Pacific Horticultural and Agricultural Market Access Program (PHAMA)

Technical Report 51: Substantiation of Australia's Requirements for Devitalisation of Taro Imports (FIJI06)

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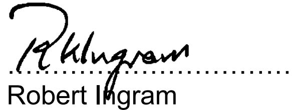

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Abbreviations

Abbreviation	Description
ACIAR	Australian Centre for International Agricultural Research
AusAID	Australian Agency for International Development
CBDV	Colocasia bobone disease rhabdovirus
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
DsMV	Dasheen Mosaic Virus
EDTA	Ethylenediaminetetraacetic acid
M-MLV	Moloney Murine Leukemia Virus
N/A	Not applicable
NSW	New South Wales
PCR	Polymerase Chain Reaction
PHAMA	Pacific Horticultural and Agricultural Market Access Program
PNG	Papua New Guinea
QUT	Queensland University of Technology
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SDS	Sodium docecyl sulfate
TaBV	Taro Bacilliform Virus
TaVCV	Taro Vein Chlorosis Virus
URS	URS Australia Pty Ltd

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Executive Summary

Biosecurity Australia has proposed that fresh taro corms be permitted into Australia subject to specific pest risk management measures. For taro corms from Fiji, two pests are of concern: *Tarophagus proserpina* and Taro vein chlorosis rhabdovirus (TaVCV). The proposed quarantine treatments for these include "removing all petiole material and apical growing points from the corms of large corm taro (*Colocasia esculenta* var. *esculenta*)". Taro corms are "topped" to remove leaf petioles and growing points and scraped to remove dormant buds.

The treatment of corms in this way seriously reduces shelf life. Because of this, growers in Fiji have requested information on the distribution of the pests of quarantine concern to Australia. The distribution of *Tarophagus* species and TaVCV is well known in Pacific Island Countries from recent surveys, the results of which have been published. However, no comparable surveys have been carried out in Australia.

In order to provide the information required, a survey was carried out from Cairns to Mackay, Queensland, and in Cudgen, New South Wales, from 5–14 May 2013. The survey was undertaken by members of Queensland University of Technology (QUT) and PestNet.

During the survey, visits were made to commercial growers, backyard hobbyists, and wild taro patches alongside streams. However, only symptoms of one virus were found: that of Dasheen mosaic virus (DsMV), a common virus that infects taro worldwide. A second virus, Taro bacilliform virus (TaBV), was found upon analysis of the samples at QUT. Both DsMV and TaBV were already known to occur in Australia. None of the plants examined showed signs of TaVCV, and tests specifically for that virus were negative.

The results suggest that TaVCV is not present in the survey area.

Only one collection of *Tarophagus* was made, and that was identified as *Tarophagus colocasiae*, a species already recorded in Australia.

1 Introduction

1.1 Background

Import conditions for fresh taro corms from Fiji into Australia require that corms are devitalised by removal of the terminal and lateral buds. The devitalisation is justified by Australia on the basis that if imported taro is propagated (rather than being consumed) then viral diseases of quarantine concern might be introduced.

Surveys of pests and diseases of taro in the Pacific, especially those of viral aetiology, were carried out in 2003 by scientists at Queensland University of Technology (QUT) and Pestnet, under projects funded by the Australian Centre for International Agricultural Research (ACIAR), Canberra. The surveys found that Dasheen mosaic potyvirus (DsMV) and Taro bacilliform badnavirus (TaBV) were in most countries, Colocasia bobone disease rhabdovirus (CBDV) was restricted to Papua New Guinea (PNG) and Solomon Islands, and Taro vein chlorosis rhabdovirus (TaVCV) was present in PNG, Solomon Islands, Vanuatu, New Caledonia, Fiji, and the Federated States of Micronesia (Revill et al., 2005).

No surveys of taro growing in Australia have been conducted to determine whether the viruses of quarantine concern are present or not. Only two viruses have been recorded in taro in Australia, namely DsMV and TaBV. However, in a recent review of import conditions for fresh taro corms into Australia (Biosecurity Australia, 2011), it was assumed that TaVCV and CBDV were absent from the country. Because of this, all taro from countries where those viruses are present are to be devitalised.

In addition to viruses, *Tarophagus* planthoppers are also of concern to quarantine authorities, as they are the putative vectors of taro rhabdoviruses. Three species have been determined to be present in the Pacific islands, but only two – *T. colocasiae*, and *T. persephone* – have been recorded in Australia. A third, *T. proserpina*, is absent. Therefore, import conditions for fresh taro corms state "all petiole material and growing points of the corm must be removed" on taro imported from countries where *T. proserpina* is present.

However, devitalisation exposes taro flesh and increases the risk of postharvest rots, which then results in further import processing delays, fumigation, and in some situations re-export or destruction of the consignment, with considerable cost implications.

1.2 Objective

The objective of the work was to make surveys of taro growing in north Queensland, south-east Queensland and northern New South Wales to determine: 1) which of the known taro viruses are present in Australia, with a specific focus on TaVCV; and 2) whether the planthopper *Tarophagus proserpina (*one of the three *Tarophagus* spp. presumed to be vectors of the two recorded taro rhabdoviruses) was present.

1.3 Scope of Work

To conduct a field survey of approximately 10 days of farms and backyard plots as well as volunteer or wild taro in north Queensland (Cairns, Deeral, Babinda, Innisfail, Tully, Cardwell, Ingham, and Mackay), south-east Queensland (Brisbane) and northern NSW (Cudgen).

To examine the plants for symptoms typically associated with virus infection / disease, and examine/test samples from a representative number of plants for:

- TaVCV;
- *Tarophagus proserpina*; and
- Any other pests or diseases encountered during the surveys that are believed to be potentially considered as quarantine pests for Australia.

Plant samples are to be collected and tested for taro viruses at QUT. Planthopper specimens are to be collected and sent to an Australian taxonomist (Dr Murray Fletcher) at the Orange Agricultural Institute, Orange, NSW, for identification.

