other ingredients were milled using a hammer mill before combination. After mixing the basal and concentrate portions warm water was added at 60 – 80% and the dough resulting from further mixing was passed through a 3mm dye mincer to form pellets. The pellets were dried in an oven at 80°C for 12hrs before feeding. Each diet was replicated three times and each replicate contained five tilapia fingerlings of the GIFT variety (Dey and Gupta 2000) in a 0.17 m^3 water tank containing rain water. The mixed sex Tilapia fingerlings weighing between 5g and 7g were bought from the Highlands Aquaculture Development Centre (HAQDEC) at Aiyura. They were initially acclimatized for a period of one week on a diet of 38% crude protein then distributed randomly to the tanks.

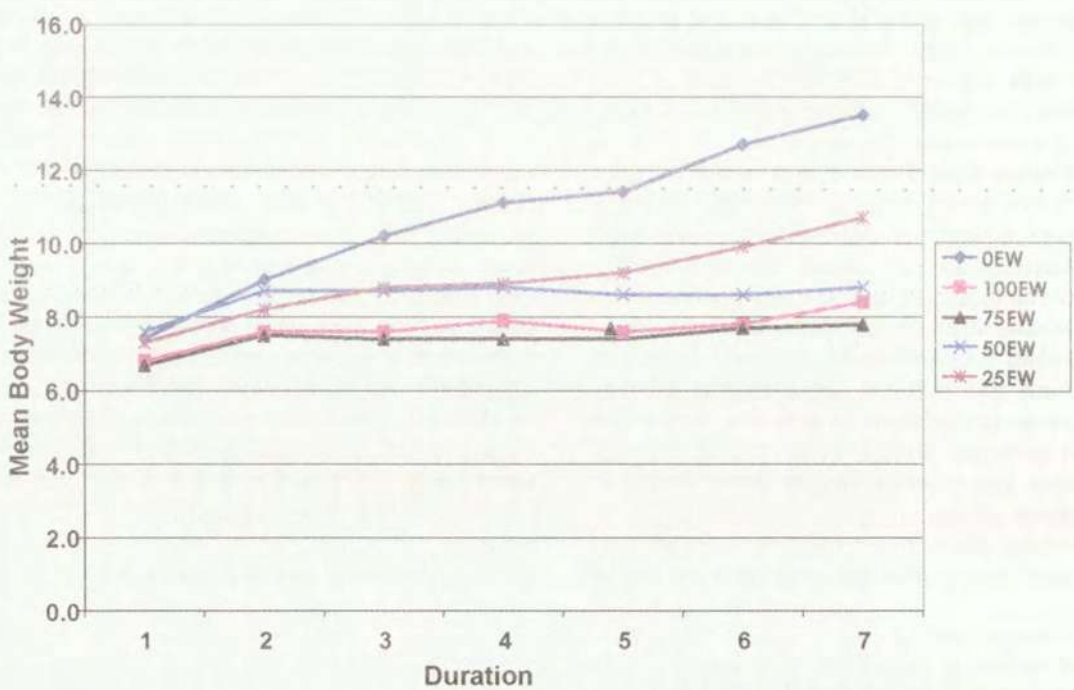
A complete randomized experimental design was used whereby the 15 treatments and replicates were randomly distributed to tanks located in a grid pattern under the shed. A compressor was used for aerating the tanks via small air hoses. The water in the experimental tanks was changed weekly.

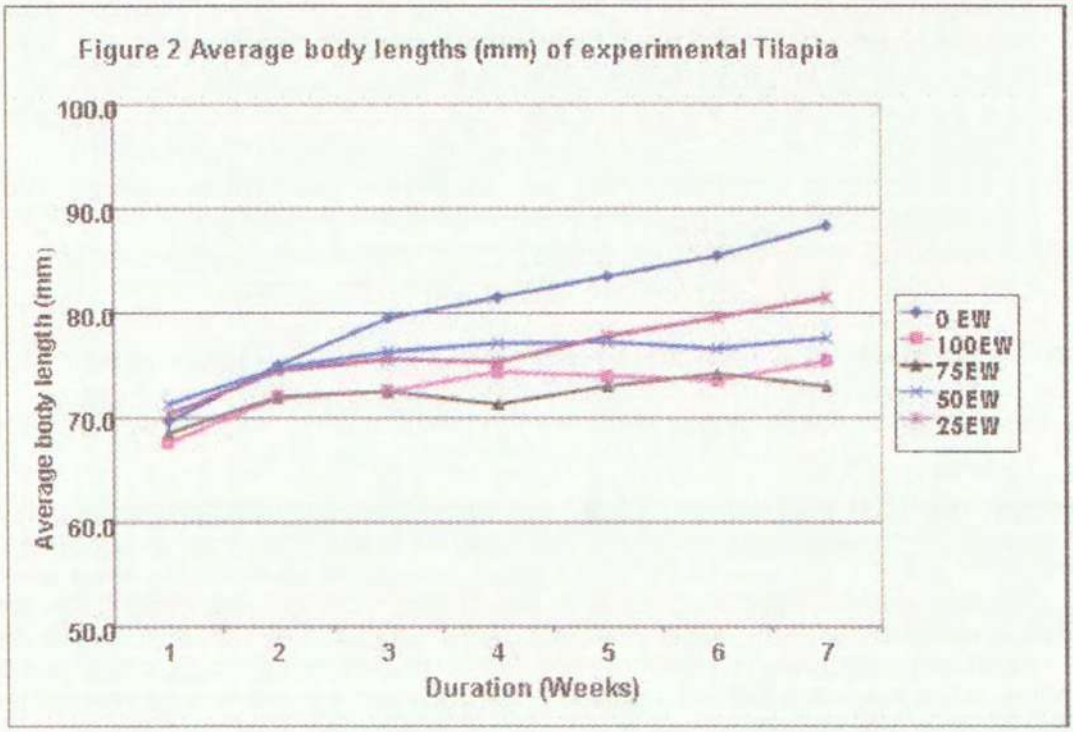
The fish were fed twice a day at a rate of 10% of their body weight adjusted on a weekly basis. Measurements made were weekly average live body weight and body length for seven weeks. Analysis of variance (ANOVA) was carried out on the two measured variables to study the effect of diets on body weight and body length of the fish.

Table 1. **Composition of experimental diets for Tilapia.**

Diet	Composition of concentrate portion of diet	Crude protein (%)	Metabolisable energy (mJ/kg)
0EW	100% fish meal	35	12.78
100EW	100% earthworm meal	35	13.83
75EW	75% earthworm meal + 25% fish meal	35	13.59
50EW	50% earthworm meal + 50% fish meal	35	13.33
25EW	25% earthworm meal + 75% fish meal	35	13.06

Figure 1 Average body weights (g) of experimental Tilapia





RESULTS

Trends in average body weight and body length of Tilapia fed the different treatment diets are shown in Figures 1 and 2 respectively. Fish on 0EW (crude protein mainly from fishmeal) had higher weekly average body weights and body length compared to all the other diets throughout the feeding period while fish on 75EW and 100EW diet had the lowest weekly average body weight and body length. It appears that as the proportion of earthworm meal increased (corresponding to a decrease in the proportion of fish meal) in the diet, tilapia tended to perform poorer in body weight and body length.

Results of analysis of variance of body weight and body length are shown in Tables 2 and 3 respectively. These results show that diet had statistically significant effect on both body weight and body length.

The results of mean separation using the least significant difference at $P<0.05$ are shown in Table 4.

