

**Surveys for PLANT DISEASES  
caused by Viruses &  
Virus-like pathogens in the  
Federated States of Micronesia,  
the Republic of Palau and  
Rotuma, Republic of  
the Fiji Islands**



SPC Land Resources Division  
Suva, Fiji

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**the Fiji Islands**

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# ABSTRACT

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Surveys for virus and virus-like plant diseases were conducted in Palau, on the islands of Babeldaob and Koror; in the Federated States of Micronesia (FSM), on the island of Yap in the State of Yap, the islands of Weno and Fefan in the State of Chuuk, the island of Kosrae in the State of Kosrae and the island of Pohnpei in the State of Pohnpei; and on the island of Rotuma in the Republic of the Fiji Islands.

New plant virus records for Palau were those of *Dasheen mosaic virus* (DsMV) in taro (*Colocasia esculenta*), *Bean common mosaic virus* (BCMV) in snakebean (*Vigna unguiculata* ssp. *unguiculata*) and *Cymbidium mosaic virus* (CymMV) in orchids (*Dendrobium* sp.), all detected by enzyme-linked immunosorbent assay (ELISA). New plant virus records for FSM were of *Taro bacilliform virus* (TaBV) in taro (*Xanthosoma* sp.) detected by polymerase chain reaction (PCR) and *Cucumber mosaic virus* (CMV) in black pepper (*Piper nigrum*), detected by ELISA. New plant virus records for Rotuma were *Zucchini yellow mosaic virus* (ZYMV) in pumpkin (*Cucurbita maxima*) and the cucurbit weed, *Benincasa hispida*, DsMV in taro (*C. esculenta*), CymMV in orchids (*Dendrobium* sp.), all detected by ELISA; TaVCV in taro (*C. esculenta*), detected by reverse transcription polymerase chain reaction (RT-PCR); and *Banana streak virus* (BSV) in banana (*Musa* sp., cv. Mysore), detected by real time PCR. These surveys also provide the first published verification of both BSV and ZYMV in the Fiji Islands.

Phytoplasmas belonging to the ‘*Candidatus* Phytoplasma aurantifolia’ (16SrII) group were detected by nested PCR in *Ipomoea batatas* (sweet potato) plants showing little leaf symptoms in both Palau and FSM.

A new virus host record for FSM was one of DsMV in *Xanthosoma* sp., detected by ELISA. Other detections using ELISA were of ZYMV in pumpkin and cucumber (*Cucumis sativas*) in FSM, and of *Papaya ringspot virus* (PRSV) in pumpkin in Palau.

In surveys of both Palau and FSM, no evidence was found for presence of citrus huanglongbing, previously known as greening disease. Twelve citrus trees in FSM and seven in Palau indexed negative by PCR for the causal agent, ‘*Candidatus Liberibacter asiaticus*’.

# INTRODUCTION

Surveys were conducted to assess the general plant virus and virus-like disease status of some of the larger islands of the Republic of Palau and the Federated States of Micronesia (FSM), and the island of Rotuma in the Republic of the Fiji Islands. These islands have in common great geographical isolation, combined with a history of particularly close linkages with distant, larger economies. This might have exposed them to the introduction of exotic plant pests. Both Palau and FSM maintained a ‘Compact of Free Association’ with the USA for many years. Palau, due to its geographical location, now maintains close contact with certain Southeast Asian countries, especially the Philippines. Whilst Rotuma is part of the Fiji Islands, it is the most outlying of Fiji’s outer islands. Virus and virus-like plant disease status has been relatively poorly documented in these regions before. There are apparently no valid published records of plant virus disease test results from Rotuma. In Palau and FSM, a focused cucurbit virus survey (Wall et al. 2006) generated a number of records, and a focused taro virus disease survey (Revill et al. 2005) provided several more for FSM only. These are summarised in Table 1 (Palau) and Table 2 (FSM).

**Table 1. Verified plant virus records from Palau**

Pathogen	Host	Citation <sup>A</sup>	Identification method
<i>Papaya ringspot virus</i> (PRSV)	<i>Cucumis sativas</i>	Wall et al. (2006)	Serology
	<i>Cucurbita maxima</i>	Wall et al. (2006)	Serology
<i>Zucchini yellow mosaic virus</i> (ZYMV)	<i>Cucumis sativas</i>	Wall et al. (2006)	Serology
	<i>Cucurbita maxima</i>	Wall et al. (2006)	Serology

<sup>A</sup>The original or earliest available citation of a reliably verified record is provided.

**Table 2. Verified plant virus records from the Federated States of Micronesia**

Pathogen	Host	State	Citation <sup>A</sup>	Identification method
<i>Cucumber mosaic virus</i> (CMV)	<i>Citrus lanatus</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucumis melo</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucurbita maxima</i>	Pohnpei	Wall et al. (2006)	Serology
	<i>Cucurbita pepo</i> ssp. <i>pepo</i> var. <i>pepo</i>	Pohnpei	Wall et al. (2006)	Serology
<i>Dasheen mosaic virus</i> (DsMV)	<i>Colocasia esculenta</i>	Kosrae	Revill et al. (2005)	RT-PCR
	<i>Colocasia esculenta</i>	Pohnpei	Revill et al. (2005)	RT-PCR
<i>Papaya ringspot virus</i> (PRSV-W)	<i>Citrus lanatus</i>	Kosrae	Wall et al. (2006)	Serology
	<i>Cucumis melo</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucumis melo</i>	Pohnpei	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Pohnpei	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Kosrae	Wall et al. (2006)	Serology
	<i>Cucurbita moschata</i>	Chuuk	Wall et al. (2006)	Serology

