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SURVEYS FOR PLANT DISEASES CAUSED BY VIRUSES and VIRUS-LIKE PATHOGENS

IN THE COOK ISLANDS



SPC Land Resources Division Suva, Fiji

SURVEYS FOR PLANT DISEASES CAUSED BY VIRUSES and VIRUS-LIKE PATHOGENS IN THE

COOK ISLANDS

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ABSTRACT

Warm and cool-season surveys for plant infecting virus diseases were conducted on the islands of Rarotonga and Aitutaki in the Cook Islands and one warm-season survey on the island of Mangaia. The first records of any banana (*Musa* sp.) infecting virus in the Cook Islands are reported. These were two strains of *Banana streak virus* (BSV-Mys and BSV-Onne), detected by specific immunocapture polymerase chain reaction (PCR), and *Cucumber mosaic virus* (CMV) detected by enzyme linked immunosorbent assay (ELISA). The first detection (by reverse transcription PCR (RT-PCR)) of CMV in kava (*Piper methysticum*) affected by kava dieback disease in the Cook Islands is reported. Most other records were of *Cucumber mosaic virus* (detected in five cucurbit species and in *Commelina diffusa* by ELISA) and of the cucurbit infecting strain of *Papaya ringspot virus* (detected in seven cucurbit species by ELISA). Other viruses detected by ELISA were *Potato virus Y* in tomato (*Lycopersicon esculentum*) and *Citrus tristeza virus* in lime and mandarin trees. In both *Colocasia esculenta* and *Xanthosoma* sp, *Taro bacilliform virus* was detected by PCR and *Dasheen mosaic virus* was detected by RT- PCR. No evidence was found for presence of *Tomato spotted wilt virus* or any other serogroup I tospovirus (ten samples tested by ELISA), or for presence of citrus huanglongbing, previously known as greening disease (five samples tested by PCR).

Introduction

General surveys for virus diseases of plants have not been conducted in the Cook Islands since the late 1970s, as reported by Mossop and Fry (1984). The listing provided by those authors also included previous plant virus records from the Cook Islands. These viruses were identified by serological tests, host range studies, or by electron microscopy (EM). Of these methods, only serology can provide a reliable, definitive identification. Host-range testing and particle morphology as observed by EM can provide some useful information, but not enough to provide a specific identification. The latter two methods are better used to provide information that complements or helps clarify results of other testing. The records listed in Mossop and Fry (1984) are again included in this paper (Table 1), together with records generated after 1984 in the broader Pacific Islands listing of Pearson and Grisoni (2002). Pearson and Grisoni (2002) include records of *Watermelon mosaic virus* in the Cook Islands which in fact refer to what was known as Watermelon mosaic virus-1 or WMV-1 (Thomas 1980). This virus is now known as Papaya ringspot virus (PRSV) and it exists as two forms (strains). One causes disease only in cucurbits and is known as the W (cucurbit infecting) strain or PRSV-W. This virus is widespread on cucurbits throughout much of the world. The papaya-infecting strain (PRSV-P) infects both papaya and cucurbits. The two viruses are very closely related and there is currently no way to distinguish them other than by host-range testing. PRSV-P has recently been recorded in papaya in the Cook Islands, but the Cook Islands Ministry of Agriculture (CIMoA) was confident of total eradication in 2004 (Davis et al. 2005a). Table 1 summarises Cook Islands plant virus records.

An important Cook Islands plant virus disease record believed to be false is an infection of tomato (*Lycopersicon* esculentum) by Tomato spotted wilt virus (TSWV) listed in Mossop and Fry (1984) and, in turn, by Pearson and Grisoni (2002). Mossop and Fry (1984), however, cite Joseph and Purea (1973) as the source of this record, stating it is unconfirmed and expressing doubt about its authenticity. TSWV is in the genus Tospovirus, a group of plant viruses that is of growing worldwide importance in cucurbits, solanaceous crops and peanuts. Twelve serogroups are known. TSWV is in serogroup I. This virus, together with Capsicum chlorosis virus (CCV) which is in serogroup IV (McMichael et al. 2002), and Iris yellow spot virus (Coutts et al. 2003), occurs in Eastern Australia. There are no verified records of these or any other tospoviruses in any of the Pacific Island countries or territories served by the SPC.