The overall mean body weight and length were 8.47g and 75.13mm respectively. Tilapia on 0EW (only fishmeal in the diet) had the highest mean body weight and body length (10.37g and 80.83mm respectively) while Tilapia on 75EW

Table 2. Analysis of variance of mean body weights (g) of Tilapia on experimental diets.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	P-value
Treatment	4	17.57	0.39	5.45	0.014
Residual	10	8.06	0.81		
Total	14	25.63			

Table 3. Analysis of variance of mean body length (mm) of Tilapia on experimental diets.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	P-value
Treatment	4	157.61	39.40	4.63	0.022
Residual	10	85.03	8.50		
Total	14	242.63			

Table 4. **Estimated mean body weights and body lengths of Tilapia on experimental diets**

Treatments diets	Mean Body Weight (g)	Mean body length (mm)
0EW	10.37^{a}	80.83^{a}
25EW	8.73^{ab}	75.53^{ab}
50EW	8.40^{b}	75.3^{b}
100EW	7.57^{b}	72.33^{b}
75EW	7.30^{b}	71.67^{b}

Legend: Within each column, values with different superscript are significantly different from each other (P<0.05) but those with the same superscript are not significantly different from each other.

had the lowest mean body weight and body length (7.3g and 71.67mm respectively).

Results of the mean separation show that, Tilapia fed on 25EW had statistically similar mean body weight and mean body length as Tilapia fed on 0EW. However Tilapia fed on the remaining diets had significantly lower mean body weight and length than fish on the 0EW diet. Tilapia on 25EW diet had similar body weights and lengths as those on the diets containing earthworm meal (i.e. 50EW, 75EW and 100EW). Generally it appears that as the percentage of earthworm meal in the diet increased, performance of the fish became poorer in terms of both body weight and body length.

DISCUSSION

The findings from this study clearly show that growth of Tilapia, in terms of body weights and lengths, was influenced by the protein sources used in their diets. Tilapia on diets containing 25% earthworm meal and only fishmeal (0EW) concentrate had similar growth performance. This suggests that although fish meal could be the best protein source for fish diets, earthworm meal could potentially be used to replace fish meal if added up to 25% or less in the concentrate. However if the amount of earthworm meal concentrate in the diet is increased above the 25% level then growth performance of the fish are likely to be adversely affected in comparison with a diet formulated solely from fishmeal.

The similarity in performance of Tilapia for body weight and body length are not surprising because both variables are measures of growth.

The generally better performance of Tilapia fed fishmeal diets may be explained in terms of the quantity and quality of protein in the diet. The quality of dietary protein depends on the amino acid profile. The amino acid profile of fish meal most closely meets the amino acid requirements of fish compared to that of earthworm (Miles and Chapman 2006). Thus from a nutritional stand point, fishmeal is often the preferred source of protein in diets for fish and shrimps. The addition of fish meal to fish diets can be expected to increases feed efficiency and growth through better digestion, nutrient uptake and utilization compared to earthworm meal.

One significant finding from this study is that 25% or less of fishmeal concentrate can be replaced by earthworm meal concentrate in the diets for Tilapia. The main advantage of using earthworms to replace fishmeal in diets for Tilapia raised by small scale farmers would be its relatively low cost and availability in even remote communities in the country compared to fishmeal. Farmers could harvest naturally existing earthworms or culture them from domestic and farm garbage and residues for this purpose. It can therefore be proposed that the process of developing earthworm meal for feeding cultured Tilapia be investigated to better understand and apply the techniques of using earthworms in Tilapia diets.

Some factors which need further investigation include the method of processing earthworms into meal, digestibility and inclusion rates of earthworm meals. Furthermore the free choice feeding system using whole earthworms could also be investigated because it is known that fish readily consume whole earthworms and this feeding system, if successful, will remove the need to process earthworms into meal.

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SCREENING OF FIVE ELITE SWEET POTATO CULTIVARS AGAINST RENIFORM NEMATODE (*Rotylenchulus reniformis*) UNDER HUMID AND SEMI-ARID CONDITIONS OF PAPUA NEW GUINEA.

Gibson Kasi and Shamsul Akanda[1].

ABSTRACT

Five elite sweet potato cultivars were tested against reniform nematode to determine the resistance/ tolerance status and the yield losses under wet coastal lowlands and dry lowland conditions of PNG. The cultivars showed varying degrees of above and underground symptoms of nematode infection. The overall nematode population per kilogram of soil was much higher at the Wawin farm than at the Unitech farm irrespective of cultivars. The cultivars B11 and RAB 36 had the highest and lowest nematodes populations but yield/plot in the reverse order respectively, irrespective of the location. Yield losses as non-marketable tuber for B11 ranged from 36.2 – 40.1%, but in case of RAB 36 it was only 4.6 – 8.22%. B11 had the highest tuber cracking of 10 – 22.3% and L676 had between 6.5 – 11.9%. The other three cultivars did not show any cracking symptoms.

Key words. *Sweet potato, resistance, reniform nematode, tuber cracking, yield loss.*

INTRODUCTION

Sweet potato *(Ipomoea batatas* (L.) Lam) is the most common staple food in the highlands and the coastal regions of Papua New Guinea. Most parts of the sweet potato are consumed directly by humans or animals. There are several hundreds of cultivars that grow well both in the highlands and coastal regions of Papua New Guinea (Bourke 1982).

The optimum growing temperature for the growth of sweet potato is 25°C with a pH of 4.3 to 8.7 and rainfall of 900 mm to 1300 mm annually (Hector *et al.* 2002). Sweet potato yields best in sandy loam soil with 25 per cent moisture content than with 40, 60 and 80 per cent moisture content (Hector *et al.* 2002). Production or yield decline in sweet potato may be due to adverse environmental and production factors, such as temperature, rainfall, soil fertility and soil pH, etc. However, diseases can also play a significant role in reducing the yield (Clark and Moyer 1988).

Plant parasitic nematodes are recognized worldwide as a potentially serious constrains to crop productivity. The reniform nematode (*Rotylenchulus spp.*), primarily occurs in tropical and sub-tropical area; and also occurs in some temperate areas. It is one of the most destructive pathogens of sweet potato affecting both yield and quality of the crop (Clark and Moyer 1988). Reniform nematode can cause substantial yield loss in sweet potato (Clark and Wright 1983; Thomas and Clark 1983), if proper management is not practiced.

Rotylenchulus reniformis is an important semi-endoparasitic pest of many crops, especially in warmer climates. It is the principal nematode damaging cotton in Egypt and in parts of the United States. This nematode also attacks tomato, soybean, and pineapple among other crops. It is also found in other tropical and sub-tropical areas of the world where sweet potato is grown (Birchfield and Martin 1965).

Evidence of root damage by *Rotylenchulus reniformis* is clear. Population can rise to very high levels; up to almost 10,000/100 cm^3 of soil and at this level the mineral balance of the host is disturbed. The reniform nematodes also cause severe cracking on roots (Clark and Wright 1983) affecting the quality and making them unsuitable for selling and human consumption.

Rotylenchulus reniformis is present in several Pacific Island nations, including Papua New Guinea and has been rated as an important and destructive nematode of sweet potato throughout the world (Birchfield and Martin 1965; Bridge 1988). In Papua New Guinea, however, there is inadequate information on this pathogen infecting sweet potato. As the high status food security crop in this nation, it is important that any pathogenic agents affecting its productivity are ade-

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quately investigated and documented so that the stakeholders, researchers and others can benefit from this information. So, two field experiments were conducted with the following objectives:

1. To screen five elite sweet potato cultivars against the reniform nematode to determine their resistance/tolerance levels.