<i>Taro vein chlorosis virus</i> (TaVVCV)	<i>Colocasia esculenta</i>	Kosrae	Revell et al. (2005)	RT-PCR
	<i>Colocasia esculenta</i>	Pohnpei	Revell et al. (2005)	RT-PCR
<i>Watermelon mosaic virus</i> (WMV)	<i>Cucumis sativas</i>	Kosrae	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Pohnpei	Wall et al. (2006)	Serology
<i>Zucchini yellow mosaic virus</i> (ZYMV)	<i>Citrulus lanatus</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Citrulus lanatus</i>	Kosrae	Wall et al. (2006)	Serology
	<i>Cucumis melo</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Kosrae	Wall et al. (2006)	Serology
	<i>Cucumis sativas</i>	Pohnpei	Wall et al. (2006)	Serology
	<i>Cucurbita maxima</i>	Pohnpei	Wall et al. (2006)	Serology
	<i>Cucurbita moschata</i>	Chuuk	Wall et al. (2006)	Serology
	<i>Cucurbita pepo</i> ssp. <i>pepo</i> var. <i>pepo</i>	Kosrae	Wall et al. (2006)	Serology
	<i>Cucurbita pepo</i> ssp. <i>pepo</i> var. <i>pepo</i>	Pohnpei	Wall et al. (2006)	Serology

<sup>A</sup>The original or earliest available citation of a reliably verified record is provided.

<sup>B</sup> RT-PCR: reverse transcription polymerase chain reaction.

Very little is known of the phytoplasma disease status of the Pacific islands. Phytoplasmas (formerly known as mycoplasma-like organisms) are unculturable bacteria found only in plant phloem vessels and in the phloem-feeding insects, mostly leafhoppers, that spread them from plant to plant. They have been associated with diseases of numerous plant species throughout the world (Seemüller et al. 1998) and up-to-date information on these pathogens and the diseases they are believed to cause in the Australian tropics can be found in Streten and Gibb (2006).

There is some question about the distribution of one of the worst diseases of citrus in the region. This is huanglongbing (HLB, formerly known as greening disease), caused by another phloem-limited bacterium, '*Candidatus Liberibacter asiaticus*'. HLB has been a problem for decades in certain Southeast Asian countries close to Palau and FSM. A report that this virus-like disease was also present in several Pacific island countries in the mid 1990s (Kiritani and Su 1999) included a record for Palau. This record is doubted by many HLB researchers because the detection method used in the study was not reliable. The disease recently reached two new locations of significance to Palau and FSM. These are the nearby island of New Guinea, where it was found in the Indonesian province of Papua in 1999 (Davis et al. 2000), then Papua New Guinea (PNG) in 2002 (Weinert et al. 2004) and in the US state of Florida (Gottwald 2006). Whilst further away, this is of significance because Palau and FSM import many goods from the USA. For these reasons, these surveys also focused on HLB.

The survey of Rotuma was conducted in May 2005 and those of Palau and FSM in August 2005.



# METHODS

## Surveys

To undertake the surveys, as many different areas as possible were visited. Crop plants of economic importance and, occasionally, also other plants, were examined at each location. When any kinds of symptoms similar to those caused by intracellular pathogens were suspected, samples were collected. Samples were returned for analyses after rapid desiccation in the field. Samples, consisting of 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. Each sample was dried over anhydrous calcium chloride (about 7 g) in sealed, 25 mL plastic vials. Samples were stored at 4°C until fully desiccated, and at -20°C thereafter. Samples were returned (under appropriate quarantine import permits) to two different laboratories for diagnostic tests.

In Palau, two days were spent on survey on the islands of Babeldaob and Koror. The survey of FSM consisted of two days spent on the island of Yap in Yap State, one day each spent on the islands of Weno and Fefan in Chuuk State, two days spent on the island of Kosrae in Kosrae State and three days on the island of Pohnpei in Pohnpei State. The survey of Rotuma was undertaken over five days.

### Enzyme-linked immunosorbent assay (ELISA) testing for viruses

Cucurbit samples from all surveys 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). Also from all surveys, aroid leaf samples (*Colocasia esculenta*, *Cyrtosperma chamissonis* and *Xanthosoma* sp.) were tested for *Dasheen mosaic virus* (DsMV) by DAS-ELISA, and citrus leaf samples were tested for *Citrus tristeza virus* (CTV) by compound direct ELISA. Orchid samples (all *Dendrobium* species) from Palau and Rotuma were tested by DAS-ELISA for CMV, *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV), and by indirect ELISA for the potyvirus group. Kava (*Piper methysticum*) and black pepper (*Piper nigrum*) leaf samples from Pohnpei and kava leaf samples from Rotuma were tested by DAS-ELISA for CMV. One snakebean (*Vigna unguiculata* ssp. *unguiculata*) sample from Palau and two kava samples from Pohnpei were tested by indirect ELISA for the potyvirus group, and the snakebean sample was also tested by indirect ELISA specifically for the potyvirus, *Bean common mosaic virus* (BCMV). Several banana leaf samples from FSM and Rotuma were tested for *Banana bunchy top virus* (BBTV), also by DAS-ELISA. All these tests were conducted at the SPC plant virology laboratory, Suva, Fiji Islands, using Agdia Inc. (Elkhart IN, USA) ELISA reagent sets. All ELISA test samples were considered positive when absorbance values exceeded three times the mean of appropriate healthy controls, which were included on each microtitre test plate.

### Reverse transcription polymerase chain reaction (RT-PCR) testing for viruses

Taro (*Colocasia esculenta*) and swamp taro (*Cyrtosperma chamissonis*) leaf samples from FSM and *C. esculenta* leaf samples from Palau and Rotuma were returned to the molecular biology laboratory of the Institute of Applied Sciences (IAS), University of the South Pacific (USP), Suva, Fiji Islands. Here, they were tested for *Taro vein chlorosis virus* (TaVCV) by RT-PCR using the methods described in Revill et al. (2005).