There are also some questions about the distribution of one of the worst diseases of citrus in the region. This is huanglongbing (HLB), previously known as greening disease and caused in much of Asia, by the phloem-limited bacterium '*Candidatus* Liberibacter asiaticus'. HLB is a major problem in tropical and sub-tropical parts of Asia, including those countries closest to the Pacific Islands (Malaysia, Viet Nam, the Philippines and Indonesia). HLB, and its vector the Asian citrus psyllid (*Diaphorina citri*), were discovered in Papua New Guinea (PNG) in 2002 (Weinert et al. 2004) and are currently the subject of a containment campaign. This virus-like disease destroys trees, has no cure, and affects all major types of citrus, making it a major new quarantine threat to the Pacific region. A report that the disease was found in the Fiji Islands, Samoa, Tonga and Palau 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 citrus leaf samples from the Cook Islands have been summarised in Davis et al. (2005b). Further details on these samples are provided in this paper.

The principal aim of the surveys reported here was to assess the general status of plant virus/virus-like diseases in the region and use this information to update the list of plant pathogens known to occur in the Cook Islands. This was done by surveying the country's two largest islands, Rarotonga and Aitutaki twice (once in the cool season and once in the warm season). They are also the two islands most frequently visited by travellers from other countries, so are considered at most risk of new plant-disease incursions. The island of Mangaia was also included in the warm-season survey.

Methods

Surveys

To undertake the surveys, as many different areas as possible were visited. Crop plants of economic importance and other plants were examined at each survey location. Samples thought to be infected by intra-cellular 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 plastic vials (25 ml in volume). 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 was undertaken over two weeks in July 2002 and the samples collected were given the collection numbers RID2821–RID2898. Survey 2 was conducted over two weeks in November/December 2003 and samples collected were given the collection numbers RID3570–RID3621.

Enzyme-linked immunosorbent assay (ELISA) testing

Cucurbit samples from survey 1 were tested at the Department of Primary Industries, Queensland (DPIQ) laboratory for *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV) and PRSV using Sanofi double antibody sandwich ELISA (DAS-ELISA) test kits. Cucurbit samples from survey 2 were tested for these viruses plus *Squash mosaic virus* (SqMV) using Agdia Inc. (Elkhart IN, USA) DAS-ELISA reagent sets at the SPC plant virology laboratory, Suva, Fiji Islands. Vanilla and other orchid samples were tested by DAS-ELISA for CMV, *Cymbidium mosaic virus* (CymMV), *Odontoglossum ringspot virus* (ORSV) and by indirect ELISA for the potyvirus group also using Agdia reagent sets at the SPC plant virology laboratory.

Following survey 1, tospovirus infection was tested for in *Capsicum annuum* samples using both the Sanofi TSWV and DSMZ tospovirus serogroup IV (WSMoV) ELISA test kits. Following survey 2, *C. annuum* samples were tested for tospovirus using the Sanofi TSWV ELISA test kit (serogroup I) and the Agdia Watermelon silver mottle virus plus Groundnut bud necrosis virus (WSMoV and GBNV) combined test kit (serogroup IV). Tospovirus was tested for in anthurium samples using only the Sanofi TSWV test kit.

Other ELISA tests conducted at the DPIQ laboratory were for *Potato virus Y* (PVY) infection using plate trapped antigen ELISA (using QDPI PVY 10 antisera produced by John Thomas, DPIQ, Australia) on two solanaceous samples (one *C. annuum*, one *L. esculentum*), for CMV infection using the Sanofi DAS-ELISA test kit in banana leaf samples, and for potyvirus infection using the Agdia potyvirus indirect ELISA test kit in a number of other samples. Citrus leaf samples were tested for *Citrus tristeza virus* (CTV) using Agdia compound direct ELISA reagent sets and extraction buffer in the laboratory of P. Jones, Rothampsted Research, UK (survey 1) and in the SPC plant virology laboratory (survey 2). 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.

Polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) testing

Aroid leaf material was sent to the laboratory of R. Harding at the Queensland University of Technology, Brisbane, Australia for testing for *Taro bacilliform virus* (TaBV) by PCR and for *Taro vein chlorosis virus* (TaVCV), *Dasheen mosaic virus* (DsMV), Taro reovirus (TaRV) and Colocasia bobone disease virus (CBDV) (only *Colocasia esculenta* samples were also tested for CBDV) by RT-PCR using the methods described in Revill et al. (2005).

Citrus leaf material was sent to the laboratory of M. Garnier, Institut Nationale de la Recherche Agronomique (INRA), Bordeaux, France (samples from survey 1) and the University of the South Pacific Institute of Applied Sciences (USP IAS) molecular biology laboratory (samples from survey 2), for testing for HLB using the PCR techniques described in Davis et al. (2005b).

Immunocapture (IC) RT-PCR

In addition to ELISA testing for CMV infection described above, banana leaf samples were also tested for *Banana streak virus* (BSV) infection at the DPIQ laboratory using specific immunocapture PCRs for a number of strains of the virus (BSV-Mys, BSV-GF, BSV-Onne and BSV–Cav) (Geering et al. 2000).