2. To estimate the yield losses due to reniform nematode under field conditions of humid lowlands and semi-arid regions of Papua New Guinea.

MATERIALS AND METHODS

Site Selection

The field experiments were conducted at two locations, namely Wawin National High School farm, in Morobe Province and Agriculture Farm of the Papua New Guinea University of Technology during 2006 to 2007.

The Wawin experimental site is located 74 kilometers west of Lae city along the Markham Highway at latitude of 6° 5' south and a longitude of 146° 8' east and an elevation of about 100 meters above sea level with grassland vegetation. The average rainfall is about 2000-3000 mm or less per year. The mean temperature ranges from 25°C-36°C and the soil is of sandy loam to loam type with a pH of 4.5 to 5.0. This area represents the dry lowland agro-ecological zone.

The agriculture farm of the Papua New Guinea University of Technology is located 10 kilometers north west of Lae City at a latitude of 6° 41' south, a longitude of 147° east and an altitude of 65 meter above sea level. The mean annual rainfall at this location is about 3800 mm and the average mean temperature ranges from 22°C to 32°C. Annual evaporation is 2139 mm, and the rainfall exceeds evaporation in each month. The soil in the farm is well drained, derived from the alluvial deposits and classed as loamy sand with a pH of 5.0-6.0 (Tumana 1986). The farm represents the wet coastal areas.

Land Preparation

An area of 200m^2 measuring 20m x 10m was selected for each of the two trials. The land was prepared for good tilth with tractor driven disc plough followed by harrowing. It was divided into five blocks and each block was divided into five plots, each measuring 4m x 2m. Drains of 50cm wide were made between blocks to drain out excess rain water. Raised bed was prepared in each plot with soil digging from the drain.

Soil samples were collected from each unit plot and soil texture, moisture content and the pH were determined. Plant parasitic nematodes associated with the soil samples collected from the experimental plots were extracted following Bearmann tray technique. Four nematode species, namely *Rotylenchulus, Meloidogyne, Paratylenchus* and *Pratylenchus* were found to be present in the experimental fields. To make the soil free from nematode infestation, marigold *(Tagetes patula* and *Tagetes erecta)* was planted in each bed. The plants were allowed to grow for two months so that roots can release the alleochemical compound called polythienyls, which kills or suppresses the nematodes present in the soil (Oostenbrink 1960). After two months of planting, the marigold plants were uprooted, cut into pieces and buried into the soil and were allowed to decompose as plant residues. Some marigold plants were left to grow at the edges of the experimental plots to prevent the movement of nematodes in/out of the field after inoculation commences.

Selection of Sweet Potato Cultivars and Planting of Cuttings

Five elite sweet potato cultivars were selected based on the tuber shape, tuber skin colour, tuber flesh colour, time of maturity, flesh texture after boiling. The flesh colour ranged from white to orange white while the flesh texture after boiling ranged from firm to soft and the maturity duration was from 4.5 to 5 months. All the cultivars were grown at the Unitech farm of PNGUT as the stock material for further plantings. All the precautions, including spraying with an insecticide (Karate) were taken to keep the stock materials pest free.

The planting of sweet potato was done four weeks after the marigold plants were mixed with the soil. Holes of 15cm deep were made into the soil of each unit plot maintaining 100cm distances from hole to hole and sweet potato cuttings of 50cm length were planted in each hole.

Experimental Design

The experiments at both the locations were carried out in randomized complete block design with five replications

Inoculation of experimental soils with *R. reniformis*

Soils were collected from *R. reniformis* infested fields of sweet potato at PNGUT Agriculture farm and Bayun High School farm. Adults and juveniles of the nematode were extracted from the soil following the Baermann's tray method (Agrios 1997) and used as inocula. The inocula were suspended in water and injected into the soil near the

base of the plant. Each plant received 10,000 inocula. Additional 4,000 nematodes were added to each bed every 10 days to maintain the population.

Inter-cultural Practices

Hand weeding was done after the second week of planting and continued throughout the five-month growing period. Fields were kept clean all the time to avoid the buildup of any pests. All the insect pests appeared in the fields were controlled by hand picking and the use of other trapping devices. No spraying of insecticide was done as this might affect the nematode survival. Disease presence, such as sweet potato scab and the little leaf were noted and the infected vines were removed from the field to minimize the spread of the diseases to other plants. No chemicals were used against the diseases. .

Data Collection

Weekly observations were made of the symptom development after the second month of planting till to the harvest. Initial symptoms in the form of yellowing of leaves, stunted growth and death of vines were observed after 60 days of inoculation. The symptoms observed were noted.

Sweet potato tubers were harvested manually with sticks. Harvested tubers were washed with tap water, air dried, graded and weighed with a weighing scale. The above ground biomass (fresh and dry weights of vines) weight of sweet potato was also recorded. The tubers were graded based on size and the quality. Tubers having small, cracked and damaged surface were graded as non-marketable ones. Good quality of tubers were graded as marketable produce and sold at the local markets.

Assessment of Parameters

Data on gall development on roots, nematode population in soils of experimental plots, tuber cracking and marketability were recorded following standard methods.

i) Root gall formation - Roots of each cultivar in each plot were washed and checked for galls on the roots. Total number of galls were counted and recorded.
ii) Tuber cracking effect – Tubers in each plot were checked for cracking, and their incidence was recorded.
iii) Marketable and non-marketable tubers - All the tubers harvested from each of the cultivars were graded as marketable and non-marketable weights (marketable ≥500g with no tuber cracks and non-marketable ≤500g with tuber cracks).
iv) Nematode population - One kilogram of soil was collected from each of the treatment plots at the time of harvest to estimate the nematode population. The nematodes were isolated following the Baermann's tray method. Nematodes were transferred into 20ml vials, observed under a compound microscope and counted to estimate the total population.

The fresh weight of the above ground biomass (leaves and vines) of each cultivar was recorded. The vines were left to dry for two weeks in the fields and dry weight of the sweet potato vines were also recorded.

Data Analysis

The data on the nematode population, fresh and dry above ground biomass weight, total tuber yield and marketable yield were analyzed using Minitab Student Release Version 12 computer software. The data on the selected parameters were subjected to ANOVA and means were compared (LSD) using the same software.

Results of the Experiment conducted at Wawin Farm

At Wawin farm, above ground symptoms of reniform nematode infection with varying degrees of severity were observed on five sweet potato cultivars (Plate 1). The leaves and vines of RAB 36, RAB 32 and DOY 2 were healthy and free from visible symptoms of reniform nematode infection. However, a few plots of RAB 32 were infected with leaf miner. Cultivar B 11 and L 676 showed moderate to severe symptoms of leaf yellowing at the base of the plants and dead vines.

Deformities/cracks appeared on sweet potato tubers due to infection of reniform nematode. Severe to moderate deformities/cracks on tubers were found in B 11, whereas tuber deformities were moderate to low in L 676. The tubers from other cultivars did not show any symptoms of deformities/cracks. Symptoms of gall formation were not found in any of the five cultivars (Plate 2).

