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Surveys for **PLANT DISEASES** caused by Viruses & Virus-like pathogens in **SAMOA & VANUATU**

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ABSTRACT

Surveys for virus and virus-like plant diseases were conducted in Samoa, on the islands of Upolu and Savai'i, and Vanuatu on the islands of Efate, Esperitu Santo, Malekula, Ambae and Tanna. New records for Vanuatu were those of *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (PRSV), detected by enzyme linked immunosorbent assay (ELISA) in pumpkin (*Cucurbita maxima)* and squash *(C. maxima* x *Cucurbita moschata*) and *Banana streak virus* (BSV) in banana, detected by real-time polymerase chain reaction (PCR). New records for Samoa were *Cymbidium mosaic virus* in orchids (*Dendrobium* sp.), detected by ELISA, and *Wisteria vein mosaic virus* in vanilla, detected by reverse transcription PCR (RT-PCR) followed by DNA sequence analysis (also reported elsewhere).

Other detections by ELISA were: *Cucumber mosaic virus* in the roadside weed *Synedrella nodiflora* and in cultivated tobacco (*Nicotiana tabaccum*) in Vanuatu; *Dasheen mosaic virus* in *Xanthosoma* sp. in Vanuatu; *Passion fruit woodiness virus* in *Passiflora foetida* and passion fruit (*Passiflora edulis*) in Samoa; ZYMV and PRSV in several cucurbits in Samoa; and *Citrus tristeza virus* in several different citrus trees in Samoa. *Taro vein chlorosis virus* was detected by RT-PCR in taro in Vanuatu, and the Mysore infecting strain of BSV (BSV-Mys) was detected by immunocapture PCR in banana (*Musa* sp. cv. Misi Luki) in Samoa.

Phytoplasmas were detected by nested PCR in sweet potato (*Ipomoea batatas)* and the related *Ipomoea pes caprae* ssp. *brasiliensis* showing little leaf symptoms in Vanuatu and in pandanus (*Pandanus tectorius*) showing white leaves in Samoa. The phytoplasmas in Vanuatu were found by DNA sequence analysis to be members of the '*Candidatus* Phytoplasma aurantifolia' (16SrII) group.

In Samoa, no evidence was found for presence of citrus huanglongbing, previously known as greening disease, in nine citrus trees indexed by PCR.

INTRODUCTION

Surveys were conducted to assess the general plant virus and virus-like disease status of Samoa and Vanuatu.
A general plant virus disease survey has been conducted once before in Samoa, in the late 1970s (Van Velsen, United Nations Development Program/Food and Agriculture Organization (UNDP/FAO) unpublished report, 1979), but one has never been conducted before in Vanauatu. The survey results from Samoa have been summarised in Mossop and Fry (1984). The viruses present in the samples collected on that survey were identified by indexing and mechanical inoculation, or by electron microscopy. The virus records from Samoa included in the listing of Mossop and Fry (1984), together with records generated after 1984, were listed again by Pearson and Grisoni (2002). In Vanuatu, a number of records resulting from various studies and specifically targeted surveys do exist and these are included in the listing of Pearson and Grisoni (2002). The known records of diseases caused by plant viruses for which there exists acceptable supportive evidence of the pathogen's identity are summarised in Table 1 (Samoa) and Table 2 (Vanuatu). There are several records from Samoa for which only inconclusive supportive evidence of pathogen identity is available. These are listed in Table 3. They include several identifications based on either symptoms only, or on presence of virus particles observed by electron microscopy only.

Very little is known of the phytoplasma disease status of Samoa and Vanuatu. Phytoplasmas (formerly known as mycoplasmalike organisms) are unculturable bacteria closely related to the genus *Acheloplasma* (Seemüller et al. 1998). They infect plant phloem vessels, are transmitted by phloem feeding insects (mostly leafhoppers) and have been associated with diseases of numerous plant species throughout the world (Seemüller et al. 1998). Sweet potato little leaf is a disease known to be associated with phytoplasmas elsewhere and its symptoms have been observed in Vanuatu (Jackson 1984). Phytoplasma test results from sweet potato (*Ipomoea batatas*) and one related plant species in Vanuatu have recently been published in Davis et al. (2006a). This information is repeated, together with further details on these samples, in this technical paper.

There is some question about the distribution of one of the worst diseases of citrus in the region. This is huanglongbing (ex-greening) disease (HLB), caused (in much of Asia) by another phloem-limited bacterium, '*Candidatus* Liberibacter asiaticus'. HLB has been spreading in certain Asian countries close to the Pacific Islands, and reached Papua New Guinea (PNG) in 2002 (Weinert et al. 2004). The disease and its vector, the Asian citrus psyllid (*Diaphorina citri*), are currently the subject of a containment campaign in PNG. A report that this virus-like disease was found in Samoa in the mid 1990s (Kiritani and Su 1999) is doubted by many HLB researchers because the detection method used in that study was not reliable. Negative HLB indexing results from four citrus leaf samples from Samoa have been summarised in Davis et al. (2005). Further details on these samples, together with more HLB test results, are provided in this paper.

Also in this paper, details are provided on a new virus infecting vanilla (*Vanilla planifolia*) in Samoa. This is a new record also included in the paper of Grisoni et al. (2006).

Upolu and Savai'i, Samoa's two main islands, were surveyed twice (once in the cool season and once in the warm season). In Vanuatu, the islands of Efate, Ambae, Esperitu Santo, Malekula and Tanna were surveyed once in the cool season.