Electron microscopy

Some other samples were examined using transmission electron microscopy after they were subjected to a miniprep virus concentration method (Geering et al. 2000) at the DPIQ laboratory.

Results

The plant virus disease records are presented in Table 2. At several locations on both Rarotonga and Aitutaki, CMV and PRSV-W were found infecting many different plant species. CMV hosts were both crop plants (Citrulus lanatus (watermelon), Cucumis sativas (cucumber), Cucurbita maxima (pumpkin), Cucumis melo (rockmelon), Piper methysticum (kava) and weeds (Momordica charantia and Commelina diffusa). PRSV-W was found on all these cucurbit species plus C. maxima x moschata (squash) and also Cucurbita pepo var. melopepo (zuchinni) and Sechium edule (choko). The other three principal cucurbit-infecting viruses (WMV, ZYMV and SqMV) were not detected. Three Cavendish banana leaf samples from Rarotonga tested positive for CMV by ELISA, and five more banana samples were infected with BSV. These were one cooking banana infected with the Red Dacca strain of BSV (BSV-Onne) and four cv. Mysore banana plants, all infected with the Mysore strain of BSV (BSV-Mys). CTV was detected by ELISA in citrus trees from both Rarotonga and Aitutaki. PVY was detected in one L. esculentum (tomato) sample from Rarotonga. DsMV and TaBV were present on Rarotonga and Aitutaki and were detected in both C. esculenta and Xanthosoma sp. (taro). TaRV and TaVCV (and also CBDV in four C. esculenta samples; Table 3) were not detected. TSWV was not detected in three anthurium samples and tospovirus serogroup I (includes TSWV) and IV were not detected in four C. annuum (chilli) samples from survey 1 showing possible tospoviruslike leaf symptoms (Table 3). However, two C. annuum (capsicum) samples from the second survey tested positive in the different tospovirus serogroup IV ELISA tests conducted following that survey (Table 4). Huanglongbing indexing returned negative results from two citrus samples from Rarotonga and three from Aitutaki (Table 3).

Figures 1–14 show the symptoms caused by the viruses found in a number of hosts.

Discussion

These surveys provided the first records of any virus infection of banana plants in the Cook Islands. The viruses detected (CMV and BSV) cause banana diseases of relatively minor importance and are widespread in the world. BSV is genomically and serologically very diverse (Lockhart and Jones 1999; Geering et al. 2000) and is mostly transmitted through infected planting material (Lockhart and Jones 1999). Some cases of infection also arise from activation of virus sequences integrated into the host genome (Ndowora et al. 1999). The effects of Queensland strains of BSV on fruit production are apparently not severe: yield losses of 7–15% have been reported in field trials (Daniels et al. 1999). However, strains of BSV in Africa have caused losses of 100% in individual plants (Tushemereirwe and Bagabe 1999).

This study also provides the first record of CMV infection of kava in the Cook Islands. CMV (alone or in combination with other factors) causes kava dieback disease. Although reports of kava dieback disease symptoms are known from several Pacific Island countries, there are verified records of CMV infection in kava for only Tonga, Fiji, Vanuatu, Samoa (Davis et al. 1996) and Hawai'i (see: http://www.ctahr.hawaii.edu/fb/). Three leaf samples from an unidentified species of *Macropiper* growing in native vegetation in central Rarotonga, collected because they were showing crinkle, chlorotic patches and vein clearing tested negative for CMV (authors' unpublished data).

DsMV and TaBV, found in aroid leaf samples at several locations on Rarotonga and Aitutaki, are widely distributed viruses of taro in the Pacific region (Revill et al. 2005). This survey also provides the first confirmation of these two viruses in *Xanthosoma* sp. in the Cook Islands. TaBV appears to be a virus of only minor importance, except when taro plants are infected with both it and CBDV. Co-infection with both viruses is believed to often (but not always) result in the lethal disease known as alomae (Revill *et al.* 2005). DsMV is thought to also cause yield losses in taro (Jackson et al. 2001) and ornamental aroids (Chase and Zettler 1982).

Overall, the diversity of plant infecting viruses recorded on these surveys was surprisingly low. The most common viruses found on Cook Islands crops and weeds were CMV and PRSV-W. CMV and PRSV-W are unrelated (CMV is a member of the genus *Cucumovirus*, PRSV a member of the genus *Potyvirus*), but both viruses share some common characteristics of great relevance to control measures. These viruses cannot survive in the soil or in decayed plant material. Both cause systemic infections, which mean that infected plants cannot be cured by spraying or by removing parts of the plant showing symptoms. Both are spread from plant to plant by many different species of aphid vectors. Both are also non-persistently transmitted by these aphids. Non persistence means that the virus is held on the insect's mouth parts only for several hours, and can be picked up from an infected plant and transmitted to another plant in a few seconds during brief feeding probes. 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. 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 to the crop). Whilst PRSV-W is thought not to be transmitted in the seed of hosts, transmission of CMV in the seed of some host species (including cucurbits) is known.