The surveys were done with the support of the AusAID-funded Pacific Horticultural and Agricultural Market Access program (PHAMA). The surveys were managed by Pestnet, in cooperation with QUT.

1.4 Duration

The survey began on 5 May 2013 in Cairns, Queensland, and was completed on 14 May 2013 at Cudgen, NSW.

1.5 Itinerary

5 May Sydney (Grahame Jackson) or Brisbane (Rob Harding) to Cairns

6 May Cairns, Yarrabah, White Rock, Deeral, Gordonvale

7 May El Arish, Bilyana, East Feluga, Babinda

8 May Carmoo, Millaa Millaa Falls (and others), Lake Eacham, Palmerston Highway

9 May Ingham, Trebonne, Journama Falls, Mutarnee

10 May Townsville, Oak Valley, Nome

11 May Bakers Creek, Bloomsbury

12 May Ashgrove

13 May Cudgen

14 May Browns Plains, Woodridge, Sydney (Grahame Jackson)

2 Methodology

2.1 Sampling

Leaf samples were taken from plants showing virus-like symptoms, as well as from symptomless leaves. The leaf samples were placed in plastic bags which were kept cool in a cooler bag containing ice-packs. Upon return to QUT, the samples were frozen at -20°C until processing. Samples were also stored desiccated under silica gel.

2.2 Extraction of Nucleic Acid

1. For each sample, collect six leaf discs (~6 mm^2) from fresh leaf tissue samples into a sterile 2 mL microfuge tube containing one lead bead. Place tube into liquid nitrogen then grind leaf tissue into a powder using the Tissue Lyser (speed 30 for 15 seconds). After shaking, place tubes back into liquid nitrogen. Repeat the shaking process once.
2. Place in liquid nitrogen and spin down in a bench-top microfuge at 18,000 g for 5 seconds to bring tissue powder to bottom of the tube.
3. Add 800µL of extraction buffer, vortex for 1 minute and centrifuge at 18,000 g for 5 minutes.
4. Remove the top layer with micropipette into a new 2 mL tube.
5. Precipitate the sample by adding 88µL of 5 M potassium acetate and 200 µL of absolute ethanol, vortex for 1 minute and centrifuge at 18,000 g for 5 minutes.
6. Remove the top layer (900µL) with a micropipette into new 2 mL tube.
7. Extract with 900µL of chloroform-isoamylalcohol (49:1 v/v), vortex and centrifuge at 18,000 g for 5 minutes.
8. Remove the top layer (800µL) with a micropipette into new 2 mL tube.
9. Extract with 800µL of chloroform-isoamylalcohol (49:1 v/v), vortex and centrifuge at 18,000 g for 5 minutes.
10. Remove the top layer (700µL) with a micropipette into new 2 mL tube.
11. Extract with 700µL of chloroform-isoamylalcohol (49:1 v/v), vortex and centrifuge at 18,000 g for 5 minutes.
12. Remove the top layer (500µL) with a micropipette into new 1.5 mL tube.
13. Add 167µL of 12M LiCl (to a final concentration of 3M). Mix gently by inversion.
14. Precipitate overnight at -20°C.
15. Centrifuge at 20,000 g at 4°C for 45 minutes.
16. Decant supernatant into a waste beaker and tap down excess on a clean tissue. Spin down for 5 seconds and remove excess liquid from tube away from the pellet with a P200 tip.
17. Wash the RNA pellet with 1 mL of chilled 70% ethanol.
18. Centrifuge at 20,000 g at 4°C for 5 minutes.
19. Decant supernatant into a waste beaker and tap down excess on a clean tissue. Spin down for 5 seconds and remove excess liquid from tube away from the pellet with a P200 tip.
20. Dry under vacuum for 10 min.
21. Resuspend the pellet in 50 µL of DEPC-treated dH_2O or RNAse free water.

Extraction buffer: 150 mM Tris base, 100 mM EDTA and 2% SDS (adjusted to pH 7.5 with conc. HCl before adding SDS), add 1% 2-mercaptoethanol just before use.

2.3 DNase Treatment of RNA

An aliquot of each extract was treated using RQ1 DNase to remove genomic DNA from the RNA preparations. A 5 µl aliquot of each extract was mixed with 1 µl of 10 x DNase reaction buffer, 2 µl of RQ1 DNase and 2 µl of nuclease free water and incubated at 37°C for 30 minutes. To terminate the reaction, 1 µl of stop solution was added and tubes incubated at 65°C.

2.4 cDNA Synthesis (for Detection of TaVCV, DsMV and 18s RNA Control)

cDNA synthesis was done using M-MLV reverse transcriptase (Promega) and primers (Table 2-1). Prepare two master mixes containing either the primers and template (mix 1) or RT reagents (mix 2) as follows:

cDNA Mix 1 (Total volume 10 µL)

- 1 µL primer TaVCVf @ 10µM
- 1 µL primer TaVCVPolA1 @ 10µM
- 1 µL primer 18Sr @ 10µM
- 0.1 µL primer oligodT @ 200µM
- 4.9 µL water
- 2 µL RNA extract.

Combine all reagents on ice then denature RNA at 95°C for 3 minutes; place on ice.

cDNA Mix 2 (Total volume 10 µL)

- 4 µL 5 x RT buffer
- 0.5 µL reverse transcriptase
- 1 µL 10 mM dNTPs
- 0.25 µL RNase inhibitor (Promega)
- 4.25 µL water.

Combine all reagents; add to denatured RNA after 3 minutes incubation on ice. Place cDNA synthesis reactions into a thermal cycler and incubate at 25°C for 5 minutes, 42°C for 60 minutes, 70°C for 15 minutes.

2.5 Polymerase Chain Reaction (PCR)

PCR for all targets was carried out using GoTaq green (Promega) in 20 µL reactions with target-specific primers (Table 2-1). For each PCR setup, a master mix was prepared as follows:

PCR mix (Total volume 20 µL)

- 10 µL GoTaq green 2x master mix
- 1 µL PCR primers @ 10µM
- 8 µL water
- 1 µL RNA extract or cDNA.