METHODS

Surveys
To undertake the surveys, as many different areas as possible were visited. Crop plants of subsistence and economic importance, and occasionally also other plants, were examined at each location. Samples thought to be infected by intracellular pathogens were returned for analyses after rapid desiccation in the field. Samples (about 1 g fresh weight of young leaves or shoot tips) showing disease symptoms were first surface-sterilised in 1% available chlorine to eliminate organisms that might have been present on external surfaces. The material was then rinsed in water, blotted dry and chopped finely. The sample was desiccated over anhydrous calcium chloride (about 7 g) in sealed, 25 mL plastic vials. They were stored at 4°C until fully desiccated, and at –20°C thereafter. Samples were returned (under appropriate quarantine import permits) to several different laboratories for diagnostic tests.
Survey 1 of Samoa was undertaken over two weeks in February 2003 and the samples collected were given the collection numbers RID3247–RID3293. Survey 2 was conducted over two weeks in July–August 2004 and samples collected were given the collection numbers RID3701–RID3765. The survey of Vanuatu was undertaken over four weeks in May–June 2003

Enzyme-linked immunosorbent assay (ELISA) testing for viruses
Cucurbit samples from both countries were tested for *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* (PRSV) and *Squash mosaic virus* (SqMV), using double antibody sandwich ELISA (DAS-ELISA). Vanilla and other orchid samples from Samoa were tested by DAS-ELISA for CMV, *Cymbidium mosaic virus* (CymMV), *Odontoglossum ringspot virus* (ORSV) and by indirect ELISA for the potyvirus group. Citrus leaf samples from Samoa were tested for *Citrus tristeza virus* (CTV) by compound direct ELISA, and taro (*Colocasia esculenta*) and *Xanthosoma* sp. leaf samples from Vanuatu were tested for *Dasheen mosaic virus* (DsMV) by DAS-ELISA. All these tests were conducted at the Secretariat of the Pacific Community (SPC) plant virology laboratory, Suva, Fiji Islands, using Agdia Inc. (Elkhart IN, USA) ELISA reagent sets. At Rothamsted Research, UK, several *Passiflora foetida* samples from Samoa, one passion fruit (*Passiflora edulis*) and one snakebean (*Vigna unguiculata* ssp. *unguiculata*) sample from Vanuatu were tested for potyvirus infection, and one tobacco (*Nicotania tabaccum*) plus two *Synedrella nodiflora* samples from Vanuatu were tested for CMV, all in the same way as described above. *Passiflora* leaf samples from Samoa that tested positive for potyvirus were then also tested for *Passionfruit woodiness virus* by DAS-ELISA (using antisera from the laboratory of J. Thomas, Department of Primary Industries and Fisheries (DPIF), Brisbane, Queensland, Australia). All ELISA test samples were considered positive when absorbance values exceeded three times the mean of appropriate healthy controls that were included on each microtitre test plate.

Reverse transcription polymerase chain reaction (RT-PCR) testing for viruses
Vanilla samples from Samoa that gave positive reactions in ELISA tests for potyvirus were forwarded to the laboratory of M. Grisoni, CIRAD/UR, Saint Pierre, Réunion Island, where they were identified further by RT-PCR using degenerate potyvirus primers followed by direct sequencing of the amplicon as described in Grisoni et al. (2006). Taro (*Colocasia esculenta*) and *Xanthosoma* sp. leaf material from Vanuatu was returned to the molecular biology laboratory of the Institute of Applied Sciences (IAS), University of the South Pacific (USP), Suva, Fiji Islands. Here, samples were tested for *Taro vein chlorosis virus* (TaVCV) by RT-PCR using the methods described in Revill et al. (2005).

Immunocapture (IC) RT-PCR testing for viruses
Banana leaf samples from Samoa were tested for *Banana streak virus* (BSV) infection at the laboratory of J. Thomas, DPIF, Queensland, using specific immunocapture PCRs for a number of strains of the virus (BSV-Mys, BSV-GF, BSV-Onne and BSV–Cav) as described in Geering et al. (2000).

Real-time PCR testing for viruses
One banana leaf sample from Samoa and one from Vanuatu were subjected to a multiplex real-time PCR test for presence of BSV, *Banana bract mosaic virus* (BBrMV), *Banana mild mosaic virus* (BMMV), CMV and *Banana bunchy top virus* (BBTV) at the Central Science Laboratory, York, UK.

Electron microscopy testing for viruses
At Rothamsted Research, UK, two maize (*Zea mays*) samples from Vanuatu were tested by immunosorbent electron microscopy using antisera for *Maize mosaic virus* (MMV) and *Maize stripe virus* (MSpV). Some other samples were examined using transmission electron microscopy only.

Phytoplasma testing
Samples from plants showing phytoplasma-like symptoms were subjected to nucleic acid extraction, followed by nested PCR at Rothamsted Research as described in Davis et al. (2006a).

Polymerase chain reaction (PCR) testing for HLB
Citrus leaf material from Samoa was tested for HLB at the USP IAS molecular biology laboratory using the PCR techniques described in Davis et al. (2005).

RESULTS

The plant virus and virus-like pathogens recorded on this survey are presented in Tables 4 (Samoa) and 5 (Vanuatu).

Viruses in Samoa

Of the five viruses screened for by ELISA in cucurbit samples from Samoa, ZYMV and PRSV were detected in *Cucurbita maxima* (pumpkin) on both islands, and marginally positive results were recorded for ZYMV and CMV in *Citrullus lanatus* (watermelon), for ZYMV in *Momordica charantia,* and for WMV in a *Crotalaria* sp. A number of cucurbit samples from Samoa showing virus-like symptoms on young leaves did not test positive for any of the five viruses indexed for. These were one *C. maxima*, one *Cucurbita pepo* var. *melopepo* (zucchini) and one *M charantia*. CTV was detected by ELISA in citrus trees from both Savai'i and Upolu (one lime, four lemons, and three mandarins). *Passion fruit woodiness virus* (PWV) was present on both Upolu and Savai'i, confirmed by ELISA in one *P. edulis* and four *P. foetida* leaf samples that had tested positive for potyvirus.

Two banana (cv. Misi luki or Mysore) leaf samples from Upolu tested positive for the Mysore strain of BSV (BSV-Mys) by immunocapture PCR. A strong real-time PCR positive result (in which a mean Ct value of two replicate wells <30 in a 40-cycle reaction was obtained) was recorded for BSV, but not the other banana infecting viruses, in a banana leaf sample from Savai'i. CymMV was detected by ELISA in orchid (*Dendrobium* sp.) leaf samples from two locations on Upolu and six vanilla samples from one plot on Upolu were potyvirus-positive also by ELISA. Three of these potyvirus-positive samples gave the expected amplicon in RT-PCR and, following sequence analysis, were most similar (93–97%) to a strain of *Wisteria vein mosaic virus* (WVMV).