The mean nematode population per kilogram of soil ranged from 172.6-986.0 (Table 1). The results show that the highest number of nematodes (986) was observed in plots with B 11 and the lowest being 172.6 in RAB 36. The difference in nematode populations between these two cultivars was significant at $p \leq 0.05$ (LSD). The nematode population in RAB 36 was also significantly lower than DOY 2 and L 676. However, there was no statistical difference between the nematode population in RAB 36 and RAB 32. The case was

Plate 1. Photographs showing above ground symptoms observed on five sweet potato cultivars due to infection with reniform nematode (*Rotylenchulus reniformis*) under field conditions at Wawin Farm.

DOY 2

B 11

L 676

RAB 36

RAB 32

similar for 32 and DOY 2.

The result shows that the fresh biomass weight was the highest in RAB 36 (17.30kg) and the lowest in L 676 (12.40kg) and this is significant at p≤0.05 (LSD). There was no significant difference in the fresh biomass weights of RAB 36 (17.30kg), RAB 32 (16.74kg) and DOY 2 (16.06kg) but were significantly higher than B 11 (13.86kg) and L 676 (12.40kg). The difference in fresh biomass weights of B 11 and L 676 cultivars was also non-significant at p≤0.05 (Table 1).

The dry biomass weight was the highest in RAB 36 (8.18kg) and the lowest in L 676 (4.50kg), which was significantly different at p≤0.05 level. The dry biomass weight of RAB 32 (6.52kg) and DOY 2 (6.16kg) was not significantly different, but was significantly higher than B 11 (4.56kg) and L 676 (4.50kg). The biomass dry weights of B 11 and L 676 were not significantly different at p≤0.05 (Table 1).

The result on total yield of tubers in Table 1 shows that it was the highest in RAB 36 (15.66kg) and the lowest in B 11 (8.72kg) and the difference was significant at p≤0.05 (LSD). The yield difference of RAB 36 (15.66kg) and RAB 32 (14.68kg) was non-significant but were significantly higher than DOY 2 (10.02kg), L 676 (9.30kg) and B 11 (8.72kg). There was also no

significant yield difference among DOY 2 (10.02kg), L 676 (9.30kg) and B 11 (8.72kg) at p≤0.05 (LSD).

Table 1 also shows the yield of marketable tubers. The highest marketable tuber yield was observed in RAB 36 (14.94kg) and the lowest in B 11 (5.22kg) and the difference was significant at p≤0.05 (LSD). The marketable tuber yield of RAB 36 (14.94kg) was significantly higher than all other cultivars. The marketable tuber yield of DOY 2 (7.2kg), L 676 (6.32kg) and B 11 (5.22kg) were non-significant at p≤0.05 (LSD), but were significantly lower than that of RAB 32.

The percentage reduction in yield as non-marketable tuber is presented in Table 1. The highest reduction in yield (40.1%) was observed in B 11 and the lowest of 4.6% in RAB 36. Meanwhile, yield reduction in L 676, DOY 2 and RAB 32 was 32.0%, 28.1% and 17.8%, respectively.

Table 1 and Plate 2 show that B 11 and L 676 had cracks in their tubers. The highest cracking

Plate 2. Photographs showing symptoms of tuber cracks caused by reniform nematode (*Rotylenchulus reniformis)* under field condition at Wawin Farm.

DOY 2

B 11

L 676

RAB 36

RAB 32

of 22.3% was observed in B 11 followed by L 676 with only 11.9% cracking, while no cracking was observed in RAB 36, RAB 32 and DOY

Results of the Experiment conducted at the Unitech Farm

The above-ground symptoms of *Rotylenchulus reniformis* infection on the five selected sweet potato cultivars at the Unitech farm are presented in Plate 3. The above-ground appearance of RAB 36 cultivar did not show any evidence of infections, however, B 11 showed symptoms of leaf yellowing and vines at the base of the plants. The cultivar RAB 32 was growing healthy, even though, a couple of plots were infected with sweet potato scab. The cultivar L 676 showed symptom of leaf yellowing and dead vines at the base of the plant, while DOY 2 did not show any symptom of infection.

The underground symptoms in Plate 4 show deformities/cracks on the tubers of B 11 and L 676, however, the other cultivars did not show any symptoms of deformities or tuber cracking.

The result in Table 2 shows that the mean nematode population was the highest in B 11 (465.2) and the lowest in RAB 36 (150.8) and this difference was significant at p≤0.05 (LSD). The nematode population in B 11 was significantly higher than the rest of the cultivars. The nematode population in RAB 36 was significantly lower than the other cultivars. The nematode population in L 676 (345) was significantly higher than RAB 32 and DOY 2 (256.8) at p≤0.05 (LSD). The difference in nematode population in RAB 32 was significantly lower than DOY 2.

The fresh weight of above-ground biomass was highest in RAB 36 (17.92kg) and the lowest in L 676 (13.12kg) and this difference was significant at p≤0.05 (LSD). There was no significant difference between RAB 36 (17.92kg) and RAB 32 (16.78kg). The above-ground fresh weight difference for B 11 and L 676 was also non-significant at p≤0.05 (LSD). The fresh weight of RAB 36 was significantly higher than DOY 2, however, the difference between the fresh weights of RAB 32 and DOY 2 was non-significant.

Table 2 also shows the mean dry weights of the five different sweet potato cultivars. The results follow the similar trend as in the fresh biomass weight.

***Table 1*. Means of parameters used to assess resistance of five sweet potato cultivars under field conditions at the Wawin Farm.**

Cultivar	Nematode population/kg soil	Rank on nematode population	Above ground Biomass (kg/plot)		Tuber yield (kg/plot)		Marketable tuber yield (kg/plot)	Percent reduction as non-marketable tubers	Tuber cracking (%)
			Fresh weight	Dry weight	Total weight	Rank			
DOY 2	386.20c	3	16.06a	6.16b	10.02b	3	7.20c	28.10	0
B 11	986.00a	1	13.86b	4.56c	8.72b	5	5.22c	40.10	22.3
RAB 36	172.60d	5	17.30a	8.18a	15.66a	1	14.94a	4.60	0
L 676	680.00b	2	12.40b	4.50c	9.30b	4	6.32c	32.00	11.9
RAB 32	267.60cd	4	16.74a	6.52b	14.68a	2	12.06b	17.80	0

All the values are means of five replications and those within the same column having a common letter(s) do not differ significantly at p≤0.05 (LSD).

The results on the total mean tuber weight is presented in Table 2. The results show that RAB 36 had the highest tuber yield of 16.78kg and the lowest being 11.6kg in B 11 and the difference was significant at p≤0.05 (LSD). The mean total yield of RAB 36 was significantly higher than L 676 (12.66kg). There were no significant differences among the total tuber yields of DOY 2, RAB 32, L 676 and B 11 at p≤0.05 (LSD). Similarly, tuber yield differences in DOY 2, RAB 32 and RAB 36 were also non-significant at p≤0.05 (LSD).

The results of marketable yield of tubers are presented in Table 2. The RAB 36 had the highest yield of 15.4kg and the lowest was in B 11 (7.4kg) and the difference was significant at p≤0.05 (LSD). The marketable yield of RAB 36 was significantly higher than the rest of the cultivars. Meanwhile, the marketable tuber yield difference for B 11 and L 676 was non-significant at p≤0.05 (LSD). The marketable tuber yield of B 11 was significantly (p≤0.05) lower than DOY 2, RAB 32 and RAB 36.

The percentage of yield reduction as non-marketable tuber in Table 2 shows that it was the highest (36.2%) in B 11 and the lowest in RAB 36 (8.22%). The reduction in cultivars DOY 2, RAB 32 and L 676 were 21.2%, 19.17% and 34.59%

Plate 3. Photographs showing above ground symptoms observed on five sweet potato cultivars due to infection with reniform nematode (*Rotylenchulus reniformis*) under field condition at Unitech.