Combine all reagents on ice, aliquot 19 µL of master mix and add 1 µL of RNA/cDNA to each reaction tube.

PCR cycling

For all primer sets except 18S RNA, cycling was carried out as follows:

- 1 cycle of 94°C for 2 minutes.
- 35 cycles of 94°C for 20 seconds, 50°C for 20 seconds and 72°C for 30 seconds.
- 1 cycle of 72°C for 2 minutes.

For 18s RNA, 35 cycles of 94°C for 5 seconds, 60°C for 5 seconds and 72°C for 1 second.

Table 2-1 Primers for cDNA synthesis and PCR

Primer	Target	Sequence 5` to 3`	Product size (bp)
18SF	18S RNA	CATCACAGGATTTCGGTCCT	500 (DNA) 190 (cDNA)
18SR		AGACAAATCGCTCCACCAAC	
TaVCV1	TaVCV	ATAATCCAGCTTTACATTCACTGAC	170
TaVCV2		TGCCTGGGCTTCCTGAGATGATCTG	
TaVCVPolA1	TaVCV	AATATGCTCTCCAGTGTTCACCC	1001
TaVCVPolA2		AGGTGCTCAAATGACTCAGCTTGTCC	
U341	DsMV	CCGGAATTCATGRTITGGTGYATIGAIAAYGG	~700
oligodT		GACTCGAGTCGACATCGTTTTTTTTTTTTTTTTT	
TaBV12F	TaBV	TCCGAATATGATGCTTTGGTCC	603
TaBVCPR		GCATTCTCGAGCAAAATGTCC	

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3 Results

3.1 Observations on Taro Virus Symptoms / Diseases

The details of the locations surveyed, grower information, and observations made at each location are provided in Table 3-1.

Table 3-1 Locations, growers and observations made during survey

Date	No.	Location	Grower	Comments
6 May	1	Cairns 35 Sperring St, Manunda	Wendy Davie and Len Specht	A hydroponics set up which includes a few taro among many other vegetables. The pink taro (probably var. Niue) were healthy. Some plants of what may be Zuiki (a species of *Colocasia* or *Xanthosoma*) growing elsewhere in the garden appeared to have symptoms of DsMV. There were no planthoppers and no symptoms of other taro viruses.
	2	Yarrabah 1685 Pine Creek Road, Glen Boughton	Denis and Irene Garvey	They are retired but still grow a few hundred taro, both Bun Long and the pink taro (cv. Niue). There were two lots of the latter as this is now the taro in demand locally, whereas Bun Long is 'exported' to the cities in Queensland and other states. There are two plots of about 200 plants. One plot was planted a month ago, the other 2 months ago. The older plants were sampled. The oldest leaves on many of the plants showed an unusual vein-clearing (faint chlorosis of the veins). This is a symptom unlike TaVCV, but one that has not been recorded previously. Some plants showed severe symptoms of Roundup (glyphosate) damage, with the first and second youngest leaves smaller than usual, severely chlorotic, and in some instances with white margins. Bun Long plants also showed herbicide damage, but no signs of the vein chlorosis.
	3	Yarrabah Pine Creek Road (next to no. 2 above) South 16 56.065 East 145 48.951	Sam Vui and sister Liz Vui	There are several hectares of taro divided into plots of different ages. Most plants were the pink taro (cv. Niue), but there were also the occasional plants of cv. Alafua, which is green and has yellow corms. Plants ready for harvest commonly showed symptoms of DsMV, and ants and aphids were present on these plants. All plants had been sprayed with insecticide against the 'small butterfly' – presumably *Spodoptera litura*, the caterpillars of which were seen occasionally.
	4	Deeral 68999 Bruce Highway South 17 13.099 East 145 54.639	Esau and Faye Suluvale	Several hectares of taro, mostly the pink variety (cv. Niue) with some rows of Tusi Tusi (Samoan name for a short variety with yellow corms). DsMV was common on cv. Niue, but rarely seen on Tusi Tusi. No *Tarophagus* seen, possibly because plants were sprayed with insecticide.

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Date	No.	Location	Grower	Comments
	5	Gordonvale 10 km south along Bruce Highway at Aloomba South 17 09.240 East 145.51.556	Sam Philipps, Secretary of the Taro Grower Association	Bun Long is grown exclusively for markets in the major cities, where it is a favourite among Asian communities and commands a higher price than cv. Niue. The taro are sold as 'organic'; it is not certified but is recognised as a high quality product, grown without the use of fertiliser or pesticides. Dipel is used 2–3 times at about 3 months against cluster caterpillar. At present, there are several long blocks of Bun Long at different ages, from 2 weeks to 8 months. Except for older blocks with ghost spot, the plants are very healthy, without signs of DsMV or *Tarophagus*. Plants are heavily mulched with dried grass. The ghost spotting is interesting. The most severely affected plot is that of the oldest plants on the windward side of the field. It appears that this is now providing inoculum for the other younger plots downwind. Samples were taken for identification.
	6	Cairns 1 Burton Close, White Rock South 16 58.122 East 145 45.066	Aunty Anna	Three PNG highland varieties, and a few cv. Niue. One plot of about 35 plants of mixed varieties, about 6–8-weeks-old, has severe DsMV; some of the plants have strap-like leaves without lobes. There are no symptoms of other viruses, and no *Tarophagus*. Aunty Anna expects all plants with DsMV to recover. No pesticides are used, nor is fertiliser applied; however, they are mulched with banana leaves and coconut husks.
7 May	7	El Arish, north of Tully South 17 52.529 East 146 01.685	Ken Lake	Two 2-hectare blocks of healthy Bun Long planted in February; there is no DsMV or *Tarophagus*. Alongside one of the blocks are six rows of 2–3-week-old suckers. Another block is near maturity, and is also healthy, except for a few plants with *Phoma* sp. shot-hole disease at the border.
	8	Percy Benn Bridge, Granadilla Road, El Arish South 17 49.178 East 146 00.582	Wild taro	A patch of purple wild taro about 30 metres downstream from the bridge. A few *Tarophagus* were present and collected, mostly early and late instars. A sample was taken from a plant showing vein chlorosis with the tissue breaking down over the veins.
	9	Bilyana, south of Tully South 18 07.012 East 145 56.549	Pat McCool	A large block of Bun Long about 6-months-old. No DsMV symptoms and no planthoppers. There is a second block adjacent to it which has been harvested. Cluster caterpillars are more common in the second block and are supplying moths for the first. The grower has used Lorsban (chlorpyriphos) against the cluster caterpillar.
	10	East Feluga East Feluga Road, north of Tully South 17 54.105 East 146 00.477	Peter Salaris	No taro are grown anymore. However, there are several varieties present in small clumps around the house and out buildings said to have come from Brian Watson when he was at Kamerungu Research Station. These were taro that Grahame Jackson sent as pathogen-indexed tissue cultures from Fiji. One is E26, which is Toakula. No symptoms of virus or *Tarophagus*.