Viruses in Vanuatu

In cucurbit samples from Vanuatu tested for five cucurbit infecting viruses by ELISA, only ZYMV and PRSV were detected. ZYMV was more common than PRSV and was found on Efate, Tanna and Malekula. PRSV was detected on Malekula and Efate. Both viruses were detected in *C. maxima* and *C. maxima* x *Cucurbita moschata* (squash). Only marginally positive results for ZYMV were obtained from *C. lanatus* and *Cucumis sativus* (cucumber) samples. One *Xanthosoma* sp. leaf sample from Espiritu Santo was positive for DsMV by ELISA, and three others plus one *C. esculenta* (taro) sample from Tanna returned only marginally positive test results. TaVCV was detected by RT-PCR in *C. esculenta* on the islands of Ambae, Esperitu Santo and Tanna. A strong real-time PCR positive result (in which a mean Ct value of two replicate wells <30 in a 40-cycle reaction was obtained) was recorded for BSV, but not the other banana infecting viruses, in one banana leaf sample from Tanna. ELISA tests detected CMV in two *S. nodiflora* on Efate and one *N. tabaccum* on Tanna, and confirmed potyvirus infection of one *Passiflora edulis* and one *Vigna unguiculata* ssp. *unguiculata* from Efate. In addition, potyvirus particles were seen by electron microscopy in one *L. esculentum* sample from Esperitu Santo.

Distinctive symptoms of Fiji leaf gall disease (chlorosis, stunting, galls on leaf) were seen in stands of *Saccharum officinarum* (sugarcane) at Pang Pang on Efate; Middle Bush on Tanna; Lambumbu and Botundir on Malekula; and Natauwa, Mavunlevu, and Fanafo on Espiritu Santo. An attempt was made to confirm these preliminary visual diagnoses by sending dried gall tissue from each location to the laboratory of BSES Limited in Brisbane, Australia. However, samples were first sent for gamma irradiation treatment as a quarantine precaution and, following treatment, failed to reach BSES.

Phytoplasmas in Vanuatu

'*Candidatus* Phytoplasma aurantifolia' (16SrII) group phytoplasmas were associated with little-leaf symptoms in sweet potato (*I. batatas*) on Tanna Island, and in *Ipomoea pes caprae* ssp*. brasiliensis* on Ambae Island.

Phytoplasma in Samoa

A phytoplasma was detected by nested PCR in one *Pandanus tectorius* tree on Savai'i that was sampled because young leaves were white. However, the amplification was weak and it was not possible to take the diagnosis further by sequence analysis of the amplicon.

Important negative results

In Samoa, HLB indexing returned negative results from seven citrus leaf samples from Savai'i and two from Upolu (Table 6). In Vanuatu (Table 7), maize leaf samples from Malekula and Esperitu Santo showing streaking and stunting tested negative for MSpV and MMV by ISEM. One vanilla support tree (*Jatrophas curcas*) on Malekula showing a strong mosaic and which was suspected to be infected with vanilla viruses tested negative for potyvirus by ELISA. Some peanut samples from Esperitu Santo and Tanna showing symptoms similar to those of peanut stripe disease were screened by electron microscopy and no virus-like particles were found.

Figures 1–17 show the symptoms caused by the viruses and phytoplasmas found in a number of hosts.

DISCUSSION

These surveys provide the first records in Vanuatu of the cucurbit infecting viruses ZYMV and PRSV, and of BSV in banana. New host records for CMV in Vanuatu (the roadside weed *S. nodiflora* and cultivated tobacco) are also noted. Moreover, *S. nodiflora* is not included in known worldwide CMV host listings (Douine et al. 1979; CABI 2002). The surveys of Samoa give the first records for CymMV and WVMV in orchids and vanilla, respectively, though the latter record is also included in the study of Grisoni et al. (2006), and confirms the presence of PWV. The phytoplasma testing conducted following these surveys provides the first two phytoplasma host records for Vanuatu (*I. batatas and I. pes caprae ssp. brasiliensis*), also included in the paper of Davis et al. (2006a). The survey of Samoa provides a preliminary phytoplasma record for this country (associated with white leaf symptoms in pandanus) though further study is needed to verify this record.

Pearson and Liyanage (1997) previously recorded presence of all the cucurbit infecting viruses identified in Samoa on these surveys, plus SqMV. The PRSV found in cucurbits in both countries is the cucurbit infecting strain of this virus (PRSV-W) which is closely related to, but distinct from, the papaya infecting strain, and causes important disease in cucurbit crops throughout much of the world (Purcifull et al. 1984). This virus, together with ZYMV and WMV are members of the genus *Potyvirus*, and share some common characteristics of significance when considering control. These viruses cannot survive in the soil or in decayed plant material. Whilst PRSV-W is not thought to be seed transmitted, there are reliable reports of low rates of cucurbit seed transmission of ZYMV in Australia (see http://www.dpi.qld.gov.au/horticulture/9575.html) and of both WMV and ZYMV in New Zealand (Burgmans and Fletcher 2000; Fletcher et al. 2000). All three cause systemic infections, meaning that infected plants cannot be cured with any spray treatment or by removing parts of the plant showing symptoms. They are spread from plant to plant by many different species of aphid vectors. They are non-persistently transmitted by these aphids, meaning they are picked up from an infected plant in a few seconds, then held on the insect's mouth parts for several hours and can be transmitted to another plant during brief feeding probes. This is most damaging if the aphids move from crop host to crop host (spreading the virus within the crop) or from weed host to crop (introducing more new infections into the crop). Because of this non persistence, spraying crops with insecticides is not a useful control measure. In fact, such sprays can increase spread because they often do not immediately kill the aphids. Instead, the insects are disturbed, fly to other nearby plants and feed and transmit virus before they die. The best method to combat these viruses is to use resistant or tolerant cultivars, which are available for several cucurbit crops. Older cucurbit crops and wild cucurbit crop plants or weeds can be significant reservoirs of mosaic virus inoculum for new plantings. Natural hosts of PRSV-W and ZYMV are mostly in the Cucurbitaceae (see: *http://image.fs.uidaho.edu/vide/sppindex.htm#S*), and WMV is known to have a wider natural host range (see *http://image.fs.uidaho.edu/vide/descr878.htm*). Whilst cucurbit volunteers and weeds should be key targets for control, other species may also be of importance. For example, this study identified one possible weed host of WMV in Samoa. This was a *Crotalaria* sp. growing adjacent to a pumpkin crop which returned a marginally positive ELISA test result.