DOY 2

B 11

L 676

RAB 36

RAB 32

respectively.

The tuber cracking defect was found only on B 11 and L 676 (Table 2). The highest tuber cracking of 10% was observed in B 11 followed by L 676 with only 6.5% cracking. No tuber cracking was observed in other four cultivars. No root gall was also observed on the tubers of any of the sweet potato cultivars.

DISCUSSION

Most sweet potato cultivars grown in Papua New Guinea are prone to diseases, most specifically to reniform nematodes (Birchfield and Martin

Plate 4. Photographs showing symptoms observed on edible roots of five sweet potato cultivars due to infection with reniform nematode (*Rotylenchulus reniformis*) under field condition at Unitech Farm.

DOY 2

B 11

L 676

RAB 36

RAB 32

Table 2. **Means of parameters used to assess resistance of five sweet potato cultivars under field conditions at the Unitech Farm.**

Cultivars	Nematode population/Kg of soil	Rank on nematode population	Above ground biomass (kg/plot)		Tuber weight (kg/plot)		Marketable tuber yield (kg/plot)	Percent reduction as Non-marketable tubers	Tuber cracking (%)
		Rank	Fresh weight	Dry weight	Total	Rank			
DOY 2	256.80c	3	16.14b	8.16b	13.84ab	3	10.90c	21.20	0
B 11	465.20a	1	13.68c	6.40c	11.60b	5	7.40d	36.20	10.0
RAB 36	150.80e	5	17.92a	11.22a	16.78a	1	15.40a	8.22	0
L 676	345.00b	2	13.12c	6.12c	12.66b	4	8.28d	34.59	6.5
RAB 32	193.00d	4	16.78ab	8.84b	14.92ab	2	12.06b	19.17	0

All the values are means of five replications and those within the same column having a common letter(s) do not differ significantly at p≤0.05 (LSD).

1965). Availability of resistant varieties can be of significant development in disease management as they are environmentally friendly, compatible with other disease management tactics, less costly and sustainable. However, with susceptible cultivars there could be substantial reduction both in terms of quality and quantity. Moreover, if the growers know the resistance status of the available cultivars they would know what to expect during the growing season. They can also plan well ahead of time as to the requirements in terms of management options and equipment, labour and costs rather than be caught by surprise. The five sweet potato cultivars that were previously tested in the greenhouse for resistance to reniform nematode were tested again under field conditions of humid lowlands and semi-arid regions of PNG to verify the resistance and estimate the yield losses in terms of quality and quantity.

Above ground symptoms of reniform nematode infection include leaf chlorosis, leaf yellowing, stunted growth and wilting (Clark and Moyer 1988). The visible effects of reniform nematodes on plants are usually subtle. The most obvious parameters to measure are yield reduction and plant stunting. In cotton, delayed flowering and fruit set are typical (Jones *et al.* 1959; Lawrence and McLean 1996). The *Rotylenchulus reniformis* also causes chlorosis in many plants and this has been shown to be related to potassium deficiency in roots as well as foliar tissues of cowpea and corn (Heffes *et al.* 1992). The test sweet potato cultivars at the Unitech and the Wawin farms showed similar symptoms including root cracks with varying degree of severity due to the difference in resistance/tolerance levels of the cultivars. The cultivars B 11 and L 676 showed leaf yellowing and vine death at both locations. The other cultivars did not show any above ground symptoms of nematode infection. Gaur and Perry (1991) also stated that in sweet potato, they might cause surface cracking of tubers. It does not cause galling of roots but causes reduction in root growth (Birchfield and Martin 1965; Bridge and Page 1982). The surface cracking was also observed in case of B 11 and L 676 in

both the locations. The other three cultivars did not show any symptoms of surface cracking and this could be due to a higher level of varietal resistance. The tested cultivars also did not show any galling as was observed by Birchfield and Martin 1965; and Bridge and Page 1982; but root growth was affected in all the five cultivars.

The severity of symptoms due to reniform nematode infection and ultimately the damage in terms of deterioration in quality and quantity depends on the nematode population level. Damage by reniform nematode is greater when population densities are high in planting time and increases during the growing season (Clark and Moyer 1988). This was clearly demonstrated in all five sweet potato cultivars tested in two locations. Overall, the nematode population was much higher in Wawin compared to the Unitech farm and this might be due to the availability of higher soil potassium at Wawin (Muneer 2004). Thomas and Clark (1983) and Robinson *et al.* (1997) reported that the presence and high levels of potassium in the soil greatly influenced the survival and growth of nematode population. Moreover, the reniform nematode population is higher in case of heavier soils, like sandy loam, sandy clay or clay loam (Kinloch and Sprenkel 1994). The soils of Wawin farm are clay loam compared to loamy sand at the Unitech farm and also high in available potassium (Muneer 2004). Nematode reproduction is greatly affected by soil texture and structure. Nematode populations tend to reproduce best in fine sand, sandy loam, well-aggregated loams and clay loam (Cook *et al.* 1997) so that might also be a reason for overall higher nematode population levels at the Wawin site. The highest nematode population level was on B 11 followed by L 676, DOY 2, BAB 32 and RAB 36, respectively. However, the ranking of the populations on the five different cultivars did not change over the locations, even though the two sites are located in two different agro-ecological zones, with the Unitech site being wet coastal lowlands and the Wawin as a dry lowlands ecosystem.

There are various factors, like morphological traits, chemical, biochemical and physiological factors in plants that contribute to resistance against the invading pathogens. The resistance mechanisms tested in the sweet potato cultivars was significantly different amongst the cultivars. This translated into the difference supporting different numbers of nematodes and ultimately different level of yields for the tested cultivars.

A number of phenolic compounds, such as chlorogenic and caffeic acids are reported to be involved in the defense mechanisms operating in plants against nematode infection (Epstein 1974; Hung and Rohde 1973; Bajaj and Mahajan 1977; Giebel 1982). The levels of preformed phenols in roots have been correlated with resistance to nematode in certain plant cultivars (Cohn 1974; Narayano and Reddy 1980).

Presence of phenolic compound called *laxative ipomoein* in the roots of sweet potatoes has been shown to be involved in providing resistance/tolerance against reniform nematode infection (Reed 1976; Kerry and Brown 1987). The amount of phenolic compounds produced by the tested cultivars differed significantly among the cultivars as evidenced from the mortality of nematodes in the phenolic extract test in the greenhouse. This in turn corresponded closely to the resistance/tolerance levels of the cultivars and might have been responsible for supporting different levels of nematode populations and ultimately affecting the tuber yield.

The total tuber yield of the five cultivars closely followed the nematode population ranking in the reverse order, i.e. RAB 36 had the highest tuber yields followed by RAB 32, DOY 2, L 676 and B 11, respectively. The yields for all the five tested cultivars were higher at Unitech farm than that at Wawin Farm. This might be due to the fact that the rainfall at the Unitech was higher than at the Wawin being 3800mm/year and the loamy sand soils with a pH of 5.0 – 6.0 that was ideal for the growth of sweet potato. Hector *et al.* (2002) reported that sweet potato tolerates a rainfall of 500mm to 1300mm per growth cycle with optimum levels of 900mm to 1300mm as this increases the root development. Clark and Moyer (1988) also documented that rainfall is one of the major contributing factors towards the growth of sweet potato. Moreover, the total nematode population per kilogram of soil was much higher for all the treatments at Wawin farm compared to the Unitech site. This difference in the nematode population also had a significant effect on the tuber yield and tuber yield reduction in terms of non-marketable tubers.