Date	No.	Location	Grower	Comments
	11	Babinda South 17 20.730 East 145 56.711	Mick Kelly	Several varieties are grown, but the most common is Alafua Sunrise. About 1.5 tonnes are sent to markets in Sydney and Melbourne each month. It is well accepted, and has been a great success over the years. There is also a green petiole variety, which is said to be a 'hybrid' (possibly a mutant selection). It has very large corms and few suckers. Drip irrigation is used and if there are any pests, spot sprays of an aerosol insecticidal are used to manage them. Herbicides are applied around the block of about 3000 plants. Cassava and a yellow-fleshed sweet potato from PNG that produces large storage roots are also grown.
8 May	13	Innisfail to Ellinjaa Falls South 17 29.626 East 145 39.322	N/A	Three taro on the river bank, 50 metres from the falls, but without DsMV or *Tarophagus*.
	14	Innisfail to Zillie Falls South 17 28.502 East 145. 39.341	N/A	Access to the falls is from above, with no easy access to the river below. The stream feeding the falls was checked but no taro were found.
	15	Innisfail to Millaa Millaa Falls	N/A	The river was followed several hundred metres from the falls, but there were no taro.
	16	Innisfail to Lakes Drive, Lake Eacham South 17 17.831 East 145 38.080	Alex Griffin	A variety of vegetables are grown for sale at the local markets; these include taro, cassava, onions, yams, bananas, chokos and many more. Alex was in PNG for 30 years working on rubber estates and in logging. He has several hundred taro, some at maturity, others recently planted. The soil is a red clay to which he adds dolomite. Sales are to Chinese and Philippine traders. Most of the taro are Tusi Tusi (Ba via in Fiji), but there are also a few others. They are badly affected by Phoma sp. shot-hole disease. Symptoms of DsMV are common, and there are aphids. Samples were taken of what looks like DsMV with necrosis over the veins. Unusual.
	17	Henrietta Creek, Palmeston Hwy, Innisfail South 17 35.886 East 145 45.487	N/A	A search was made for taro along the river for about 200 metres from the waterfall. Unfortunately, none were found.
	18	Carmoo, north of Tully (Corner of Mission Beach Road and East Feluga Road) South 17 55.310 East 146 00.590	Tanu and Naomi Wulf	Two large blocks; one has already been harvested where suckers remain among weeds. The large block contains about 1000 plants in six rows. There is severe glyphosate spray damage along the edge of one block. Each block contains cv. Niue and Alafua Sunrise. DsMV is present and so are aphids, but both are uncommon. Corms of cv. Niue have a condition that is of concern: about 70% of the corms contain hard, irregular, light brown inclusions of various sizes (some several cm). These affect market value as even after cooking they retain their firmness and are an unattractive feature.
9 May	19	Neame Street, Ingham South 18 38.608 East 146 09.586	Jeff Bow and Alice	A few plants of three taro varieties (Bun Long, Niue and Tusi Tusi) are present in a rather overgrown garden. Jeff and Alice gave up growing taro commercially about 7 years ago. No *Tarophagus* are present, although there are aphids. DsMV was not seen.

Date	No.	Location	Grower	Comments
	20	Trebonne, Ingham (Abergowrie Road and Sheahams Road). South 18 30.894 East 145 59.410	Paul Koy	A large planting of several thousand mature (but small) Bun Long, and several other varieties in an area overgrown with weeds. A row of a very white taro is probably a Japanese variety. No *Tarophagus* were found, and aphids were present although rare. DsMV was absent. A block of much younger Bun Long were present adjacent to the main planting, with symptoms of cluster caterpillar. The large block of Bun Long showed symptoms of fertiliser burn. Youngest leaves were healthy, the second showed small white irregular lesions above, and brown, roughly round, spots below, 2–3 mm diameter; however, the above and below spots were independent of each other. Generally, lesions were more pronounced on the left side, the side that unfurled first. The brown spots were similar to symptoms of ghost spot, except the spots were not present on the upper leaf surface. Mostly, the lesions on the upper surface remained small, irregular and purple. Younger plants of Bun Long among trellised fruit trees showed brown lesions similar to those seen at Sam Philipps.
	21	Volk Road, Matarnee, south of Ingham South 18 57.584 East 146 15.887	Herbert Fehrenbacher	Herbert grew taro previously but has given up growing the crop.
	22	Jourama Falls, south of Ingham (nr Bruce Hwy)	N/A	A small patch of perhaps 20 green wild taro with suckers were seen at the bridge before the picnic area. No DsMV, aphids or *Tarophagus* were present.
10 May	23	Mackay Community Gardens, Railway Estate South 19 17.096 East 146 49.300	Several	Met Roger Winton and Andrew Ygosse at the Department of Agriculture, Fisheries and Forestry office in Townsville, and with Andrew went to the Mackay Community Gardens at the Railway Estate. Numerous people were growing vegetables of various kinds, including a few taro. Plants showed occasional symptoms of DsMV in island taro and many aphids. There were no *Tarophagus*.
	24	Oak Valley, Greta Road, and Alligator Creek and Bentley Drive, Nome, Townsville	Several	Farmers were met at all these places but none was growing taro; one said that returns were not sufficient to make taro an attractive crop.
11 May	25	Bakers Creek, Mackay 71 Main Street South 21 12.447 East 149 08.838	Doug Mooney	A descendent of a marriage between a Solomon Islands woman and a man from Vanuatu. He has been growing taro for many years in his garden. Most of the plants are Bun Long. Next year there is the 150th anniversary of the South Sea Islander Association, when it is expected that people will increase their taro production. The taro were healthy: there were no symptoms of DsMV, and no *Tarophagus*. Some aphids were seen.