The most effective control method for these viruses is use of resistant or tolerant cultivars. However, depending on the crop, the virus and the country (import restrictions can prevent new introductions) these are often not available. Certain cultural control strategies can reduce incidence of non-persistent viruses in crops. Most achieve this by reducing initial inoculum levels, before the virus spreads through the crop. Some of these are particularly applicable to small-scale production systems like those of the Cook Islands. Removal of alternative hosts in and around the crop is a key one of these. PRSV-W hosts are mostly Cucurbitaceae and certain members of the Chenopodiaceae (see: http://image.fs.uidaho.edu/vide/sppindex.htm#S). Natural infection of papaya by PRSV-W (and movement of PRSV-P to cucurbits) appears to be relatively unusual (Bateson et al. 1994, 2002; Tetsuo 2003). This means that cucurbit volunteers and weeds should be key targets for control of PRSV-W. Whilst no *M. charantia* samples tested positive for PRSV in these surveys, this cucurbit vine has been shown to be a host of the virus in the Cook Islands (Thomas 1980). CMV is much harder to combat in this way, as this virus has an extremely broad host range; it infects over 800 plant species in 85 families (Gallitelli 1998). These are mostly herbaceous plants and the

list includes many weeds common in the Cook Islands. Importantly, mechanical inoculation experiments (Fiji Ministry of Agriculture, Sugar and Land Resettlement, unpublished data) have recently shown that the extremely common weed *Mikania micrantha* is a symptomless host of CMV. Reflective mulches can also reduce initial inoculum levels (Summers et al. 1995), because the reflected light repels newly arriving winged aphids, reducing the number of new infectious from elsewhere. If this is effective in the early stages of growth, before the plants start to produce fruit, production losses can be minimised.

Intercropping with non-hosts plants can also be a valuable control technique for non persistent plant viruses. Since aphids lose the virus from their mouthparts when they probe, feeding on non-host leaves eliminates the danger to susceptible hosts. In the same way, tree canopies above or around the crop can protect susceptible crop plants underneath from incoming infectious winged aphids. This can be especially important in the case of CMV as the choice of non-host intercrop plants may be limited, and most tree species are not known to be hosts of this virus.

There are a number of other plant diseases caused by viruses or virus-like agents that are currently of particular quarantine concern in the Pacific region because they are found on only some islands. The apparent absence from the Cook Islands of banana bunchy top disease (caused by Banana bunchy top virus or BBTV) is of particular importance. By late 2003, laboratory test records confirming presence of BBTV in the 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 being seen in the field in Tuvalu and American Samoa. These records are not known to have been confirmed by diagnostic testing. Many banana plants were examined during these surveys and no bunchy top-like symptoms were seen. The tospovirus screening reported here (no tospovirus serogroup I found) provides further evidence that the early report of TSWV causing a "bronze top of tomato" in the Cook Islands (Joseph and Purea 1973) may indeed be erroneous, as suggested in Mossop and Fry (1984). However, two capsicum samples from the second survey reported here tested positive in the ELISA test for tospovirus serogroup IV used. This test gave lower background readings and stronger positive reactions than the test used for survey 1 samples (authors' unpublished data). However, PCR testing using CCV-specific primers returned negative results (L. McMichael, unpublished data). Studies to identify this virus are continuing. Whilst TaBV is widespread across the Pacific, CBDV and alomae disease are known only in Papua New Guinea (PNG) and Solomon Islands. CBDV is therefore a virus of enormous guarantine concern in the Pacific and all efforts should be made to ensure it does not spread with taro planting material. No virus was found in the small number of vanilla/orchid samples tested, but the relatively unimportant CymMV and ORSV, plus the more damaging Vanilla mosaic virus (VanMV, a potyvirus), have been recorded before in the Cook Islands during a survey focusing on vanilla viruses (Pearson et al. 1993). The negative citrus HLB screening results reported here, and in Davis et al. (2005b), support the widely held belief that this disease is not present in the Cook Islands.