### Polymerase chain reaction (PCR) testing for Taro bacilliform virus (TaBV)

*Colocasia esculenta* and *Xanthosoma* sp. leaf samples were tested for TaBV by PCR at the USP IAS laboratory. A modification of the diagnostic test of Revill et al. (2005) was used. This modification was the use of the REDExtract-N-Amp Plant PCR kit (Sigma, USA) for DNA extraction and PCR. This was done according to the manufacturer's instructions, except for the addition of 1 mM MgCl<sub>2</sub> to the PCR reaction mix.

### Real-time PCR testing for viruses

Two banana leaf samples from Rotuma were subjected to a real-time PCR test for presence of *Banana streak virus* (BSV) at the Central Science Laboratory (CSL), York, UK.

### PCR testing for HLB

Citrus leaf material from Palau and FSM was tested for HLB at the USP IAS molecular biology laboratory using the PCR techniques described in Davis et al. (2005b). The PCR integrity of DNA extracts that tested negative for HLB was verified by amplifying 16S rDNA of other bacteria present in the preparations using the PCR primers rP1/fD1 (Weisburg et al. 1991)

### Phytoplasma testing

Samples from sweet potato (*Ipomoea batatas*) plants showing little leaf symptoms in Palau and FSM were subjected to nucleic acid extraction, followed by nested PCR and DNA sequence analysis at CSL, UK, as described in Davis et al. (2006a).

# RESULTS

The results of the survey of FSM are presented in Table 3, of Palau in Table 4 and of Rotuma in Table 5.

**Table 3. Plant virus and phytoplasma records from the Federated States of Micronesia, August 2005**

Host plant Family Genus, species	Field collection number	Approximate location	Symptoms <sup>A</sup>	Pathogen <sup>B</sup>
<b>Araceae</b>				
<i>Xanthosoma</i> sp. (taro)	4039	Dhol Pomi, Yap,	WOGM	DsMV
	4041	Dhol Pomi, Yap,	WOGM	DsMV
	4060	Weno, Chuuk,	WOGM, distortion	DsMV
	4057	Ruu Gagil, Yap	Feathery WOGM	TaBV
<b>Convolvulaceae</b>				
<i>Ipomoea batatas</i> (sweet potato)	4056	Ruu Gagil, Yap,	Little leaf	Phytoplasma in 'Ca. P. aurantifolia' (16SrII) group
	4058	Ruu Gagil, Yap,	Little leaf	Phytoplasma in 'Ca. P. aurantifolia' (16SrII) group
<b>Cucurbitaceae</b>				
<i>Cucurbita maxima</i> (pumpkin)	4033	Agric. Station, Yap	Mild YOGM	ZYMV
	4034	Agric. Station, Yap	Mild YOGM	ZYMV
	4043	Dhol Pomi, Yap	Patchy YOGM	ZYMV
	4044	Dhol Pomi, Yap	YOGM	ZYMV
	4045	Dhol Pomi, Yap	Diffuse YOGM	ZYMV
	4046	Dhol Pomi, Yap	Diffuse YOGM	ZYMV
	4062	Tunnuk, Weno, Chuuk	Very faint YOGM	ZYMV
	4063	Tunnuk, Weno, Chuuk	Very faint YOGM	ZYMV
<i>Cucumis sativas</i> (cucumber)	4072	Fefan, Chuuk	Strong YOGM	ZYMV
	4073	Fefan, Chuuk	Strong YOGM	ZYMV
	4074	Fefan, Chuuk	Strong YOGM	ZYMV
	4075	Fefan, Chuuk	Strong YOGM	ZYMV
	4103	Palikir, Pohnpei	None	ZYMV +m
<b>Piperaceae</b>				
<i>Piper nigrum</i> (black pepper)	4094	Mwatalenine, Pohnpei	WOGM	CMV

<sup>A</sup>WOGM, white on green mosaic; YOGM, yellow on green mosaic

<sup>B</sup>Viruses detected were: CMV, *Cucumber mosaic virus*; DsMV, *Dasheen mosaic virus*; TaBV, *Taro bacilliform virus*; ZYMV, *Zucchini yellow mosaic virus*.

Cucurbit leaf samples were screened by ELISA for the five most common cucurbit-infecting viruses (*Papaya ringspot virus*, *Zucchini yellow mosaic virus*, *Watermelon mosaic virus*, CMV and *Squash mosaic virus*). CMV and DsMV were detected by ELISA. Phytoplasmas were detected by nested PCR and identified by sequence analysis.

ELISA test results were considered positive (+) when absorbance readings (405 nm) exceeded three times the mean of healthy controls. Marginally positive test results (+m) were those that exceeded twice the mean of the healthy controls, but were less than three times the mean.