Date	No.	Location	Grower	Comments
	26	Mackay Midge Point Road, Bloomsbury South 20 41.081 East 148 36.801	Robert and Rebecca Hold	A large plot is present (about 50 x 40 m), containing several thousand Bun Long taro. The taro are sold at the Show Ground Markets on Sundays. Some rows have been ratooned and fertilised (Nitrophoska) and the plants have formed a canopy over the ground. In other areas, the plants have been harvested with one or two plants per stand still remaining. The plants have been planted through black polythene to control weeds. None of the plants showed symptoms of DsMV, although aphids were present. No *Tarophagus* were seen. Many small grasshoppers were present, and there were signs of cluster caterpillars, but neither pest required control measures.
12 May	27	Ashgrove, Brisbane South 27 26.777 East 152 57.568	N/A	Patches of taro were examined along the creek that runs through the Ashgrove Golf Course. They have deep purple petioles and leaves with purple patches, similar to those at the Percy Benn Bridge. Aphids were common, but there was no sign of DsMV. However, one leaf showed dark green areas bordering the main veins on the top surface. Below, these areas are clearly defined: the smaller veins – those branching from the thick main veins – were purple, giving a feather-like appearance.
13 May	28	Cudgen, NSW South 28 26.221 East 153 55.624	Ross Julien	About 9 hectares of Japanese taro, cv. Ishikawa Wase, were present; botanically, this taro is *Colocasia esculenta* var. *antiquorum*, although modern molecular methods have found no difference between the variety and Pacific taro, *C. esculenta* var. *esculenta*. However, *antiquorum* taro produce a central corm with many cormels which are "blind", meaning that they are without leaves. By contrast, *esculenta* taro produces suckers with leaves above ground. The *antiquorum* cormels can be stored, and in many countries storage lasts several months, perhaps through winter or a seasonal dry period. When conditions are right for growth, the cormels are used as planting material for the next crop. DsMV was common in Ishikawa Wase, but no other symptoms of virus were seen. Older leaves showed lesions of ghost spot. *Tarophagus* were not seen. A block of "wild" *C. esculenta* taro in a swampy area nearby was without DsMV, but showed symptoms of shot-hole, caused by *Phoma* sp.
14 May	29	Browns Plains, Brisbane South 27 39.480 East 153 02.419	Paulo	A grower of Samoan ethnicity. A small backyard plot of about 75 taro, mostly cv. Niue, with some Bun Long. Aphids were present, and DsMV was common. Mites, too, were present along the veins, but no *Tarophagus*.
	30	Woodridge, Brisbane South 27 38.332 East 153 05.938	Starling	A garden of perhaps 30 plants of cv. Niue and a few Alafua Sunrise. DsMV and aphids were present on most plants. By contrast, a small number (approximately 12) of wild taro, used for their youngest leaves, growing next to the plot, showed no aphids and no symptoms of DsMV.

3.2 Virus Testing of Plant Samples

A total of 24 leaf samples were taken from plants showing virus-like symptoms, as well as from symptomless leaves. Information on the location of the plants that were sampled and any symptoms they were showing is provided in Table 3-2. Representative symptoms are depicted in Appendix A.

Table 3-2 Details of leaf samples collected for virus testing

Sample	Location (refer to Table 3-1 above)	Symptoms
1	1	DsMV-like
2	2	DsMV-like
3		DsMV-like
4		DsMV-like
5	7	DsMV-like
6	8	Unusual
7	16	DsMV-like
8		DsMV-like
9		DsMV-like
10		DsMV-like
11		Symptomless
12	18	DsMV-like
13		DsMV-like
14	20	Symptomless
15	25	Symptomless
16	26	Symptomless
17	27	Unusual
18		Symptomless
19	28	DsMV-like
20		DsMV-like
21		Symptomless
22	29	DsMV-like
23		Symptomless
24	30	Unusual

Although the major objective of this study was to test for the presence of TaVCV, plants were also tested for the potyvirus, Dasheen mosaic virus (DsMV), and the badnavirus, Taro bacilliform virus (TaBV). Nucleic acids were extracted from fresh leaf material for each of the 24 samples. As a positive control for TaVCV, nucleic acid was also extracted from a plant known to be infected with TaVCV (provided by the Secretariat of the Pacific Communityin Fiji). Positive controls were also included for DsMV and TaBV testing. As a quality control for the extraction of RNA, reverse transcription polymerase chain reaction (RT-PCR) was carried out using primers that detect the 18S ribosomal RNA gene sequence. This same primer set was used to detect the presence of genomic DNA using PCR directly from extracts. RT-PCR assays were then performed using two different primer sets for the detection of TaVCV and one set of primers for the detection of potyviruses (DsMV). In addition, PCR was carried out for the detection of Taro bacilliform virus (TaBV).

Prior to testing the 24 samples for the presence of the RNA viruses (TaVCV and DsMV), the presence and quality of RNA in each of the nucleic acid extracts was examined. This was done by testing the extracts for the presence of 18S RNA (encoded by the house-keeping 18S DNA gene) by RT-PCR. However, to ensure that the PCR primers are specifically amplifying 18S RNA, and not contaminating 18S DNA, the presence of DNA in the extracts was checked by PCR. As shown in the gel below

(Figure 3-1), when PCR was carried out on the 24 nucleic acid extracts (lanes 1–24) to detect the presence of any contaminating DNA, PCR products (amplicons) of the expected size (500 bp) were amplified from all samples except 11, 20 and 21. This indicated that almost all extracts contained contaminating DNA.