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TABLES

Table 1. Previously published plant virus and viroid records from the Cook Islands

Pathogen	Host	Citation [▲]	Identification method ^B
Citrus enation/woody gall	Citrus spp.	Meister (1976) ^c	Indicator hosts ^D
Citrus exocortis viroid (CEVd)	Citrus spp.	Meister (1976) ^c	Indicator hosts ^D
Citrus psorosis virus (CPsV, plus other viruses in a complex)	Citrus spp.	O'Conner (1969) ^c	Indicator hosts ^D
Citrus tristeza virus (CTV)	Citrus spp.	Meister (1976) ^c	Indicator hosts ^D
Cucumber mosaic virus (CMV)	Capsicum annuum	Thomas (1978) ^c	
	Commelina diffusa	Mossop and Fry (1984) ^c	Host range studies/serology/ EM - not stated which
	Cucurbita pepo var. melopepo (zuchinni)	Mossop and Fry (1984) ^c	Host range studies/serology/ EM - not stated which
	Passiflora edulis	Thomas (1978) ^c	
Cymbidium mosaic virus (CymMV)	Vanilla planifolia	Pearson et al. (1993)	Serology and EM
	Vanilla	Pearson et al. (1993)	Serology and EM
Dasheen mosaic virus (DsMV)	tahitensis Colocasia esculenta,	Revill et al. (2005)	PCR
	Xanthosoma sp.	Gollifer et al. (1977)	EM
Odontoglossum ringspot virus (ORSV)	Vanilla planifolia	Pearson et al. (1993)	Serology and EM
	Vanilla tahitensis	Pearson et al. (1993)	Serology and EM
Potato virus Y (PVY)	Capsicum annuum	Thomas (1978) ^c	
Taro baciliform virus (TaBV)	Colocasia esculenta	Revill et al. (2005)	PCR
	Xanthosoma sp.	Gollifer et al. (1977)	EM
Tobacco mosaic virus (TMV)	Lycopersicon esculentum	Thomas (1978) ^c	
Vanilla mosaic virus (VanMV)	Vanilla tahitensis	Pearson <i>et al</i> . (1993)	Serology and EM
Papaya ringspot virus type W (PRSV-W)	Citrullus lanatus	Thomas (1980) ^E	Host range, EM and serology
	Cucurbita maxima	Thomas (1980) ^E	Host range, EM and serology
	Cucurbita pepo	Thomas (1980) ^E	Host range, EM and serology
	Cucumis sativas	Thomas (1980) ^E	Host range, EM and serology
	Momordica charantia	Thomas (1980) ^E	Host range, EM and serology
Papaya ringspot virus type P ^F (PRSV-P)	Carica papaya	Davis <i>et al</i> . (2005a)	Serology, PCR

^AThe original or earliest available citation of the most reliable record available is provided.

^BEM: Particles of similar description seen with electron microscopy, therefore not a definitive identification method if not combined with some other technique; PCR: polymerase chain reaction.

^cOriginal paper not available to verify identification methods used, or precise identification methods not stated.

^DBill Hoskins, retired Head of Research, Totokoitu Research Station, personal communication

^EListed as WMV-1^FBelieved to be eradicated by early 2005.

 Table 2. Plant viruses detected in samples collected from the Cook Islands in 2002 and 2003

Host plant	Field			
family, genus, species (common name)	collection number	Approximate location	Symptoms ^A	Virus ^B
Araceae				
Colocasia esculenta (taro)	RID 2840	Totokoitu, Rarotonga	Strong YOGM	DsMV ^c
	RID 2866	Matavera, Rarotonga	Strong feathery WOGM	DsMV ^c , TaBV ^c
	RID 2890	Titikaveka, Rarotonga	Symptomless sample	DsMV ^c , TaBV ^c
	RID 2881	Vaipae, Aitutaki	Feathery WOGM	DsMV ^c , TaBV ^c
Xanthosoma sp. (taro)	RID 2841	Totokoitu, Rarotonga	Severe feathery YOGM	DsMV
	RID 2867	Matavera, Rarotonga	Very strong feathery YOGM	DsMV
	RID 2868	Matavera, Rarotonga	Feathery YOGM and leaf distortion	DsMV
	RID 2897	Matavea, Rarotonga	Severe WOGM and leaf distortion	DsMV
	RID 2877	Tautu, Aitutaki	Feathery YOGM and extreme leaf distortion	DsMV
	RID 2878	Tautu, Aitutaki	Feathery YOGM	DsMV
	RID 2882	Vaipae, Aitutaki	Feathery YOGM and leaf distortion	DsMV
	RID 2884	Amuri, Aitutaki	Feathery WOGM and severe leaf distortion	DsMV, TaBV
Commelinaceae				
Commelina diffusa				
	RID 2827	Totokoitu, Rarotonga	YOGM	CMV
	RID 2847	Kavare, Rarotonga	Strong and streaky YOGM	CMV
	RID 2861	Aroranga, Rarotonga	Strong and streaky YOGM	CMV
	RID 2870	Matavera, Rarotonga	Strong and streaky YOGM	CMV
	RID 2891	Titikaveka, Rarotonga	Strong and streaky YOGM	CMV
	RID 2892	Arerangi, Rarotonga	Strong and streaky YOGM	CMV