Rodoni et al. (1999) reported the presence of BBrMV in Samoa. BBrMV is not known anywhere else in the Pacific, but does cause a serious banana disease in India, Sri Lanka and the Philippines. Typical symptoms of infection in these countries are streaks on the bracts of the inflorescence and the leaf petioles, and mottling on the pseudostem. The banana plants in Samoa sampled in the study of Rodoni et al. (1999) were showing abnormal symptoms for BBrMV. They were apparently similar to those caused by infection with BSV. These are chlorotic streaks on leaves with brown/black colouration, and a co-infection with BSV was suspected in that case. To date, banana bract mosaic disease has not been recognised as a production problem in Samoa. During the surveys reported here, BSV-like symptoms were extremely common in cv. Misi Luki, but no symptoms similar to bract mosaic disease were seen. It was possible to test only one banana leaf sample for both viruses by real-time PCR and this gave a positive result only for BSV. Although BSV is widespread in Samoa, losses may not be serious since it is known that Queensland strains of this virus, which include BSV-Mys, cause yield losses of only 7–15% (Daniels et al. 1999). In contrast to Samoa, BSV was apparently very rare in Vanuatu, with only one plant observed with banana streak symptoms on the entire survey. Although BSV has mealybug vectors, plant to plant spread is not great, with most virus transmission occurring through infected planting material (Lockhart and Jones 1999). In addition, infection can also arise from activation of virus sequences integrated into the host genome (Ndowora et al. 1999). For these reasons, incidence of banana streak disease in Vanuatu could remain low, so long as planting material is selected carefully.

Of the orchid-infecting viruses found in Samoa, CymMV is thought to be of little importance to vanilla production and is commonly found in ornamental orchids. The report of WVMV in Samoan vanilla is of more interest, as the symptoms were seemingly identical to those caused by Vanilla mosaic virus (VanMV). VanMV is a tentatively named species closely related to DsMV that infects only *Vanilla* spp. and causes a major disease problem in several Pacific Islands (Pearson et al. 1993). WVMV naturally infects fabaceous hosts, but is capable of infecting members of other families (see http://image.fs.uidaho.edu/vide/descr902.htm). It is therefore likely that the potyvirus infections of vanilla observed in the Samoan plot originated from WVMV-infected fabaceous weeds or the leguminous support trees used in the plot. Further testing of these potential hosts is planned.

The plot of vanilla where this new disease was found was intended as a planting material source for future expansion of production in Samoa. Symptomatic plants were identified and removed (rogued) before planting material was distributed to growers.

TaVCV and DsMV are widespread across the Pacific and both have been detected before in *C. esculenta* from Vanuatu (Revill et al. 2005) The Vanuatu survey adds a record of TaVCV on Ambae and the first confirmation of DsMV infection of *Xanthsoma* sp. in the country. Whilst the importance of TaVCV is still unclear (Revill et al. 2005), DsMV has been implicated in causing yield losses in taro (Jackson et al. 2001) and ornamental aroids (Chase and Zettler 1982).

The phytoplasmas associated with little-leaf symptoms of *Ipomoea* spp. in Vanuatu were found to be closely related to '*Ca.* P. aurantifolia' which is in the 16SrII group according to the IRPCM Phytoplasma/Spiroplasma Working Team (2004). Similar phytoplasmas are known to be associated with sweet potato little leaf in Australia (Gibb et al. 1995; Davis et al. 1997; Schneider et al. 1999) and on the island of New Guinea (Davis et al. 2003). The phytoplasma associated with white leaf symptoms in pandanus in Samoa deserves further investigation as possible phytoplasma infections of Pandanus species have not been well studied.

Stands of sugarcane apparently affected by Fiji leaf gall disease were found in several village gardens during the Vanuatu survey. Symptoms of this disease are extremely distinctive and it is unlikely that another pathogen could be involved. However, it was impossible to confirm these visual observations with any laboratory testing following this survey. Moreover, it is not certain if previous records of the disease in Vanuatu (see http://image.fs.uidaho.edu/vide/descr769.htm) are based on anything more than visual observation. In commercial sugarcane production, timely removal of infected plants (rogueing) can be an effective Fiji leaf gall disease control strategy (Egan et al. 1989). Similarly, this disease can be readily controlled in village gardens by removing affected plants and carefully selecting disease free (symptomless) planting material for propagation.

It was possible to confirm PWV infection in wild and cultivated *Passiflora* spp. at several locations in Samoa. PWV can be an important constraint to passion fruit production, and co-infections of PWV with an as yet unassigned potyvirus (tentatively named *Passiflora virus Y,* PaVY) have recently been investigated in Australia and the island of New Guinea (Parry et al. 2004). The symptoms shown by the PWV-positive *P. foetida* leaves in Samoa are identical to those of PaVY in *P. foetida* found in northern Australia (author's unpublished data). Potyvirus infection of passion fruit was also confirmed in Vanuatu, but no virus-specific testing was undertaken.

The CMV detected in two *S. nodiflora* plants is worth noting because this appears to be the first record of this species as a CMV host and because the plants were growing adjacent to kava plants showing dieback disease symptoms. Kava dieback is a disease believed to be caused by this virus in combination with other factors, and a management strategy is now being promoted which includes control of key alternative hosts (Davis et al. 2006b). *S. nodiflora* may be an important alternative host for dieback causing strain(s) of CMV. Further investigations into the CMV status of *S. nodiflora* growing in kava dieback disease infection foci are needed.

There are a number of other plant diseases caused by viruses, of quarantine concern in the Pacific region because they are found on some islands only. One that is of particular importance because it is believed to be absent from Vanuatu is banana bunchy top disease, caused by BBTV. By late 2005, laboratory test records confirming presence of BBTV in Fiji Islands, Tonga, Samoa (Karan et al. 1994) and New Caledonia (Kagy et al. 2001) had been published. There are also unpublished laboratory test records of BBTV in Guam and on Wallis Island (but not Futuna, the other main island in the Territory of Wallis and Futuna). In addition, there are reliable reports, dating back many years, of the distinctive symptoms of the disease seen in the field in Tuvalu and American Samoa. Whether a diagnostic test to confirm these records has been performed is not known. Many banana plants were examined during the survey of Vanuatu and no bunchy top-like symptoms were seen, and one banana leaf sample also tested negative for BBTV by real-time PCR. Peanut stripe disease, which is caused by the peanut stripe strain of *Bean common mosaic virus,* is present on the island of New Guinea, but not in PNG (Davis et al. 2002). As the virus is seed borne at high rates, it is a quarantine threat to the region. Peanut leaf samples showing similar symptoms in Vanuatu appeared to be free of virus when screened by electron microscopy. The negative citrus HLB screening results reported here, together with preliminary data presented in Davis et al. 2005, adds support to the widely held belief that this disease is not present in Samoa.