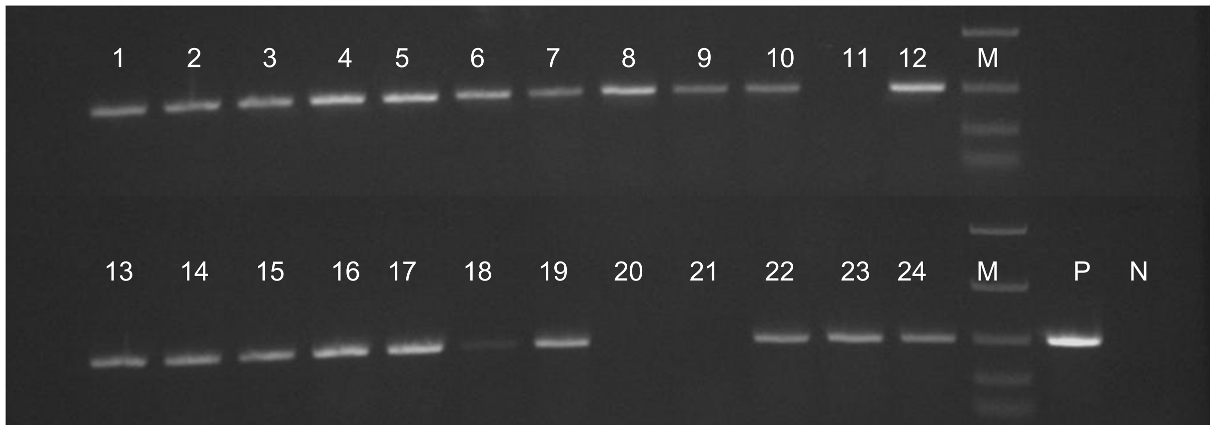

Figure 3-1 18S PCR on nucleic acid extracts to check for the presence of contaminating DNA

Molecular weight marker = M; positive control = P; no template control = N

To remove the contaminating DNA, the 24 extracts were treated with DNase and then the PCR was repeated. As shown in Figure 3-2, no products were amplified, indicating that the contaminating DNA had been digested by the DNase treatment.

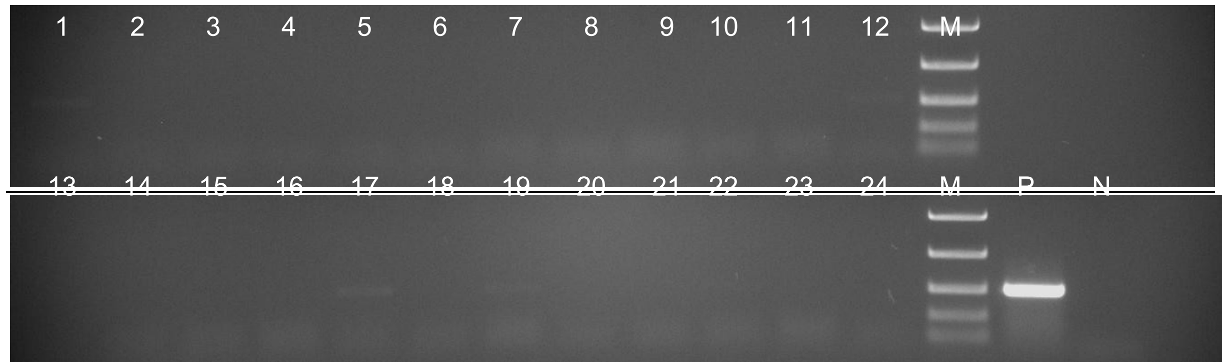

Figure 3-2 18S PCR on DNase-treated extracts to check for the presence of contaminating DNA

Molecular weight marker = M; positive control = P; no template control = N

The DNase-treated extracts were subsequently used to test for the presence of 18S RNA by reverse-transcription PCR (RT-PCR). As shown in Figure 3-3, amplicons of the size expected for 18S RNA (190 bp) were present in all extracts except sample 20. These results indicated that RNA was successfully extracted for all samples except sample 20. The extracts were subsequently used for the detection of TaVCV and DsMV.

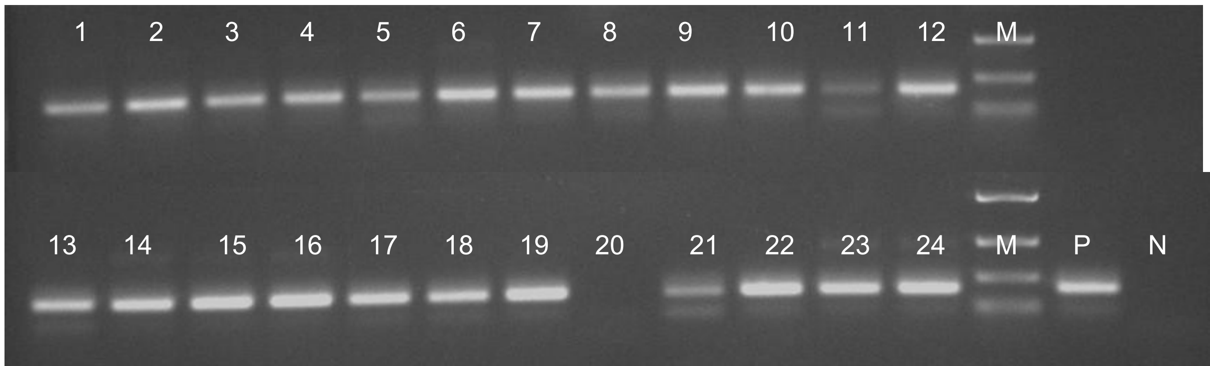

Figure 3-3 RT-PCR to check for the presence of 18s RNA

Molecular weight marker = M; positive control = P; no template control = N

Testing for TaVCV

PCR was done on cDNA prepared from the 24 RNA extracts using two different virus-specific primer sets, TaVCV1/2 and TaVCVPolA1/A2. These were designed to analyse different regions of the TaVCV genome and were used to maximise the chances of detecting any TaVCV isolate that may have been present.