Cucurbitaceae				
Citrullus lanatus (watermelon)	RID 2846	Kavare, Rarotonga	Mild and blotchy YOGM	PRSV
	RID 2893	Titikaveka, Mild and blotchy YOGM Rarotonga		PRSV
	RID 2894	Titikaveka, Rarotonga	YOGM plus strap-like leaves	PRSV, CMV
	RID 3611	Ambala, Rarotonga	YOGM	PRSV
	RID 3620	Nikao, Rarotonga	YOGM	PRSV
	RID 3621	Nikao, Rarotonga	YOGM	PRSV
	RID 2874	Tautu, Aitutaki	YOGM of older leaves	PRSV
	RID 2875	Tautu, Aitutaki	YOGM of younger leaves	PRSV
	RID 2883	Amuri, Aitutaki	YOGM	PRSV
	RID 3584	Tautu, Aitutaki	Strong YOGM	PRSV
	RID 3585	Tautu, Aitutaki	YOGM	PRSV
Cucurbita maxima (pumpkin)	RID 2863	Matavera, Rarotonga	Mild and patchy YOGM on older leaves	PRSV
	RID 2864	Matavera, Rarotonga	Diffuse YOGM	PRSV
	RID 2869	Matavera, Rarotonga	Severe YOGM	PRSV, CMV
	RID 2889	Titikaveka, Rarotonga	None (symptomless sample)	PRSV
	RID 3586	Araura, Aitutaki	YOGM and distortion	PRSV
	RID 3587	Araura, Aitutaki	Strong YOGM	PRSV
	RID 3589	Amuri, Aitutaki	Mild YOGM	PRSV
Cucurbita maxima x moschata (squash)	RID 2851	Ambala, Rarotonga	Mild YOGM on youngest leaves.	PRSV

Cucumis melo (rockmelon)	RID 2822	Totokoitu, Rarotonga	YOGM	PRSV, CMV
	RID 2824	Totokoitu, Rarotonga	YOGM	PRSV , CMV
	RID 2825	Totokoitu, Rarotonga	None (symptomless sample)	PRSV
	RID 2826	Totokoitu, Rarotonga	Mild YOGM	PRSV
	RID 2852	Ambala, Rarotonga	YOGM	CMV
	RID 3603	Ngatangiia, Rarotonga	YOGM	PRSV
	RID 3614	Totokoitu, Rarotonga	YOGM	PRSV
Cucurbita pepo var. melopepo (zuchinni)	RID 2854	Ambala, Rarotonga	Chlorosis, strap-like leaves	PRSV
	RID 2855	Ambala, Rarotonga	Chlorotic patches and leaf curl	PRSV
	RID 2856	Ambala, Rarotonga	Chlorosis, strap-like leaves	PRSV
	RID 2858	Aroranga, Rarotonga	Strap-like leaves, leaf curl	PRSV
Cucumis sativus (cucumber)	RID 2845	Totokoitu, Rarotonga	YOGM	PRSV , CMV
	RID 2865	Matavera, Rarotonga	Diffuse and blotchy YOGM	PRSV
	RID 2895	Matavera, Rarotonga	None (symptomless sample)	PRSV
	RID 3619	Tokerau, Rarotonga	Yellow on green mosaic	PRSV
Momordica charantia	RID 2821	Te Kou, Rarotonga	YOGM	CMV
	RID 2862	Aroranga, Rarotonga	Mild YOGM	CMV
	RID 2871	Matavera, Rarotonga	Mild YOGM	CMV
	RID 3583	Tautu, Aitutaki	YOGM	CMV
	RID 3588	Araura, Aitutaki	YOGM	CMV
	RID 3590	Amuri, Aitutaki	YOGM	CMV
Sechium edule (choko)	RID 3600	Avirua, Rarotonga	YOGM	PRSV
Musaceae Musa sp. (AAA,	RID 3580	Avaavaroa, Rarotonga	Chlorotic streaks on most leaves	CMV
Cavendish group)	RID 3581	Avaavaroa, Rarotonga	Chlorotic streaks on most leaves	CMV
	RID 3604	Totokoitu, Rarotonga	Chlorotic streaks on most leaves	CMV