**Table 4. Plant virus and phytoplasma records from Palau, August 2005**

Host plant Family Genus, species	Field collection number	Approximate location	Symptoms <sup>A</sup>	Pathogen <sup>B</sup>
<b>Araceae</b>				
<i>Colocasia esculenta</i> (taro)	4006	Ngerceheiong, Babaldaob	WOGM	DsMV
	4007	Ngerceheiong, Babaldaob	WOGM	DsMV
	4030	Koror	WOGM	DsMV
<b>Convolvulaceae</b>				
<i>Ipomoea batatas</i> (sweet potato)	4021	Aimeliki, Babeldaob	Little leaf	Phytoplasma in 'Ca. P. aurantifolia' (16SrII) group
<b>Cucurbitaceae</b>				
<i>Cucurbita maxima</i> (pumpkin)	4018	Aimeliki, Babeldaob	Faint chlorotic blotch	PRSV
	4020	Aimeliki, Babeldaob	Chlorotic blotch	PRSV
<i>Momordica charantica</i> (bitter gourd)	4028	Aimeliki, Babeldaob	YOGM	WMV +m
<b>Fabaceae</b>				
<i>Vigna unguiculata</i> ssp. <i>sesquipedalis</i> (snake bean)	4029	Aimeliki, Babeldaob	Mild YOGM	BCMV
<b>Orchidaceae</b>				
<i>Dendrobium</i> sp.	4024	Aimeliki, Babeldaob	Ringspots	CymMV
	4025	Aimeliki, Babeldaob	Chlorotic spots	CymMV
	4026	Aimeliki, Babeldaob	Chlorotic spots	CymMV

<sup>A</sup>WOGM, White on green mosaic; YOGM, yellow on green mosaic

<sup>B</sup>Viruses detected were: BCMV, *Bean common mosaic virus*; CMV, *Cucumber mosaic virus*; DsMV, *Dasheen mosaic virus*; PRSV, *Papaya ringspot virus*; WMV, *Watermelon mosaic virus*; ZYMV, *Zucchini yellow mosaic virus*.

Cucurbit leaf samples were screened by ELISA for the five most common cucurbit-infecting viruses (*Papaya ringspot virus*, *Zucchini yellow mosaic virus*, *Watermelon mosaic virus*, CMV and *Squash mosaic virus*). The snake bean leaf sample was screened for BCMV (genus *Potyvirus*) and the potyvirus group by ELISA, and was positive in both tests. Orchid samples were screened by ELISA for *Cymbidium mosaic virus* (CymMV), *Odontoglossum ringspot virus* (ORSV), BCMV, CMV, and the potyvirus group. Phytoplasmas were detected by nested PCR and identified by sequence analysis.

ELISA test results were considered positive (+) when absorbance readings (405 nm) exceeded three times the mean of healthy controls. Marginally positive test results (+m) were those that exceeded twice the mean of the healthy controls, but were less than three times the mean.

**Table 5. Plant virus records from Rotuma, May 2005**

Host plant Family Genus, species	Field collection number	Approximate location	Symptoms <sup>A</sup>	Pathogen <sup>B</sup>
<b>Asteraceae</b>				
<i>Synedrella nodiflora</i>	3961	Gesta	YOGM	ZYMV +m
<i>Mikania micrantha</i>	3945	Hafafa	None	ZYMV +m
<b>Araceae</b>				
<i>Colocasia esculenta</i> (taro)	3941	Luta	WOGM	DsMV
	3950	Tapana	WOGM	DsMV +m
	3940	Luta	Chlorotic and necrotic veins	TaVCV
	3971	Pepsei	Chlorotic veins	TaVCV
<b>Cucurbitaceae</b>				
<i>Benincasa hispida</i>	3956	Malahaha	YOGM	ZYMV
	3957	Malahaha	YOGM	ZYMV
	3963	Gesta	YOGM	ZYMV
	3964	Gesta	YOGM	ZYMV
	3966	Gesta	YOGM	ZYMV
	3973	Pepsei	YOGM	ZYMV +m
<i>Cucurbita maxima</i> (pumpkin)	3946	Savaea	YOGM	ZYMV
	3967	Gesta	YOGM	ZYMV +m
	3968	Gesta	YOGM	ZYMV
	3969	Gesta	YOGM	ZYMV
	3977	Juju	YOGM	ZYMV
<b>Musaceae</b>				
<i>Musa</i> sp. (Mysore AAB)	3955	Malahaha	Chlorotic streaks plus brown black markings	BSV
<i>Musa</i> sp. (Mysore AAB)	3979	Lopta	Chlorotic streaks plus brown black markings	BSV
<b>Orchidaceae</b>				
<i>Dendrobium</i> sp.	3954	Mojito's GH	Chlorotic blotch	CymMV
	3981	Town	Slight chlorotic blotch	CymMV

<sup>A</sup>WOGM, white on green mosaic; YOGM, yellow on green mosaic

<sup>B</sup>Viruses detected were: BSV, *Banana streak virus*; CymMV, *Cymbidium mosaic virus*; DsMV, *Dasheen mosaic virus*; TaVCV, *Taro vein chlorosis virus*; ZYMV, *Zucchini yellow mosaic virus*,

Cucurbit leaf samples were screened by ELISA for the five most common cucurbit-infecting viruses (*Papaya ringspot virus*, *Zucchini yellow mosaic virus*, *Watermelon mosaic virus*, *Cucumber mosaic virus* (CMV) and *Squash mosaic virus*). Orchid samples were screened by ELISA for CymMV, *Odontoglossum ringspot virus*, CMV, and the potyvirus group. ELISA test results were considered positive when absorbance readings (405 nm) exceeded three times the mean of healthy controls. Marginally positive test results (+m) were those that exceeded twice the mean of the healthy controls, but were less than three times the mean.

TaVCV was detected by reverse transcription polymerase chain reaction (RT-PCR) and BSV was detected by real-time PCR.

### *Viruses in FSM*

Of the five viruses screened for by DAS-ELISA in cucurbit samples from FSM, ZYMV was detected in *Cucurbita maxima* (pumpkin) at two locations in Yap and one location in Chuuk, and in *Cucumis sativas* (cucumber) at one location in Chuuk. DsMV was detected in *Xanthosoma sp.* by DAS-ELISA in both Yap and Chuuk, at one location each and TaBV was detected in *Xanthosoma sp.* at one location in Yap. CMV was detected in one *Piper nigrum* (black pepper) plant on Pohnpei.