As shown in Figure 3-4 and Figure 3-5, no products were amplified from any of the 24 samples using either of the primers pairs. As expected, the extract from the known TaVCV-infected plant included as a positive control tested positive, while the no template control tested negative.

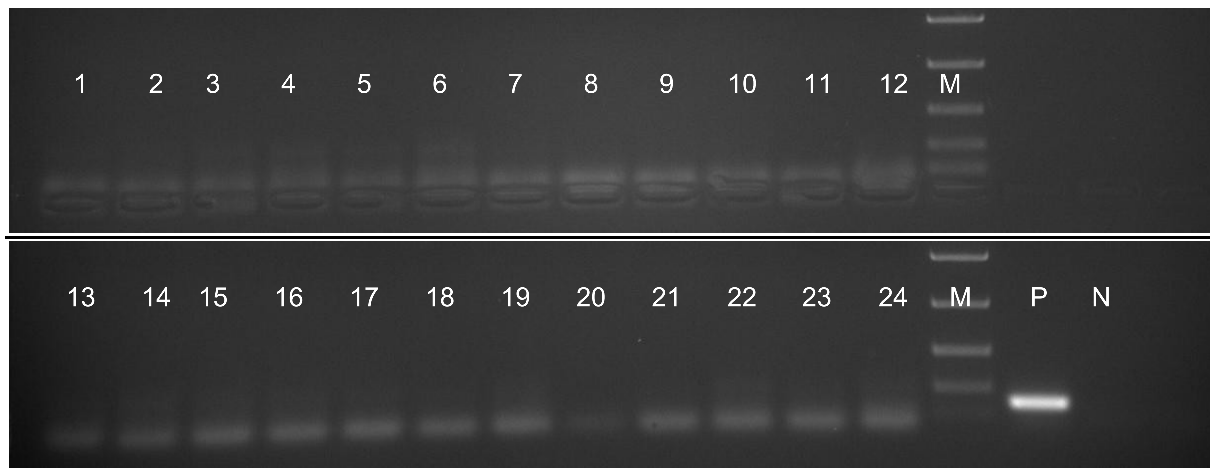

Figure 3-4 PCR, using primers TaVCV1/2, to detect the presence of TaVCV

Molecular weight marker = M; positive control = P; no template control = N

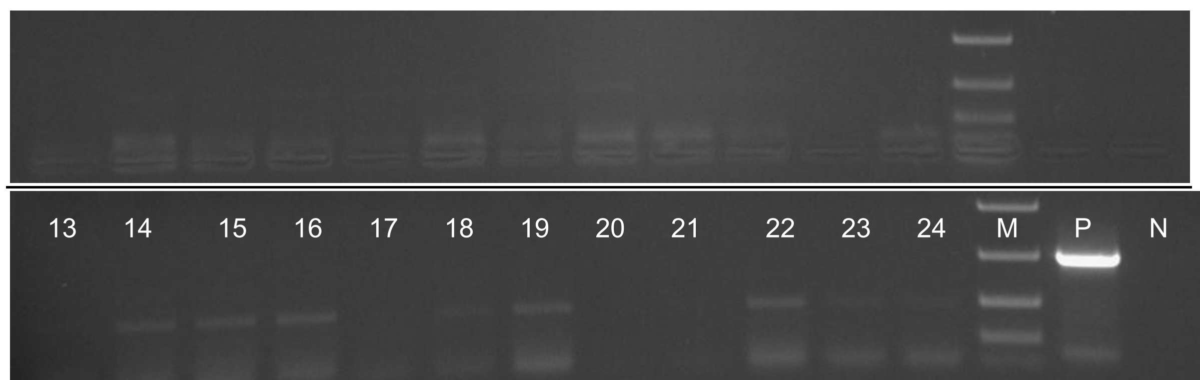

Figure 3-5 PCR, using primers TaVCVPolA1/A2, to detect the presence of TaVCV

Molecular weight marker = M; positive control = P; no template control = N

Testing for DsMV

PCR was carried out on cDNA prepared from the 24 RNA extracts to detect the presence of Dasheen mosaic virus using primers U341/oligodT. These primers are designed to amplify any potyvirus, including DsMV. As shown in Figure 3-6, a product of the expected size of approximately 700 bp was amplified from samples 1–4, 7–10, 12–13, 19, 22 and 24.

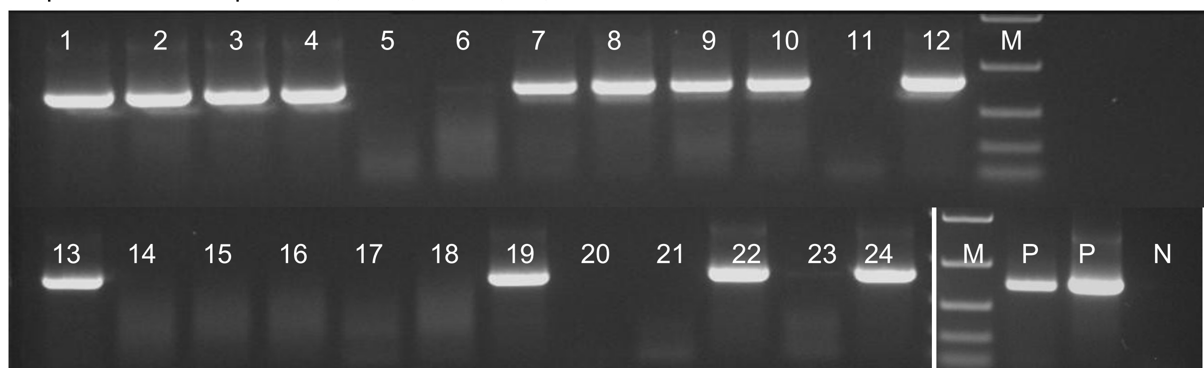

Figure 3-6 PCR, using primers U341/oligodT, to detect the presence of DsMV

Molecular weight marker = M; positive control = P; no template control = N

To confirm that the amplified product was DsMV, amplicons from samples 2, 19 and 22 were cloned and sequenced (see Appendix B to Appendix D). Analysis of the sequences showed that they were 92–95% similar to published DsMV sequences, thus confirming their identity as DsMV.