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Table 1. Verified plant virus and virus-like disease records from Samoa

Pathogen	Host	Citation[A]	Identification method[B]
Banana bract mosaic virus[C] (BBrMV)	*Musa* sp.	Rodoni et al. (1999)	Serology, EM and PCR
Banana bunchy top virus (BBTV)	*Musa* sp.	Karan et al. 1994	PCR
Banana streak virus (BSV)	*Musa* sp.	Thomas et al. (1994)	ISEM
Citrus vein enation / woody gall virus (?Luteoviridae)	*Citrus aurantifolia*	Thomas (1978)	Not available[D]
--	*Citrus limon*	Thomas (1978)	Not available[D]
--	*Citrus reticulata*	Thomas (1978)	Not available[D]
Citrus exocortis viroid (CEVd)	*Citrus limon*	Van Velsen (1979)	Not available[D]
Citrus psorosis virus (CPsV)[E]	*Citrus reticulata*	Thomas (1978)	Not available[D]
--	*Citrus sinensis*	Thomas (1978)	Not available[D]
Citrus tristeza virus (CTV)	*Citrus aurantifolia,*	Van Velsen (1979)	Not available[D]
	Citrus paradisi	Thomas (1978)	Not available[D]
Cucumber mosaic virus (CMV)	*Benincaspa hispida*	Pearson and Liyanage (1997)	Serology
	Capsicum annuum	Thomas (1978)	Not available[D]
	Cucumis sativas	Van Velsen (1979)	Not available[D]
	Musa sp.	Kiritani and Su (1999)	Serology
	Passiflora edulis	Thomas (1978)	Not available[D]
	Piper methysticum	Davis et al. (1996)	Serology
	Psophocarpus tetrogonolobus	Pearson and Liyanage (1997)	Serology
Dasheen mosaic virus (DsMV)	*Colocasia esculenta,*	Revill et al. (2005)	PCR
Fiji disease virus (FDV)	*Saccharum officinarum*	Van Velsen (1979)	Not available[D]
Papaya ringspot virus-W (PRSV-W)	*Cucumis sativas*	Pearson and Liyanage (1997)	Serology
	Cucurbita maxima	Pearson and Liyanage (1997)	Serology
	Citrullus lanatus	Pearson and Liyanage (1997)	Serology
Peanut mottle virus (PeMoV)	*Arachis hypogaea*	Thomas (1978)	Not available[D]
Potato virus Y (PVY)	*Capsicum annuum*	Thomas (1978)	Not available[D]
Squash mosaic virus (SqMV)	*Citrullus lanatus*	Pearson and Liyanage (1997)	Serology
	Cucurbita maxima	Pearson and Liyanage (1997)	Serology
	Trichosanthes cucumerina	Pearson and Liyanage (1997)	Serology
Taro baciliform virus (TaBV)	*Colocasia esculenta*	Revill et al. (2005)	PCR
Tobacco mosaic virus (TMV)	*Lycopersicon esculentum*	Thomas (1978)	Not available[D]
	Nicotiana tabacum	Van Velsen (1979)	Not available[D]
Watermelon mosaic virus (WMV)	*Citrullus lanatus*	Thomas (1978)	Not available[D]
--	*Cucurbita maxima*	Thomas (1978)	Not available[D]
--	*Cucurbita pepo*	Thomas (1978)	Not available[D]
Zucchini yellow mosaic virus (ZYMV)	*Citrullus lanatus*	Pearson and Liyanage (1997)	Serology
--	*Cucumis sativas*	Pearson and Liyanage (1997)	Serology
--	*Cucurbita maxima*	Pearson and Liyanage (1997)	Serology

[A]The earliest citation of a reliably verified record is provided.

[B]EM: Electron microscopy; ISEM: Immunosorbent electron microscopy; PCR: Polymerase chain reaction, following reverse transcription in the case of RNA viruses.

[C]The BBrMV record came from leaves showing BSV-like symptoms and the disease is not known as a production problem in Samoa.

[D]These records are listed in Mossop and Fry (1984) as reliable identifications achieved using host range studies and/or serology and/or electron microscopy, with no further specification provided. It was impossible to cite the original survey reports (Thomas 1978; Van Velsen 1979) at the time of preparation of this technical paper. This means no further comment on validity or otherwise (as judged by modern standards) of these diagnostic techniques employed in the late 1970s is possible.

[E]Not a virus specific verification, as psorosis disease is thought to be caused by a complex of several viruses.

Table 2. Verified plant virus records from Vanuatu

Pathogen	Host	Citation[A]	Identification method[B]
Coconut foliar decay virus (CFDV)	*Cocos nucifera*	Randles and Hanold (1989)	EM, molecular methods
Cucumber mosaic virus (CMV)	*Piper methysticum*	Davis et al. (1996)	Serology
Dasheen mosaic virus (DsMV)	*Colocasia esculenta*	Revill et al. (2005)	PCR
Digitaria streak virus (DSV)	*Digitaria sanguinalis*	Dollet et al. (1986)	EM, serology
Hibiscus chlorotic ringspot virus (HCRSV)	*Abelmoschus manihot*	Brunt and Spence (2000)	EM, serology
Odontoglossum ringspot virus (ORSV)	*Vanilla planifolia*	Pearson et al. (1993)	EM, serology
Vanilla mosaic virus (VanMV)	*Vanilla planifolia*	Pearson et al. (1993)	EM, serology
Watermelon mosaic virus (WMV-Vanilla necrosis strain)	*Vanilla planifolia*	Pearson et al. (1993)	EM, serology
Taro baciliform virus (TaBV)	*Colocasia esculenta*	Revill et al. (2005)	PCR
Taro vein chlorosis virus (TaVCV)	*Colocasia esculenta,*	Revill et al. (2005)	PCR
Taro reovirus (TaRV)	*Colocasia esculenta,*	Revill et al. (2005)	PCR

[A]The original or earliest available citation of a reliably verified record is provided.
[B]EM: Electron microscopy; PCR: polymerase chain reaction, following reverse transcription in the case of RNA viruses.