### *Viruses in Palau*

Cucurbit virus testing by DAS-ELISA of leaf samples from Palau gave records of PRSV in pumpkin at one location and one marginally positive result for WMV in a *Momordica charantia* (bitter melon) leaf sample. DsMV DAS-ELISA testing detected this virus in *C. esculenta* (taro) at two locations. One *V. unguiculata* ssp. *unguiculata* (snakebean) leaf sample from Palau tested positive to BCMV and also the potyvirus group, by ELISA. In ELISA tests on orchid leaf samples from Palau, CymMV was detected in three *Dendrobium* species at one location.

### *Viruses on Rotuma*

Cucurbit virus testing by DAS-ELISA of leaf samples from Rotuma detected only ZYMV. Four pumpkin plants at two locations plus five *Benincasa hispida* plants (a cucurbit weed) at two locations gave positive test results. Each of these species also returned one marginally positive test result. Marginally positive ZYMV test results were also obtained when two weed species, mile-a-minute (*Mikania micrantha*) and *Synedrella nodiflora* were included in the screening. DsMV DAS-ELISA testing detected this virus in *C. esculenta* (taro) at one location plus a marginally positive result at a second location. In ELISA tests on orchid samples, CymMV was detected in two *Dendrobium sp.* leaf samples at two locations. TaVVCV was detected by RT-PCR in *C. esculenta* from two locations and BSV was detected by real time PCR in banana leaf samples from two locations.

### *Phytoplasmas in FSM and Palau*

Phytoplasmas belonging to the ‘*Candidatus Phytoplasma aurantifolia*’ (16SrII) group were associated with sweet potato plants showing little leaf symptoms at one location each in Yap State, FSM and in Palau.

### *Important negative results*

HLB indexing returned negative PCR test results from five citrus leaf samples from Kosrae, three from Chuuk, and two each from Pohnpei and Yap in FSM (Table 6), and from seven citrus leaf samples from Palau (Table 7). Three banana (*Musa sp.*) leaf samples from Pohnpei and two from Kosrae in FSM, plus three from Rotuma (Table 8) tested negative by ELISA for BBTV. Three of the banana plants in FSM and two on Rotuma were showing an unusual, upright growth habit. A number of aroid leaf samples showing various faint feathery mosaic symptoms from all four states in FSM tested negative for both DsMV and potyvirus by DAS-ELISA (Table 6). Negative results for TaVVCV by RT-PCR were obtained for two *C. esculenta* each from Palau and Chuuk in FSM, plus two *C. chamionissis* and one *C. esculenta* from Yap in FSM (Tables 6 and 7). Kava leaf samples from both Pohnpei and Rotuma tested negative for CMV by ELISA (Table 6 and Table 8).

**Table 6. Notable samples in which no pathogen was detected in specific tests in the Federated States of Micronesia**

Host plant Family Genus, species	Field collection number	Approximate location	Symptoms <sup>A</sup>	Tested negative for <sup>B</sup>
<b>Araceae</b>				
<i>Colocasia esculenta</i> (taro)	4040	Dhol Pomi, Yap	Feathery WOGM	DsMV, Poty, CMV
	4088	Tafunsak, Kosrae	Faint YOGM	DsMV, Poty, CMV
	4089	Tafunsak, Kosrae	Faint YOGM	DsMV, Poty, CMV
	4105	Palikir, Pohnpei	Faint feathery YOGM	DsMV, Poty, CMV
	4064	Nepukus, Weno, Chuuk	Chlorotic veins	TaVCV
	4071	Fefan, Chuuk	Chlorotic veins	TaVCV
	4054	Malai, Yap	Chlorotic veins	TaVCV
	4060	Weno, Chuuk	WOGM, distortion	TaBV
<i>Cyrtosperma chamissonis</i> (swamp taro)	4036	Arngel, Yap	Faint, feathery YOGM	DsMV, Poty, CMV
	4048	Dhol Pomi, Yap	YOGM	DsMV, Poty, CMV
	4050	Ngarly, Yap	Feathery WOGM	DsMV, Poty, CMV
	4068	Fefan, Chuuk	Feathery YOGM	DsMV, Poty, CMV
	4095	Mwatalenine, Pohnpei	Faint YOGM	DsMV, Poty, CMV
	4051	Ngariy Yap	Chlorotic veins	TaVCV
	4052	Ngariy Yap	Chlorotic veins	TaVCV
	<i>Xanthosoma</i> sp. (taro)	4057	Ruu Cagil, Yap	Feathery WOGM
4039		Dhol Pomi, Yap	WOGM	TaBV
4041		Dhol Pomi, Yap	WOGM	TaBV
<b>Musaceae</b>				
<i>Musa</i> sp. (AAA)	4091	Mwatalenine, Pohnpei	Upright posture	BBTV
<i>Musa</i> sp. cv. Mysore (AAB)	4085	Tafunsak, Kosrae	Upright posture	BBTV
	4099	Mwatalenine, Pohnpei	BSV-like symptoms only	BBTV
	4102	Kolonia, Pohnpei	BSV-like symptoms only	BBTV
<i>Musa</i> sp. (AAB)	4077	Malem, Kosrae	Upright posture	BBTV
<b>Piperaceae</b>				
<i>Piper methysticum</i> (kava)	4100	U, Pohnpei	Marginal chlorosis	CMV, Poty
	4101	U, Pohnpei	Marginal chlorosis	CMV, Poty
<b>Rutaceae</b>				
<i>Citrus × aurantifolia</i> (lime)	4083	Island Cafe, Kosrae	Chlorosis and corky veins	HLB <sup>B</sup> , CTV
	4087	Tafunsak, Kosrae	Chlorotic blotch and prominent veins	HLB <sup>B</sup> , CTV
<i>Citrus × limon</i> (lemon)	4081	Walung, Kosrae	Chlorotic blotch	HLB <sup>B</sup> , CTV
	4114	Likinlohng, Pohnpei	Chlorotic blotch	HLB <sup>B</sup> , CTV
<i>Citrus × microcarpa</i> (calamondin)	4115	FSM college, Pohnpei	GOYVB	HLB <sup>B</sup> , CTV
<i>Citrus japonica</i> (kumquat)	4049	Dhol Pomi, Yap	Chlorotic blotch	HLB <sup>B</sup> , CTV