The ranking of the cultivars for the total tuber yield did not change over the locations. This might be due to the fact that the resistance levels of the cultivars are quite stable.

Critical decision making in agriculture in terms of resource allocation, long term research goals and disease management requires the information on plant disease incidence, severity and yield losses. Studies on yield loss estimation are of special importance in terms of disease management decisions. All the cultivars tested in two different locations showed different levels of yield losses rang-

ing from as low as 4.6% in case of RAB 36, the most resistant cultivar to as high as 40.1% in case of B 11, the most susceptible cultivar at the Wawin farm. These yield reductions as non-marketable tubers were in line with the ranking of the total nematode populations on each of the cultivars. Yield losses due to reniform nematodes on cotton were reported to be 9.5 17.4% in India and 40-60% in Egypt. However, in Louisiana and Mississippi, USA, yield losses as high as 40-60% and an average as little as 15-30% was reported (Bridge 1988; Robinson *et al.* 1997). Jones *et al.* (1959) and Overstreet (1996) reported a yield loss as low as 25% to as high as 60% from the reniform infested cotton fields in USA.

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INVESTIGATION OF PEANUT STRIPE VIRUS DISEASE AT MARKHAM-RAMU VALLEY IN PAPUA NEW GUINEA

Madeline Iko[1], Shamsul Akanda[1] and Birte Komolong[2]

ABSTRACT

A survey was conducted to investigate the presence of Peanut Stripe Virus in the Markham-Ramu Valley of the Morobe Province, the major peanut growing areas of Papua New Guinea.

Peanut leaf samples with apparent virus-like symptoms, infected soybean leaves and peanut seed samples were collected from different fields from the survey sites and tested through indirect ELISA using polyclonal antibody. Only four out of 59 samples showed positive reaction to the presence of viruses. The observation of symptoms did not indicate the presence of PStV even though the presence of Aphis craccivora was confirmed.

These preliminary findings need to be investigated further by collecting larger numbers of samples including alternative hosts from all over the peanut growing areas of the country, and more specifically, the areas bordering Indonesia, using monoclonal antibody and/or polymerase chain reaction (PCR).

Keywords: *Peanut, Peanut Stripe Virus*, Aphis craccivora, *ELISA, PCR, polyclonal antibody.*

INTRODUCTION

Peanut (*Arachis hypogaea*) is an important seed legume widely grown in Papua New Guinea (PNG) mostly as a cash crop by the smallholders and are sold through informal road side markets. It is an economically important crop because of its protein and edible oil content.

In PNG, peanut is cultivated on 14000 ha of land with an estimated annual gross income of about K30, 000.00 (Wemin and Geob 2004).

The commercial production of this crop has ceased since the 1980s due to various constraints, such as poor seed quality, improper agronomic practices, lack of proper machinery, non-availability of markets and land tenure problems (Rachaputi *et al.* 2006). However, it is estimated that by 2010, the peanut industry in PNG will increase significantly due to the increase in land use for this crop (Rachaputi *et al.* 2006).

Pests and diseases are one of the major constraints to peanut production. A recent survey has revealed 17 groups of insects and 13 different diseases in major peanut growing areas of PNG (Wightman *et al.* 2005) with early and late leaf spots caused by *Cercospora arachidicola* and *C. personata*, respectively; and rusts caused by *Puccinia arachidis* are more prevalent.

Peanut stripe virus, a strain of the Bean Common Mosaic Virus (Berger *et al.* 1997; cited in CABI 2006), is a quarantinable disease because of its severe impact on peanut yields in major peanut growing countries of the world, such as Indonesia, China, India and the United States of America. The yield reduction in Indonesia was reported to be 30-60% (Manzila 2005) and 40% under field conditions in China (Xu *et al.* 1989). In USA, yield loss of up to 20% was reported to occur under greenhouse condition (Demski and Lovell 1985). The PStV is seed-borne both in peanuts and soybean and, therefore, are the main source of primary inoculum (Demski and Lovell 1985; Xu and Zhang 1986; Zettler *et al.* 1993; cited in CABI 2005). The peanut stripe virus disease is seed borne and the transmission by infected peanut seeds is very high and only 2-5% seed transmission can culminate in an epidemic. The secondary spread of the disease is mostly by the aphids, which spread the infection in a non-persistent manner. The *Aphis craccivora* is assumed to be the major vector for the PStV (Sreenivasulu and Demski 1988; Wongkaew *et al.* 1988; Xu 1988).

There are several variants of PStV as described according to the symptoms in older leaves. Generally, the symptom begins as chlorotic flecks or rings on young quadrifoliates with slight stunting. The early symptom begins with discontinuous striping along the lateral veins (Demski *et al.* 1984).

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The control of PStV is mostly achieved through the use of PStV-free certified peanut seeds. In addition, early detection of the disease would most likely enable complete eradication.

There is no report of the presence of PStV in PNG, eventhough CABI (2005) indicated the presence of the major insect vector, *Aphis craccivora* in PNG. The study by Whitman *et al.* 2005 could not however confirm the presence of the aphid.

Despite being absent in PNG, there is considerable risk of incursion and rapid establishment of PStV due to various predisposing factors. The virus is pandemic in Indonesia and this could be one of the pathways for the entry. Alternative hosts, such as soybean (*Glycine max*), *Lupin albus*, Lucerne (*Medicago sativa*), *Calopogonium caeruleum*, *Pueraria* spp are being regularly imported from overseas. Furthermore, others, such as mungbean *(Vigna radiata*), cowpea (*Vigna unguiculata*), *Stylosanthes*, are widely grown in the country. The presence of these species would most likely enable the rapid spread with the subsequent dissemination by the aphids. With these views in mind, a study was conducted with the following objectives:

1. To establish the status of PStV in the Markham-Ramu valley through field inspection and ELISA testing.
2. To confirm the status of *Aphis craccivora* in PNG.

MATERIALS AND METHODS

Survey Sites and Sample Collection

The peanut plots at Ramu Sugar Ltd, Trukai Industries Ltd. at Erap, NARI - Bubia, Philip Rhalda Farm- Markham and Waritzian village in Markham valley in the Morobe Province of PNG were selected as the study areas as these are the major peanut production areas.

The peanuts varieties grown in Ramu Sugar and Trukai Industries Ltd are improved varieties imported from Australia and India (International Centre for Crops Research for Semi-Arid Tropics - ICRISAT) under the Australian Centre for International Agricultural Research (ACIAR) funded project for trial purposes, while the local variety, Yarang is grown in the other locations.

Peanut rows in the selected plots in the survey sites were inspected for peanut stripe virus-like symptoms. Any plant that displayed virus-like symptoms were uprooted and put into wet plastic bags individually, labeled and packed in iced containers. This was done to keep the samples fresh for the ELISA testing which was conducted in the Biotech Centre at the University of Technology. The number and types of samples collected from these sites are presented in Table 1. Samples were stored at 4^0C and processed within 3 days after collection.

Aphid Collection

Aphids were collected only from Ramu Sugar Ltd. and could not be collected from other areas either because of heavy rainfalls or sprayed with insecticides at or before the time of sample collection. Aphids were collected from the growing shoots of peanuts, stored in 70% ethanol, labeled and sent to NARI-Insectory in Port Moresby for identification.