Musa sp. (ABB)	RID 3605	Totokoitu,Chlorotic streaks plusRarotongabrown black markingson some leaves		BSV-Onne
Musa sp. (AAB, cv. Mysore)	RID 3606	Totokoitu, Rarotonga	Chlorotic streaks plus brown black markings on some leaves	BSV-Mys
	RID 3507	Totokoitu, Rarotonga	Chlorotic streaks plus brown black markings on some leaves	BSV-Mys
	RID 3608	Totokoitu, Rarotonga	Chlorotic streaks plus brown black markings on some leaves	BSV-Mys
	RID 3613	Maire Nui, Rarotonga	Chlorotic streaks plus brown black markings on some leaves	BSV-Mys
Piperaceae				
Piper methysticum (kava)	RID 2828	Totokoitu, Rarotonga	Chlorosis and crinkle	CMV
	RID 2829	Totokoitu, Rarotonga	Diffuse chlorotic blotch plus early dieback on same stem	CMV
	RID 2830	Totokoitu, Rarotonga	Crinkle, pucker and chlorotic patches	CMV
	RID 2831	Totokoitu, Rarotonga	Crinkle, pucker and internal necrosis of stem	CMV
	RID 2834	Totokoitu, Rarotonga	YOGM	CMV
Rutaceae				
Citrus aurantifolia (lime)	RID 2835	Totokoitu, Rarotonga	Sectoral chlorosis	СТУ
<i>Citrus reticulata</i> (mandarin) mandarin, tangerine	RID 3595	Avirua, Rarotonga	Chlorotic blotch and prominent veins	СТУ
-	RID 2885	Amuri, Aitutaki	General chlorosis	СТV
	RID 3582	Tautu, Aitutaki	General chlorosis	СТУ
Solanaceae				
Lycopersicon esculentum (tomato)	RID 2860	Aroranga, Rarotonga	Diffuse yellow on green mosaic	PVY

^AWOGM: white on green mosaic, YOGM: yellow on green mosaic

^BViruses were: BSV: Banana streak virus, CMV: Cucumber mosaic virus, CTV: Citrus tristeza virus, DsMV: Dasheen mosaic virus, PRSV: Papaya ringspot virus, PVY: Potato virus Y, TaBV: Taro bacilliform virus.

^cResults reported previously in Revill et al. (2005).

DsMV, and CMV in kava were detected by RT-PCR, Ta BV was detected using PCR, BSV was detected using specific immunocapture PCRs for a number of strains of BSV. CMV (in cucurbits and banana), PRSV, CTV, and PVY were identified by ELISA. ELISA test results were considered positive when absorbance readings (405 nm) exceeded three times the mean of healthy controls).

Collection numbers RID2821-RID2898 were from survey 1 (July 2002), and RID3570-RID3621 were from survey 2 (November/ December 2003).

Host plant family, genus, species (common name)	Field collection number	Approximate location	Symptoms ^A	
Araceae				
Anthurium andraenum (anthurium)	RID 3596	Avirua, Rarotonga	Chlorotic markings	TSWV [®] and NVD by EM
	RID 3597	Avirua, Rarotonga	Chlorotic markings	TSWV ^B and NVD by EM
	RID 3617	Aroroangi, Rarotonga	Chlorotic blotch	TSWV ^B and NVD by EM
Colocasia esculenta (taro)	RID 2840	Totokoitu, Rarotonga	Strong YOGM	CBDV ^c
	RID 2866	Matavera, Rarotonga	Strong feathery WOGM	CBDV ^c
	RID 2890	Titikaveka, Rarotonga	Symptomless sample	CBDV ^c
	RID 2881	Vaipae, Aitutaki	Feathery WOGM	CBDV ^c
Orchidaceae				
Dendrobium sp.	RID 3615	Avavaroa, Rarotonga	Chlorotic blotching	Potyvirus group, CymMV, ORSV and CMV ^D
	RID 3616	Avavaroa, Rarotonga	Chlorotic spots distinct	Potyvirus group, CymMV, ORSV and CMV ^D
Vanilla planifolia	RID 3573	Oneroa, Mangaia	Chlorotic blotch -slight	Potyvirus group, CymMV, ORSV and CMV ^D
Rutaceae				
Citrus aurantifolia (lime)	RID 2835	Totokoitu, Rarotonga	Extreme chlorosis of half of canopy	HNegative for huanglongbing ^E
<i>Citrus reticulata</i> (mandarin) mandarin, tangerine	RID 2885	Amuri, Aitutaki	General chlorosis	HNegative for huanglongbing ^E
	RID 3582	Tautu, Aitutaki	Chlorotic blotch and very slightly corky veins	HNegative for huanglongbing ^E
	RID 3595	Avirua, Rarotonga	Chlorotic blotch and very slightly prominent veins	HNegative for haunglongbing ^E
Citrus sinensis (orange)	RID 2886	Amuri, Aitutaki	Green on yellow vein banding, thin, upright leaves	HNegative for huanglongbing ^E
Solanaceae				
Capsicum annuum var. annuum (chilli)	RID 2842	Vaimaanga, Rarotonga	YOGM, crinkle, distortion, chlorosis, and small leaves chlorosis, small leaves Tospoviruses? Possibly damage from sap	Tospovirus ^F
Capsicum annuum var. annuum (chilli)	RID 2843	Vaimaanga, Rarotonga	YOGM, crinkle, distortion, chlorosis, small leaves	Tospovirus ^F