	4067	Fefan, Chuuk	Chlorotic blotch	HLB <sup>B</sup> , CTV
	4070	Fefan, Chuuk	Chlorosis and corky veins	HLB <sup>B</sup> , CTV
<i>Citrus reticulata</i> (mandarin)	4055	Amun Cagil Yap	Chlorotic blotch and corky veins	HLB <sup>B</sup> , CTV
	4061	Sapuk, Weno, Chuuk	Chlorosis and corky veins	HLB <sup>B</sup> , CTV
	4082	Utwe, Kosrae	Chlorotic blotch and corky veins	HLB <sup>B</sup> , CTV
	4084	Tafunsak, Kosrae	Chlorotic blotch and prominent veins	HLB <sup>B</sup> , CTV

<sup>A</sup>GOYVB, green on yellow vein banding; WOGM, white on green mosaic; YOGM, yellow on green mosaic

<sup>B</sup>Aroid leaf samples were tested by RT-PCR for *Taro vein chlorosis virus* (TaVVCV) or by ELISA for *Dasheen mosaic virus* (DsMV), *Cucumber mosaic virus* (CMV) and the potyvirus group (Poty). Banana leaf samples were tested by ELISA for *Banana bunchy top virus* (BBTV). Citrus samples were tested by polymerase chain reaction for presence of '*Candidatus Liberibacter asiaticus*', the cause of HLB, and by ELISA for *Citrus tristeza virus* (CTV).

**Table 7. Notable samples in which no pathogen was detected in specific tests in Palau**

Host plant Family Genus, species	Field collection number	Approximate location	Symptoms <sup>A</sup>	Tested negative for <sup>B</sup>
<b>Araceae</b>				
<i>Colocasia esculenta</i>	3998	Airai, Babeldaob	Chlorotic veins	TaVVCV
	3999	Airai, Babeldaob	Chlorotic veins	TaVVCV
<b>Rutaceae</b>				
<i>Citrus japonica</i> (kumquat)	4001	Airai, Babeldaob	GOYVB and chlorotic blotch	HLB, CTV
	4004	Ngchesar, Babeldaob	Chlorotic blotch	HLB, CTV
	4005	Ngiwal, Babeldaob	GOYVB and chlorotic blotch	HLB, CTV
<i>Citrus reticulata</i> (mandarin)	4008	Korore	GOYVB and chlorotic blotch	HLB, CTV
<i>Citrus × aurantium</i> (orange)	4009	Korore	GOYVB	HLB, CTV
	4016	Lekken, Babeldaob	Chlorotic blotch and corky veins	HLB, CTV
	4017	Lekken, Babeldaob	Chlorotic blotch and corky veins	HLB, CTV

<sup>A</sup>GOYVB, green on yellow vein banding

<sup>B</sup>Aroid leaf samples were tested by RT-PCR for *Taro vein chlorosis virus* (TaVVCV). Citrus samples were tested by polymerase chain reaction for presence of '*Candidatus Liberibacter asiaticus*', the cause of HLB and by ELISA for *Citrus tristeza virus* (CTV).

**Table 8. Notable samples in which no pathogen was detected in specific tests in Rotuma**

Host plant Family Genus, species	Field collection number	Approximate location	Symptoms	Pathogen <sup>A</sup>
<b>Musaceae</b>				
<i>Musa</i> sp. (AAA)	3943	Hafafa	Upright sucker habit	BBTV
<i>Musa</i> sp. (ABB)	3980	Lopta	Slight choke throat	BBTV
<i>Musa</i> sp. (AAA)	3982	Lumao	Upright habit	BBTV
<b>Piperaceae</b>				
<i>Piper methysticum</i> (kava)	3959	Gesta	Crinkle	CMV
	3960	Gesta	Crinkle	CMV

<sup>B</sup>Banana leaf samples were tested by ELISA for *Banana bunchy top virus* (BBTV). Kava leaf samples were tested by ELISA for *Cucumber mosaic virus* (CMV)

Figures 1–11 show the symptoms caused by the viruses and phytoplasmas found in a number of hosts.

# DISCUSSION

These surveys provide the first records of DsMV, BCMV, and CymMV in Palau; TaBV and CMV in FSM; and DsMV, TaVCV, ZYMV, CymMV and BSV on Rotuma. This is also the first time that a phytoplasma-associated disease has been verified in both Palau and FSM. Additionally, the survey of FSM records, for the first time, DsMV in *Xanthosoma* sp., and lists more records of ZYMV in pumpkin and cucumber, and of PRSV in pumpkin in Palau. Surprisingly, the BSV and ZYMV records are the first published for these viruses in the Fiji Islands. Both viruses are generally regarded as common in Fiji and had been verified in ELISA testing of leaf samples from plants growing near Suva (SPC and Fiji College of Agriculture, unpublished data).