Table 3. Other records indicating possible presence of plant infecting viruses and virus-like pathogens in Samoa

Pathogen	Host	Citation	Identification method
Bean yellow mosaic virus (BYMV)	*Phaseolus vulgaris*	Thomas (1978)	Symptoms observed
Cucumber mosaic virus (CMV)	*Lycopersicon esculentum*	Thomas (1978)	Symptoms observed
Dasheen mosaic virus (DsMV)	*Alocasia macrorrhiza*	Van Velsen (1979)	EM[A]
--	*Caladium bicolor*	Van Velsen (1979)	EM[A]
--	*Xanthosoma sp.*	Van Velsen (1979)	EM[A]
Passionfruit woodiness virus (PWV)	*Passiflora edulis*	Gerlach (1988)	No diagnostic test methods stated
	Passiflora foetida	Gerlach (1988)	No diagnostic test methods stated
Presumed virus			
Citrus blind pocket/concave gum pathogen	*Citrus aurantifolia*	Thomas (1978)	Not available[B]
--	*Citrus limon*	Thomas (1978)	Not available[B]
--	*Citrus reticulata*	Thomas (1978)	Not available[B]

[A]Original report unavailable to cite, but observation of particles of correct size and shape by electron microscopy (EM) was the principal diagnostic method at that time. EM alone is not considered a definitive identification method unless combined with some other technique.
[B]It was impossible to cite the original survey reports (Thomas 1978; Van Velsen 1979) at the time of preparation of this technical paper.

Table 4. Plant virus and virus-like pathogen records from surveys of Samoa in 2003 and 2004

Host plant Family Genus, species	**Field collection number**	**Approximate location**	**Symptoms[A]**	**Pathogen[B]**
Cucurbitaceae				
Citrullus lanatus (watermelon)	3741	Tamumalala, Upolu	YOGM	ZYMV +m, CMV +m
Cucurbita maxima (pumpkin)	RID 3251	Siusega, Upolu	YOGM	PRSV, ZYMV +m
--	RID 3265	Lata, Savai'i	YOGM	PRSV, ZYMV
--	RID 3267	Salelologa, Sava'i	YOGM	PRSV, ZYMV +m
	RID 3271	Salelavalu, Savai'i	Severe YOGM	ZYMV , PRSV +m
	RID 3291	Saleimoa, Upolu	Mild YOGM	PRSV
	RID 3731	Falefa, Upolu	YOGM	ZYMV
	RID 3732	Falefa, Upolu	YOGM	ZYMV
	RID 3733	Falefa, Upolu	YOGM	ZYMV
	RID 3742	Tamumalala, Upolu	YOGM	ZYMV
	RID 3743	Tamumalala, Upolu	YOGM	ZYMV
Cucurbita maxima x *Curcurbita moschata* (squash)	RID 3749	Aleisa, Upolu	YOGM	ZYMV
Momordica charanta	RID 3282	Nu'u, Upolu	Slight YOGM	ZYMV +m
Fabaceae				
Crotalaria sp.	RID 3263	Lata, Savai'i	YOGM	WMV +m
Musaceae				
Musa sp. AAB (cv. Misiluki)	RID 3250	Suisega, Upolu	Chlorotic streaks plus brown black markings on some leaves	BSV -Mys
Musa sp. AAB (cv. Misiluki)	RID 3273	Nafanua, Upolu	Chlorotic streaks plus brown black markings on some leaves	BSV -Mys
Musa sp. AAB (cv. Misiluki)	RID 3710	Sataua, Savai'i	Chlorotic streaks plus brown black markings on some leaves	BSV
Orchidaceae				
Dendrobium sp. (Dendrobium)	RID 3745	Tamumalala, Upolu	Ringspots	CymMV
	RID 3751	Alafua, Upolu	Chlorotic spots	CymMV
Vanilla planifolia (vanilla)	RID 3758	Nu'u, Upolu	Indented chlorotic markings	Potyvirus
	RID 3759	Nu'u, Upolu	Chlorotic markings	Potyvirus
	RID 3762	Nu'u, Upolu	Chlorotic markings	Potyvirus
	RID 3763	Nu'u, Upolu	Chlorotic blotch	WVMV[C]
	RID 3764	Nu'u, Upolu	Strong white markings – mosaic-like, not indented	WVMV[C]
	RID 3765	Nu'u, Upolu	Indented chlorotic markings	WVMV[C]
Pandanaceae				
Pandanus tectorius	RID 3718	Asina, Savai'i	White leaf	Phytoplasma
Passifloraceae				
Passiflora edulis (passion fruit)	RID 3255	Falealupo, Savai'i	YOGM	PWV
Passiflora foetida	RID 3258	Asau, Savai'i	YOGM	PWV
	RID 3268	Salelologa, Savai'i	YOGM	PWV
	RID 3277	Nafanua, Upolu	YOGM	PWV
	RID 3288	Malaelu, Upolu	YOGM	PWV

Passiflora quadrangularis (granadilla)	RID 3266	Lata, Savai'i	YOGM	Potyvirus
Rutaceae				
Citrus x *aurantifolia* (lime)	RID 3724	Vaiola, Savai'i	Chlorotic blotch	CTV
Citrus x *limon* (lemon)	RID 3725	Vaipouli, Savai'i	Chlorotic blotch	CTV
	RID 3735	Sauniatu, Upolu	Chlorotic blotch	CTV
	RID 3705	Salailua, Savai'i	Chlorotic blotch and corky veins	CTV
	RID 3712	Lata, Savai'i	General chlorosis	CTV
Citrus reticulata (mandarin)	RID 3706	Faleaupo, Savai'i	Yellow on green vein banding	CTV
	RID 3736	Mulivai, Upolu	Chlorotic blotch	CTV
	RID 3711	North West Savai'i	Yellow on green vein banding and chlorosis	CTV

[A]WOGM: white on green mosaic; YOGM: yellow on green mosaic.
[B]Viruses were: BSV: *Banana streak virus*; CMV: *Cucumber mosaic virus*; CTV: *Citrus tristeza virus*; CymMV: *Cymbidium mosaic virus*; PRSV: *Papaya ringspot virus*; PWV: *Passion fruit woodiness virus*; WMV: *Watermelon mosaic virus*; WVMV: *Wisteria vein mosaic virus*; ZYMV: *Zucchini yellow mosaic virus*. Potyvirus: identified to genus only.
[C]Data also published in Grisoni et al. (2006).