Capsicum annuum var. annuum (chilli)	RID 2843	Vaimaanga, Rarotonga	None	Tospovirus ^F
Capsicum annuum (capsicum)	RID 2849	Tikioki, Rarotonga	Diffuse chlorotic mosaic	Tospovirus ^F

^AWOGM: white on green mosaic, YOGM: yellow on green mosaic.

^BTested using ELISA for *Tomato spotted wilt virus* (TSWV)

^cResults reported previously in Revill et al. (2005).

^DTested using ELISA for potyvirus group, ORSV and CymMV.

^ENegative for haunglongbing disease by PCR: results summarised in Davis et al. (2005b).

^FTested using ELISA for TSWV and Tospovirus serogroup IV.

EM: electron microscopy, NVD: No virus detected.

Table 4. Samples subjected to ongoing studies

Host plant family, genus, species (common name)	Field collection number	Approximate Location	Symptoms ^A	Comments ^B
Fabaceae				
Desmodium sp.	RID 2836	Totokoitu, Rarotonga	Strong YOGM	Flexuous rods seen by EM, negative for potyvirus by ELISA
	RID 2850	Tikioki, Rarotonga	Strong YOGM	Few straight rods seen by EM
Lileaceae				
Hippeastrum sp.	RID 2857	Kauare, Rarotonga	Chlorotic streak/ mosaic	Few rods seen by EM, negative in potyvirus group ELISA
Orchidaceae				
Unknown orchid	RID 2898	Avarua, Rarotonga	Ringspots - yellow on green	Flexuous to straight rods seen by EM
Solanaceae				
Capsicum annuum (capsicum)	RID 2872	Tautu, Aitutaki	Strap-like leaves and distortion and chlorosis	Rods seen by EM, negative by PVY ELISA
	RID 3598	Avirua, Rarotonga	Yellow on green mosaic	Tospovirus serogroup IV positive, studies ongoing
	RID 3599	Avirua, Rarotonga	Necrosis at veins	Tospovirus serogroup IV positive, studies ongoing

^AYOGM: yellow on green mosaic.

^BEM: electron microscopy.

FIGURES



Fig. 1. RID 2840: Colocasia esculenta (taro) and RID 2841: Xanthosoma sp. infected with Dasheen mosaic virus (DsMV).



Fig. 2. RID 2867: Xanthosoma sp. infected with Dasheen mosaic virus (DsMV).



Fig. 3. RID 2868: Xanthosoma sp. infected with Dasheen mosaic virus (DsMV).



Fig. 4. RID 2847: Commelina diffusa infected with Cucumber mosaic virus (CMV).



Fig. 5. RID 3587: Cucurbita maxima (pumpkin) infected with Papaya ringspot virus (PRSV).



Fig. 6. RID 2822: Cucumis melo (rockmelon) infected with Papaya ringspot virus (PRSV) and Cucumber mosaic virus (CMV).



Fig. 7. RID 3603: Cucumis melo (rockmelon) infected with Papaya ringspot virus (PRSV).



Fig. 8. RID 2858: Cucurbita pepo var. melopepo (zuchinni) infected with Papaya ringspot virus (PRSV).



Fig. 9. RID 3583: Momordica charantia infected with Cucumber mosaic virus (CMV).



Fig. 10. RID 3600: Sechium edule (choko) infected with Papaya ringspot virus (PRSV).



Fig. 11. RID 3604: *Musa* sp. (Cavendish group, AAA genotype) infected with *Cucumber mosaic virus* (CMV).



Fig. 12. RID 3605: *Musa* sp. (ABB genotype) infected with *Banana streak virus* (BSV) strain BSV-Onne.



Fig. 13. RID 3608: *Musa* sp. (cv. Mysore, AAB genotype) infected with *Banana streak* virus (BSV) strain BSV-Mys.



Fig. 14. RID 2834: Piper methysticum (kava) infected with Cucumber mosaic virus (CMV).