The viruses causing mosaic diseases in cucurbits in these surveys were the potyviruses ZYMV in FSM and Rotuma and PRSV-W, the cucurbit-infecting strain of PRSV, in Palau. This virus is different, but very closely related to the papaya-infecting strain, PRSV-P, which causes one of the worst diseases of papaya on certain other islands of the Pacific. The survey of Wall et al. (2006) also found these potyviruses in both FSM and Palau, as well as the potyvirus WMV at a lower incidence in FSM only. Members of the genus *Potyvirus* share some characteristics of significance when considering control. They cause systemic infections, meaning that infected plants cannot be cured with any spray treatment or by removing parts of the plant showing symptoms. They also cannot survive in the soil or in decayed plant material. These potyviruses are all spread non-persistently from plant to plant by many different species of aphid vectors. This means they are picked up from an infected plant in a few seconds, then held on the insect's mouthparts for several hours and can be transmitted to another plant during brief feeding probes. In this way, aphids move from crop host to crop host and spread the virus within the crop. They can also introduce virus from weed hosts 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. Older susceptible crops and wild susceptible crop plants can be significant reservoirs of potyvirus inoculum for new plantings. For this reason, the incidence of mosaic disease in cucurbit crops tends to be higher on more intensively cropped islands.

Certain weeds can also be important initial inoculum sources. Natural hosts of PRSV-W and ZYMV are mostly in the Cucurbitaceae (see: <<http://image.fs.uidaho.edu/vide/sppindex.htm#S>>). Importantly, these surveys suggest that the mile-a-minute and *S. nodiflora* weeds may be worth further investigation as alternative hosts of ZYMV, as leaf samples of each from Rotuma returned marginally positive ELISA test results. Other weeds found on Pacific islands that returned marginally positive ELISA test results for ZYMV include *Desmodium* sp. and *Macroptilium atropurpureum* in Tonga (Davis et al. 2006d) and *Momordica charantia* in Samoa (Davis et al. 2006b). Alternatively, it may be that the ZYMV antiserum is not specific enough and other potyviruses are being detected. There is no indication from the manufacturers of the antiserum that such cross reactivity has been encountered before, however (see: <[http://www.agdia.com/cgi\\_bin/catalog.cgi/77700](http://www.agdia.com/cgi_bin/catalog.cgi/77700)>).

Of the cucurbit-infecting viruses, ZYMV was found to be the most prevalent on the surveys reported here and this has been the case on earlier surveys of Tonga and New Caledonia (Davis et al. 2006d), as well as Vanuatu and Samoa (Davis et al. 2006b). ZYMV has spread throughout the world in recent years, and it is speculated that this long distance spread may have been via infected seeds (Desbiez and Lecoq 1997). 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 New Zealand (Burgmans and Fletcher 2000; Fletcher et al. 2000). In contrast, PRSV-W is not thought to be seed transmitted. The best method to combat these viruses is to use resistant or tolerant cultivars, which are available for several cucurbit crops.

During the survey of Rotuma, BSV-like symptoms were fairly common in banana cv. Mysore, and virus presence was confirmed by real-time PCR in representative leaf samples from two plants. In Australia, Queensland strains of this virus, including the one usually infecting cv. Mysore (BSV-Mys), cause yield losses of only 7–15% (Daniells et al. 1999). The incidence of banana streak disease can be kept low if planting material is selected carefully. This is because most virus transmission occurs through infected planting material and plant-to-plant spread by mealybug vectors is not great (Lockhart and Jones 1999). It is worth noting that very convincing BSV-like symptoms were also found on many cv. Mysore plants during the survey of FSM. One banana leaf sample from Yap, two from Chuuk, two from Kosrae and three from Pohnpei were collected because they were showing such symptoms. However, they were tested in a different multiplex PCR at CSL, UK, and all gave negative results.

The phytoplasmas implicated in sweet potato little leaf disease in both FSM and Palau are members of the '*Ca. Phytoplasma aurantifolia*' (16SrII) group. Members of this same group are also associated with sweet potato little leaf disease in Australia (Gibb et al. 1995, Davis et al. 1997; Schneider et al. 1999; Davis et al. 2003), the Island of New Guinea (Davis et al. 2003) and Tonga, New Caledonia and Vanuatu (Davis et al. 2006a). Importantly the '*Ca. Phytoplasma aurantifolia*' (16SrII) group



phytoplasmas found on other Pacific islands (Davis et al. 2006a,c) are unrelated to some of the principal phytoplasma quarantine threats to the Pacific region. Perhaps ranking highest amongst these are the phytoplasmas associated with lethal diseases of coconuts belonging to the Coconut lethal yellowing (16SrIV) group. This group includes the phytoplasma associated with coconut lethal yellowing disease tentatively named '*Candidatus Phytoplasma palmae*' and phytoplasmas associated with similar diseases of coconut on the African continent, tentatively named '*Candidatus Phytoplasma cocostanzaniae*' and '*Candidatus Phytoplasma cocosnigeriae*' (IRPCM 2004). Coconut lethal yellowing disease has devastated coconut populations in Central America and the Caribbean (Bourdeix et al. 2004), resulting in a significant economic impact on tourism and other industries. The phytoplasma findings in Palau and FSM, like those made on other islands before, reinforce the importance of maintaining a high level of quarantine vigilance in the Pacific region.

These surveys provide some useful negative qualitative data on certain diseases of concern in the region. For example, there was no testing done for viruses, viroids or phytoplasmas on betel nuts or coconuts, because no disease symptoms were found. In addition, the surveys of FSM and Palau provide further quantitative data that support the widely held belief that HLB is not present in the Pacific islands east of New Guinea. The negative citrus HLB screening results reported here add to the body of evidence published by Davis et al. (2005c, 2006b,c,d) who targeted citrus trees for testing because of their disease-like symptom expression but, using molecular methods, indexed them negative to HLB.