BSV-Mys was detected in two banana samples using specific immunocapture polymerase chain reactions (PCRs) for a number of strains of BSV; BSV (no strain designation) was detected in one sample by real-time PCR; WVMV was detected by DNA sequence analysis following potyvirus-specific reverse transcription-PCR; the phytoplasma in *P. tectorus* was detected by nested PCR (but strain characterisation was not possible because amplification was weak); and the potyvirus identification in *P. quadrangularis* was achieved by electron microscopy only. CMV, CTV, CymMV, PRSV, PWV, ZYMV, potyvirus (in vanilla) were identified by enzyme linked immunosorbent assay (ELISA). ELISA test results were considered positive when A405 > 3 x mean of healthy controls (marginal positive results (+m) were those of A405>2 x mean but < 3x mean).

Table 5. Plant virus and virus-like pathogen records from a survey of Vanuatu, May/June 2003

Host plant Family Genus, species	**Field collection number**	**Approximate Location**	**Symptoms[A]**	**Pathogen[B]**
Araceae				
Colocasia esculenta (taro)	3379	Lovunivili, Ambae	Chlorotic veins	TaVCV
	3384	Job farm, Santo	Chlorotic veins	TaVCV
	3442	White Sands, Tanna	Chlorotic veins	TaVCV
	3451	Green Hill, Tanna	Feathery WOGM	DsMV +m
Xanthosoma sp. (taro)	3386	Natauwa, Santo	Feathery WOGM	DsMV +m
	3402	Rose Point, Santo	Feathery WOGM	DsMV
	3406	Mavunlevu, Santo	Feathery WOGM	DsMV +m
	3415	Malo Is., Santo	Feathery WOGM	DsMV +m
Asteraceae				
Synedrella nodiflora	3361	Pangpang, Efate	Strong YOGM	CMV
	3362	Pangpang, Efate	Diffuse YOGM	CMV
Convulvulaceae				
Ipomoea batatas (sweet potato)	3441	White Sands, Tanna	Little leaf and chlorosis	Phytoplasma in '*Ca*. P. aurantifolia' 16SrII group[C]
Ipomoea pes-caprae ssp. *brasiliensis*	3377	Saratamata, Ambae	Little leaf and chlorosis	Phytoplasma in '*Ca*. P. aurantifolia' 16SrII group[C]
Cucurbitaceae				
Citrullus lanatus (watermelon)	3373	Mele, Efate	YOGM	ZYMV +m
Cucurbita maxima (pumpkin)	3346	Port Vila, Efate	YOGM	ZYMV
	3354	Teuma, Efate	YOGM	ZYMV+m
	3365	Pag Pag, Efate	YOGM	ZYMV
	3371	Mele, Efate	Strong YOGM	ZYMV
	3372	Mele, Efate	Diffuse YOGM	ZYMV
	3429	Lambumbu, Malekula	Mild YOGM	PRSV, ZYMV
	3446	Mt Yasur, Tanna	Mild YOGM	PRSV +m, ZYMV +m
	3453	Green Hill, Tanna	Mild YOGM	ZYMV
	3467	Middle Bush, Tanna	Mild YOGM	ZYMV +m
	3468	Middle Bush, Tanna	Strong YOGM	ZYMV
Cucurbita maxima x moschata (squash)	3474	Rainbow Garden, Efate	Strong YOGM[D]	ZYMV
	3475	Rainbow Garden, Efate	Strong YOGM[D]	PRSV, ZYMV
Cucumis sativus (cucumber)	3352	Snake Hill, Efate	Strong YOGM	ZYMV +m
Fabaceae				
Vigna unguiculata ssp. *sesquipedalis* (snakebean)	3374	Mele, Efate	Strong YOGM	Potyvirus
Musaceae				
Musa sp. (ABB)	3456	Green Hill, Tanna	Yellow streaks on leaves	BSV

Passifloraceae				
Passiflora edulis (passionfruit)	3356	Teouma, Efate	YOGM	Potyvirus
Solanaceae				
Lycopersicon esculentum (tomato)	3414	Malo Is. Santo	Mild yellow on green mosaic	Potyvirus
Nicotiana tabacum (tobacco)	3450	Green Hill, Tanna	YOGM	CMV

[A]WOGM: White on green mosaic, YOGM: yellow on green mosaic
[B]Viruses were: BSV: *Banana streak virus*, CMV: *Cucumber mosaic virus, DsMV: Dasheen mosaic virus,* PRSV: *Papaya ringspot virus*, TaVCV: *Taro vein chlorosis virus*, ZYMV: *Zucchini yellow mosaic virus*. Potyvirus: identified to genus only.
[C]Data also published in Davis et al. (2006a).
[D]Incidence at this location was very high: most plants affected

TaVCV was detected by reverse transcription polymerase chain reaction (RT-PCR); phytoplasmas were detected by nested PCR, then identified by sequence analysis; BSV was detected by real-time PCR; and the potyvirus identification in *L. esculentum* was achieved by electron microscopy only. CMV, DsMV, PRSV, ZYMV, potyvirus (in two cases) were identified by ELISA. ELISA test results were considered positive when A405 > 3 x mean of healthy controls (marginal positive results (+m) were those of A405>2 x mean but < 3x mean).

Table 6. Notable samples from Samoa in which no pathogen was detected in specific tests

Host plant family, genus, species (common name)	Field collection number	Approximate location	Symptoms	Tested negative for
Rutaceae				
Citrus x *aurantifolia* (lime)	RID 3724	Vaiola, Savai'i	Chlorotic blotch	Huanglongbing (HLB[A]) disease
Citrus x *limon* (lemon)	RID 3704	Vaiafai, Savai'i	Chlorotic blotch	HLB[A]
	RID 3705	Salailua, Savai'i	Chlorotic blotch and corky veins	HLB[A]
	RID 3712	Lata, Savai'i	General chlorosis	HLB[B]
	RID 3725	Vaipouli, Savai'i	Chlorotic blotch	HLB[B]
	RID 3735	Sauniatu, Upolu	Chlorotic blotch	HLB[B]
Citrus reticulata (mandarin)	RID 3706	Faleaupo, Savai'i	Yellow on green vein banding	HLB[A]
	RID 3711	North West Savai'i	Yellow on green vein banding and chlorosis	HLB[B]
	RID 3736	Mulivai, Upolu	Chlorotic blotch	HLB[B]

[A]Tested by PCR for presence of '*Candidatus* Liberibacter asiaticus', data summarised in Davis et al. (2005).
[B]Tested by PCR for presence of '*Candidatus* Liberibacter asiaticus', data not previously published.