Indirect ELISA Testing

The indirect ELISA testing was done following the protocol by Hobbs *et al.*, (1987). A basic ELISA kit containing the rabbit polyclonal antibody and antisera was obtained from ICRISAT (generosity of Dr. Farid Waliyar). The content of the protocol included 200 µl of Anti-rabbit IGg ALP polyclonal antibody (used at 1:2000). Addi-

Table 1. **The number of samples collected from the various survey sites.**

Location	Type of sample collected	No. of samples
Ramu Sugar Ltd	Peanut leaves	27
Trukai Industries Ltd (Erap)	Stored Peanut Seeds Peanut leaves	11 5
NARI-Bubia	Soybean leaves	6
Philip Rhalda farm-Markham Valley	Peanut leaves	6
Waritzian Village – Markham Valley	Peanut leaves *Pueraria* spp leaflet	3 1

tional materials were 1 ml of Antisera for PStV (used at 1:5000); 15mg of Paranitrophenyl phosphate PNPP tablets (used at 1 tablet for 20 ml of substrate buffer) and 0.1 g of PStV positive control.

The negative controls were healthy peanut leaves collected from the PNG University of Technology farm grown from the seeds distributed by Ramu Sugar Ltd.

The carbonate buffer was prepared from and distilled water was added to make a one liter solution. The phosphate buffer saline (PBS) was produced by mixing 2.38 g Na_2HPO_4, 0.4 g KH_2PO_4 and KCl, 16.0 g of NaCl and distilled water was added to make a 2 liter solution. The pH of the carbonate buffer and PBS were not adjusted. The antibody buffer (PBS-TPO) was made up of 100ml PBS, 2.0 g ovalbumin and 2.0 g PVP (Polyvinyl Pyrrolidone 40,000MW). Two drops of Tween 20 was added. Finally, the substrate buffer (Diethanolamine buffer) was made with 450 ml of distilled water and 50 ml of diethanolamine. The pH of this solution was adjusted to 9.8.

The test peanut leaves/seeds were ground in carbonate buffer (1.59 g/L of Na_2CO_3, 2.93 g/L of $NaHCO_3$) at 1/50 dilution and 150µl of this mixture was dispensed in each well of the ELISA micro-plate (96 wells) and incubated overnight in the refrigerator at 4°C. Healthy leaves weighed at 1g were ground in 20 mls of antibody buffer and filtered into an empty container. Then, 8µl of PStV antibody was added to this healthy sap and allowed to incubate for 1 hour at 37°C.

The refrigerated micro-plate was then washed with three changes of PBS-T (PBS buffer plus Tween 20), with 3 minutes between each wash. After this, 150µl of the healthy sap was dispensed into each well of the ELISA plate and incubated for 1 hour at 37°C, followed by three washes with PBS-T as described above. At the end of the washing, 10µl of anti-rabbit ALP-Conjugate was diluted in 20µl of antibody buffer and 150µl of this mixture was dispensed in each well. This was again incubated for another hour at 37°C and washed with PBS. Finally, 15mg of Paranitrophenyl phosphate PNPP tablet was dissolved in 20 ml of substrate buffer (50 ml of diethanolamine in 450ml H2O) and 150µl of this solution was dispensed into each well and the color development was observed and the ELISA readings were taken 15-20 minutes after the positive control changed colour.

The plates were read at wavelength 1 and optical density of 405nm in a Maxline Micro-plate reader using the software SoftMax-Pro to take the readings.

Using the respective means of the samples from the reading, samples were considered positive when the readings exceeded three times the mean of the negative control. All samples with mean values less than twice the mean of the negative control were considered negative. Marginally positive values were more than twice but less than three times the mean of the negative control. However, marginally positive values were not considered as true values of the recording of viruses. This determination of values was recommended by the Australian Quarantine and Inspection Services (Richard Davis: Personal communication)

RESULTS

A number of peanut plants with virus like symptoms were observed at the survey sites. Some of the most common symptoms are presented in Plates 1-5.

Severe stunting with smaller and narrower leaves was observed at Ramu Sugar site and also at Philip Rhalda Farm and Trukai Industries (Plates 3 and 5). Furthermore, inter-veinal wrinkling with downward curling from the leaf tip and margin depicting mild mottling was the most common symptom found in all the locations.

The symptom shown in plate 1 was quite common and confined to the peanut trial locations at Ramu Sugar. Inter-veinal wrinkling with downward curling from leaf tip and margin depicting the mild mottling were also observed.

In general, the plants at Trukai Industries looked healthier than those in Ramu Sugar Ltd. Symptoms of stunting with reduced size of leaves

Plate 1. Ramu Sugar sample indicating a possible symptom of Peanut Mottle Virus (PeMoV). Note the depression of inter-veins as indicated at the leaf margin. This symptom has also been referred to as the blotch isolate of PStV. Note the blotch is surrounded by light green rings (see arrows).

(Plate 3) were found occasionally in the field. It was explained that these plants were the volunteer plants from the seeds which had been left in the field from previous harvest.

The soybeans that were inspected at NARI-Bubia, were highly infested with various insects including the jassids and ladybirds. Almost 70% of the soybean plants in this area showed mosaic symptoms. There were only two plants which were severely stunted with curling leaves.

The symptoms of mild mottling were common at Philip Rhalda farm and Waritzian village. The *Pueraria* sp. had green mosaic symptoms.

ELISA Test

The results of ELISA readings for the samples collected from Ramu Sugar, Trukai Industries, NARI, Philip Rhalda Farm and Waritzian village in the Markham Valley are presented in Table 2. The results show that all of the peanut leaf samples collected from the Ramu Sugar trial sites

Plate 2. The mild mottling effect observed on this plant from Ramu Sugar. Leaf curling and chlorotic areas on the leaves are visible. The plant was also stunted.

Plate 3. The whole plant was stunted with reduced size of leaves

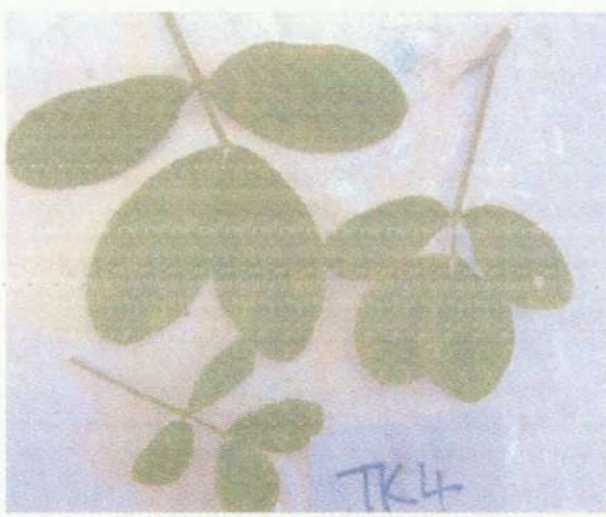

Plate 4. Symptom which resembles the striping effect. However, is NOT discontinuous as shown in Plate 1. This symptom was not common in all the fields inspected.

Plate 5. The leaflet at bottom left shows greatly reduced leaf lamina, folded together to the midrib. Leaflet at bottom right shows vein clearing.