It was surprising that every HLB leaf sample from FSM and Palau also tested negative for tristeza disease by ELISA. The CTV ELISA test was repeated with a different antisera reagent set sometime after the first negative test was undertaken and the same result was obtained. Citrus tristeza disease is exceptionally widespread across citrus growing areas of the world (see: <http://image.fs.uidaho.edu/vide/descr222.htm>). In contrast to these results, CTV was readily detected in similar samples collected for HLB screening from other islands in the Pacific (Davis et al. 2005c, 2006b,c). A wider CTV-focused survey is needed to confirm if these islands are free of tristeza disease.

BBTV is a virus of extreme quarantine concern in the Pacific. This is because its distribution is patchy: it is present on some islands but absent from others. By late 2006, laboratory test records confirming the presence of BBTV in the Fiji Islands, Tonga, Samoa (Karan et al. 1994), New Caledonia (Kagy et al. 2001) and Wallis Island in the French Territory of Wallis and Futuna (Davis et al. 2005a) had been published. There are also unpublished laboratory test records of BBTV in Guam, plus reliable reports, collected over 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 all three surveys and no bunchy-top-like symptoms were seen. The nearest things found to such symptoms were in banana plants on Rotuma and in FSM. These were one plant in Pohnpei State and two in Kosrae State, plus three on Rotuma, showing an unusual growth habit. Samples were collected and included in the BBTV screening that followed and all tested negative. BBTV moves most readily from island to island in infected planting material. For Palau and FSM, perhaps the most immediate quarantine threat would be posed by illegal importation of banana planting material from Guam. As Rotuma has strong links with the other islands of Fiji and no inter-island quarantine restrictions operate, the apparent absence of bunchy top disease may be an important domestic quarantine concern for Fiji. If BBTV is indeed not on Rotuma, movement of banana planting material (suckers) from any other island in the Fiji group to Rotuma should be prevented.

In the taro virus survey of Revill et al. (2005), TaVCV and DsMV were both found to be widespread across the Pacific. DsMV was found in every country and territory visited, while TaVCV was more unevenly distributed, occurring on just over half of the countries / territories surveyed. Results of the surveys reported here add Palau and Rotuma and the FSM States of Yap and Chuuk to the DsMV distribution map. However, the surveys reported here failed to find convincing DsMV-like symptoms or to detect the virus in either Pohnpei or Kosrae, the two States in FSM where the virus was detected by Revill et al. (2005). TaVCV symptoms were seen often on Rotuma, and virus presence there was confirmed. In contrast, leaf samples collected because they were showing chlorotic veins in Palau and FSM tested negative for TaVCV. RNA was extracted from all of these leaf samples and RT-PCR testing was conducted as a single batch, suggesting the failure to detect was not an experimental error. Whilst the importance of TaBV and 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).

Kava dieback disease, which is caused by CMV in combination with other factors (Davis et al. 1996), is the worst production problem for this crop in the Pacific. The surveys of both Pohnpei and Rotuma suggest an absence of dieback. This may be an important observation, as kava is a major commodity on both islands. No symptoms of kava dieback disease or leaf symptoms typical of infection by CMV (yellow on green mosaic and leaf distortions) were found on any kava plants examined on Pohnpei or Rotuma. This conclusion was supported by ELISA screening of leaf samples from both islands that showed symptoms slightly similar to those of CMV infection.

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# FIGURES



**Fig. 1. RID 4021:** *Ipomoea batatas* (sweet potato) showing little leaf symptoms in Palau, in which a phytoplasma in the ‘Candidatus Phytoplasma aurantifolia’ (16SrII) group was detected. Normal sized leaves can be seen on other plants towards the edges of the photograph.



**Fig. 2. RID 4024:** Orchid (*Dendrobium* sp.) infected with *Cymbidium mosaic virus* (CymMV), showing blotchy ringspot symptoms in Palau.



**Fig. 3. RID 4056:** *Ipomoea batatas* (sweet potato) showing little leaf symptoms in Yap, FSM, in which a phytoplasma in the ‘Candidatus Phytoplasma aurantifolia’ (16SrII) group was detected. Normal sized leaves can be seen on the right.



**Fig. 4. RID 4058:** Field view of an *Ipomoea batatas* (sweet potato) plant showing little leaf symptoms in Yap, FSM amongst normal plants. A phytoplasma in the '*Candidatus Phytoplasma aurantifolia*' (16SrII) group was detected.



**Fig. 5. RID 4072:** *Cucumis sativas* (cucumber) infected with *Zucchini yellow mosaic virus* (ZYMV) in Chuuk, FSM.



**Fig. 6. RID 4074:** *Cucumis sativas* (cucumber) infected with *Zucchini yellow mosaic virus* (ZYMV) in Chuuk, FSM.



**Fig. 7. RID 3940:** *Colocasia esculenta* (taro) infected with *Taro vein chlorosis virus* (TaVCV) on Rotuma.



**Fig. 8. RID 3956:** The cucurbit weed, *Benincasa hispida* infected with *Zucchini yellow mosaic virus* (ZYMV) on Rotuma.



**Fig. 9. RID 3957:** The cucurbit weed, *Benincasa hispida* infected with *Zucchini yellow mosaic virus* (ZYMV) on Rotuma.



**Fig. 10. RID 3979:** *Musa* sp. (banana, cv. Mysore) infected with *Banana streak virus* on Rotuma.



**Fig. 11. RID 3971:** *Colocasia esculenta* (taro) infected with *Taro vein chlorosis virus* (TaVCV) on Rotuma. Chlorotic vein symptoms are visible on the leaf on the right only.

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