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).

[1]Department of Agriculture, PNG University of Technology, Lae 411, Papua New Guinea
[2] National Agricultural Research Institute, Lae 411, Papua New Guinea

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

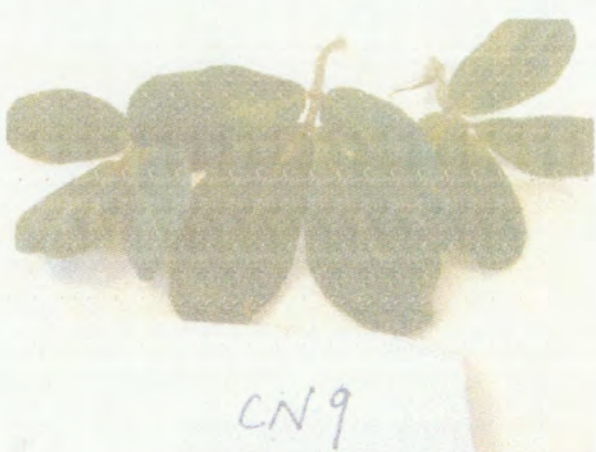

Plate 1. Ramu Sugar sample indicating a possible symptom of Peanut Mottle Virus (PeMoV). Note the depression of inter-veins as indicated at the leaf margin. This symptom has also been referred to as the blotch isolate of PStV. Note the blotch is surrounded by light green rings (see arrows).

(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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