Table 2. **Showing the results of ELISA for samples collected from Ramu Sugar Ltd, Trukai Industries, NARI, Philip Rhalda farm and Waritzian village.**

Location	Type of sample collected	No. of Samples	Virus reaction		
			Positive	Marginally positive	Negative
Ramu Sugar Ltd	Peanut leaves	27	0	0	27
Trukai Industries Ltd	Stored Peanut Seeds	11	0	2	9
	Peanut leaves	5	4	0	1
NARI-Bubia	Soybean leaves	6	0	0	6
Philip Rhalda farm	Peanut leaves	6	0	0	6
Waritzian Village	Peanut leaves	3	0	1	2
	Pueraria spp leaflet	1	0	0	1

was negative. Out of the 11 peanut stored seed samples collected from Trukai Industries, only two showed marginally positive reactions and the rests were negative. But, four of the five leaf samples collected from the same location showed positive reaction and the other one was negative.

The symptoms of the positive reactions are those of the samples shown in Plates 3 to 5. One of the samples also had the curling on the leaves. All the six soybean leaf samples collected from NARI-Bubia showed negative reactions to virus presence. Similar is the case with all the peanut leaf samples collected from the Philip Rhalda farm.

Out of the three peanut leaf samples collected from Waritzian village, one showed marginally positive reaction and the other two were negative. Moreover, the lone leaf sample of the *Pueraria* spp. collected from the same location showed negative reaction.

The symptoms of the positive results are those of the samples shown in Plates 3, 4 and 5 collected from the Trukai Industries Ltd.

Aphids Identification

The aphids were identified and confirmed to be *Aphis craccivora*, the major vector for the transmission of PStV.

DISCUSSION

Peanut Stripe Virus is an important seed borne disease of peanut affecting the industry throughout the world. Because of the seed borne nature of the disease, a rigorous quarantine protocol is in place that affects the trade including the exchange of germplasms. So, early detection and timely intervention are essential to help protect the emerging peanut industry of PNG.

Most of the infected plants at the trial sites at Ramu Sugar and Trukai Industries showed severe stunting. This might be due to the fact that these sites received very little rain during the growing season. The situation had further been aggravated with nutritional deficiency. Similar situation was also observed in the Philip Rhalda farm

It was expected that the ELISA results obtained would have been the same for the samples from Ramu Sugar and Trukai Industries because the origin of the seed stock was the same. Similarly, the Waritzian village peanuts are the same varieties (local) that are grown in the Philip Rhalda Farm. However, some samples tested positive for Trukai Industries were found to be negative for Ramu Sugar. Even if the seed source was the same, but they were not the first generation seeds that is, not planted for the first time in both locations. The seeds used in these trials were the carryover seeds from the previous trials and during the growth periods they might have been infected with viruses through the insect vectors leading to the different ELISA readings for the samples from the different sites. One of the positive samples showed the striping symptom which was obtained from Trukai Industries, but the striping was not discontinuous as is the characteristic feature of PStV (Plate 1). Furthermore, no initial chlorotic rings were also observed on the same plant and no apparent stunting. This might be due to the environment and strain of the virus as was reported in CABI 2005.

Similar symptoms were observed on plants at

Waritzian village and Ramu Sugar. The village is also a trial area for the Ramu Sugar peanut project. It is quite possible because of inter-transmission between the peanut plots by the aphids.

The other samples that showed positive reactions to ELISA were severely stunted with reduced leaf lamina. However, there were no mosaics or mottling found in the leaves.

Symptoms resembling to PeMoV were sighted but the same symptoms could also be described as the blotch isolate of PStV. In addition, the ELISA result indicated negative response to this sample, thereby raising the possibility to be PeMoV. This is based on the fact that PeMoV does not cross react with the antisera to PStV. A positive reaction was expected if the sample were the blotch isolate of PStV, thereby ruling out the symptom as the possible strain of PStV. However, this needs to be confirmed with further tests.

It is worth mentioning that there is no commercial ELISA kit for testing peanut stripe virus and thus, it was not possible to obtain the monoclonal antibody for PStV. There have been problems with non-specificity due to the use of polyclonal antibodies. The viruses, especially of the Potyvirus group may cross react with this antiserum and, therefore, leading to the positive results.

At this point in time, therefore, the question remains as to the status of Peanut Stripe Virus in Papua New Guinea, specifically, in these areas of intensive peanut farming. The imported peanuts may introduce this disease and it will spread rapidly because of the presence of the vectors and the alternative hosts, particularly, under the congenial environmental conditions of Ramu Sugar.

Apparently, the use of polyclonal antibody for the detection of PStV is not recommended due to the problems outlined above. Thus, other methods, in particular, PCR must be considered in this respect. The monoclonal antibody is also another method that can be adapted for this purpose because of its specificity.

One of the most outstanding findings of this study is the identification and confirmation of the presence of *Aphis craccivora* in the survey sites. This might raise the alarm that once the virus is introduced in to the country, it would disseminate quickly because of the availability of susceptible host plant throughout the year and that could be a disaster for the emerging peanut industry of PNG.

The out come of this preliminary study formed the foundation to continue further studies. Further studies need to be conducted collecting a large number of samples including the alternative hosts from all over the country, particularly the areas bordering Indonesia using monoclonal antibody and/or PCR.

CONCLUSIONS

This study identified and confirmed the presence of *Aphis craccivora*, the major vector for the peanut stripe virus in the survey sites. This is a worrying development as the introduction of the virus could have a devastating effect on the emerging peanut industry of PNG because of the presence of insect vector and the availability of susceptible hosts through the year. The outcome of this study formed the foundation to continue further studies, collecting a large number of samples including the alternative hosts from all over the country, particularly the areas adjacent to Indonesian borders using monoclonal antibody and/or PCR.

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Internal reports, communications and memoranda are not valid references. The criteria for valid publications (in the scientific world) are that publications are distributed widely among those interested in the subject and are available to the international public in major libraries and from the publisher. This therefore excludes reports circulated only within a department and to a few outsiders and conference documents available only to those who attended the conference and the like.

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Special care should be take to see that every reference in the text is included in the list of references and vice versa, and that there is consistency in the spelling of author's names and the citation of the dates throughout the paper.

12. Review of papers - All papers will be submitted to suitable professional referees. Major changes will be referred to the author for consideration. Minor editorial changes will be made without consultation but will be presented to the author(s) at proof stage. The final decision to accept or reject a paper, rests with the Editor.

13 Offprints - Twenty-five free off-prints are given to the author. Where there are several authors, the first author will be sent the off-prints. Extra off-prints may be ordered at the time the galley proofs are returned to the editor. Costs will be determined at the time of printing.

14. Recognised abbreviations in this journal are:

g	- gram
kg	- kilogram
t	- tonne
l	- litre
ml	- millilitre
ha	- hectare
mm	- millimetre

cm - centimeter
M - metre
a.s.l. - above sea level
yr - year
wk - week
h - hour
min - minute
s - second
k - kina
n.a. - not applicable or not available
n.r. - not recorded
var - variance
s.d. - standard deviation
s.e.m.- standard error of difference
d.f. - degrees of freedom

Levels of significance

n.s. - not significant
* - 0.01 $p < 0.05$
** - 0.001 $p < 0.01$
*** - $p < 0.001$

Either kg/ha or kg.ha is acceptable, but large combinations of units should be in the form kg.ha to avoid possible mathematical ambiguity.

15. Submission of manuscripts - All correspondence should be addressed to: Editor, PNG Journal of Agriculture, Forestry and Fisheries, Agricultural Information Branch, Publication Section, Department of Agriculture and Livestock, P.O. Box 2033, Port Moresby, Papua New Guinea or e-mail to dalit@daltron.com.pg and chrisdekuku@yahoo.co.uk