Table 7. Notable samples from Vanuatu in which no pathogen was detected in specific tests

Host plant Family Genus, species	Field collection number	Approximate Location	Symptoms	Details[A]
Fabaceae				
Arachis hypogaea	3410	Fanafo, Santo	Green on yellow blotch	NVD
	3448	Lenaken, Tanna	Green on yellow blotch	NVD
	3452	Green Hill, Tanna	Green on yellow blotch	NVD
Jatropha curcas	3426	Lambumbu, Malekula	Strong YOGM[B]	Potyvirus
Poaceae				
Zea mays	3412	Fanafo, Santo	Streaky mosaic and stunt	Maize mosaic virus (MMV) and maize stripe virus (MSpV)
	3420	Loone, Malekula	Streaky mosaic and leaf stripe	MMV and MSpV

[A]NVD: no virus was detected by electron microscopy, potyvirus was negative by ELISA, and MMV/MSpV negative tests were conducted using immunosorbent electron microscopy.
[B]YOGM: yellow on green mosaic.

FIGURES

Fig. 1. Vanilla planifolia (vanilla) leaves showing sunken white on green mosaic symptoms, caused by Wisteria vein mosaic virus (WVMV) in Samoa.

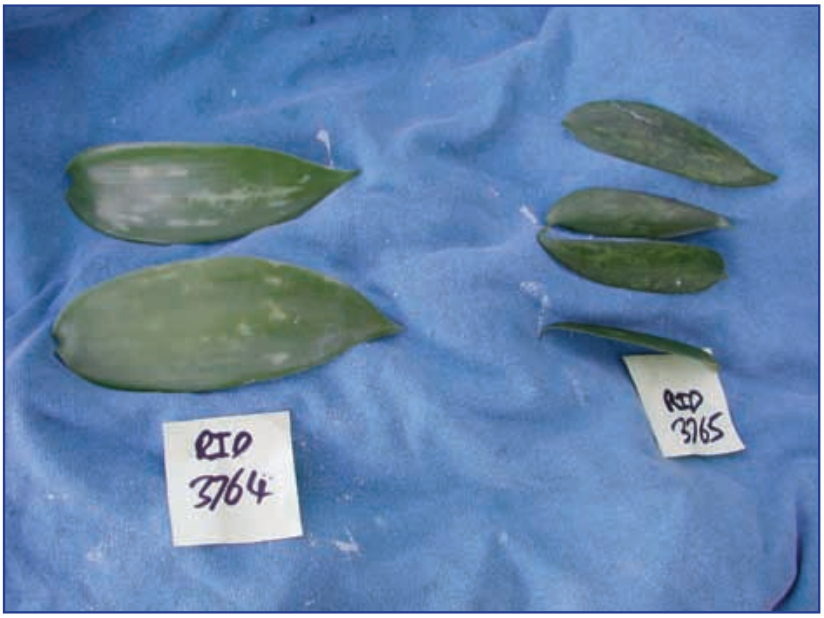

Fig. 2. RID 3764 and 3765: Vanilla planifolia (vanilla) infected with Wisteria vein mosaic virus (WVMV) in Samoa.

Fig. 3. RID 3255: Passiflora foetida infected with Passionfruit woodiness virus (PWV) in Samoa.

Fig. 4. RID 3263: Crotalaria sp. possibly infected (marginally positive ELISA test result obtained) with Watermelon mosaic virus (WMV) in Samoa.

Fig. 5. RID 3265: Cucurbita maxima (pumpkin) infected with Zucchini yellow mosaic virus (ZYMV) and Papaya ringspot virus (PRSV) in Samoa.

Fig. 6. RID 3271: Cucurbita maxima (pumpkin) infected with Zucchini yellow mosaic virus (ZYMV) and possibly also (marginally positive ELISA test result obtained) Papaya ringspot virus (PRSV) in Samoa.

Fig. 7. RID 3718: Pandanus tectorius (pandanus) showing white leaf symptoms in Samoa, in which an unknown phytoplasma was detected.

Fig. 8. RID 3731: Cucurbita maxima (pumpkin) infected with Zucchini yellow mosaic virus (ZYMV) in Samoa.

Fig. 9. RID 3732: Cucurbita maxima (pumpkin) infected with Zucchini yellow mosaic virus (ZYMV) in Samoa.

Fig. 10. RID 3733: Cucurbita maxima (pumpkin) infected with Zucchini yellow mosaic virus (ZYMV) in Samoa.

Fig. 11. RID 3361: Synedrella nodiflora infected with Cucumber mosaic virus (CMV) showing strong yellow on green mosaic symptoms in Vanuatu.

Fig. 12. RID 3377: Ipomoea pes caprae ssp. brasiliensis showing little-leaf symptoms in Vanuatu, in which a phytoplasma in the 'Candidatus Phytoplasma aurantifolia' (16SrII) group was detected. The little-leaf symptom is visible in the background, with normal sized leaves in the foreground.

Fig. 13. RID 3379: Colocasia esculenta (taro) infected with Taro vein chlorosis virus (TaVCV) in Vanuatu.

Fig. 14. RID 3456: Musa sp. (banana, ABB genotype) infected with Banana streak virus (BSV) in Vanuatu. Symptoms were atypical for banana streak disease as yellow streaking, rather than being patchy and occasional on only some leaves, was uniform across all leaves of all suckers and most leaves of the main plant. In addition, no black/brown markings were superimposed on the yellow streaks.

Fig. 15. RID 3468: Cucurbita maxima (pumpkin) infected with Zucchini yellow mosaic virus (ZYMV) in Vanuatu.

Fig. 16. Stand of Saccharum officinarum (sugarcane) showing symptoms similar to those of Fiji leaf gall disease, caused by Fiji disease virus (FDV) in Vanuatu.

Fig. 17. Close-up of leaf gall symptoms thought to be those of Fiji leaf gall disease in Saccharum officinarum (sugarcane) in Vanuatu.