Testing for TaBV

Since TaBV is a DNA virus, PCR was carried out directly from the 24 nucleic acid extracts using TaBV-specific primers, TaBV12F/TaBVCPR. As shown in Figure 3-7, a product of the size expected for TaBV was amplified from samples 7–10, 12–13 and 22–24.

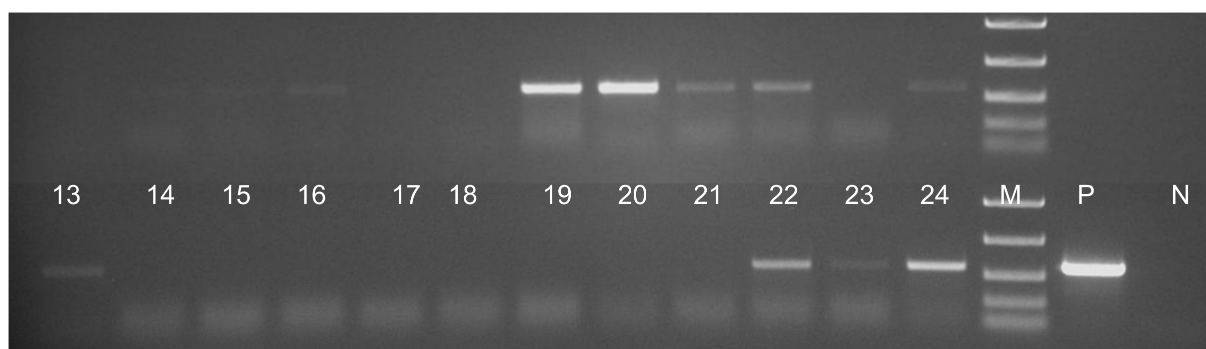

Figure 3-7 PCR, using primers TaBV12F/TaBVCPR, to detect the presence of TaBV

Molecular weight marker = M; positive control = P; no template control = N

To confirm that the amplified product was TaBV, the amplicon from sample 8 was cloned and sequenced (see Appendix E). Analysis of the sequences showed that it was 92% similar to the published TaBV sequence, thus confirming its identity as TaBV.

Re-testing of sample 20

As mentioned previously, the control reactions for sample 20 were negative, indicating either that the nucleic acid extraction from this sample was unsuccessful or that the extracted nucleic acid was of a poor quality. Therefore, fresh extractions were done for sample 20 and the testing was repeated as described above. Using these fresh extracts, the 18S RNA housekeeping gene was amplified as expected, indicating that RNA was successfully extracted. The extracts were subsequently tested for TaVCV and DsMV, along with the known positive controls. The known positive controls that were included amplified the correct size product as expected. However, no products of the size expected for TaVCV or DsMV were amplified from sample 20 using the primers to detect these two viruses.

A summary of the entire virus testing is provided in Table 3-3.

3.3 *Tarophagus* collections

Tarophagus planthoppers were found on one occasion only: at Percy Benn Bridge, Granadilla Road, El Arish. This was unexpected, considering the ease with which a previous report found *Tarophagus* on wild taro (Matthews, 2003). None were found in commercial plantings. There was no obvious reason for their absence, although one could speculate that their absence was due to routine applications of pesticides in commercial plots for cluster caterpillar control. There is also the possibility that floods in Queensland in recent years have washed out wild taro patches, as visits to several of the localities where Matthews (2003) previously recorded wild taro failed to find the plants during the present survey.

The sample of *Tarophagus* collected at Percy Benn Bridge was identified as *T. colocasiae*, which has been previously reported in Queensland.

Table 3-3 Summary of PCR testing of taro samples for TaVCV, DsMV and TaBV

Sample	Location	Symptoms	TaVCV	Potyvirus (DsMV)	TaBV
1	1	DsMV-like	-	+	-
2	2	DsMV-like	-	+	-
3		DsMV-like	-	+	-
4		DsMV-like	-	+	-
5	7	DsMV-like	-	-	-
6	8	Unusual	-	Faint	-
7	16	DsMV-like	-	+	+
8		DsMV-like	-	+	+
9		DsMV-like	-	+	+
10		DsMV-like	-	+	+
11		Symptomless	-	-	-
12	18	DsMV-like	-	+	Faint
13		DsMV-like	-	+	Faint
14	20	Symptomless	-	-	-
15	25	Symptomless	-	-	-
16	26	Symptomless	-	-	-
17	27	Unusual	-	-	-
18		Symptomless	-	-	-
19	28	DsMV-like	-	+	-
20		DsMV-like	-	-	Not tested
21		Symptomless	-	-	-
22	29	DsMV-like	-	+	+
23		Symptomless	-	Faint	Faint
24	30	Unusual	-	+	+

4 Conclusions

- During the survey, no symptoms typical of those associated with TaVCV infection, alomae or bobone disease were observed on any taro plants.
- The only virus symptoms observed during the survey were those normally associated with DsMV.
- In almost all cases, plants showing DsMV-like symptoms tested positive for this virus as expected.
- Several plants were found to be infected with both DsMV and TaBV (mixed infections are not uncommon and have been reported elsewhere).
- The presence of DsMV and TaBV in representative positive samples was confirmed by sequencing. These viruses are already known to occur in Australia.
- A number of taro plants exhibiting unusual, virus-like symptoms were observed. These symptoms may not necessarily be due to virus infection but could be due to nutritional deficiencies, physiological disorders or some other cause. Samples from plants showing unusual symptoms were collected from three locations (samples 6, 17 and 24) and were tested for viruses. Sample 6 tested positive for DsMV, sample 24 tested positive for DsMV and TaBV, and sample 17 tested negative for all three viruses.
- The lack of typical TaVCV symptoms observed on any taro plants, together with the negative results for TaVCV in all samples tested using the sensitive molecular test known as PCR, suggests that TaVCV is not present in the regions surveyed.
- A single collection of *Tarophagus* planthoppers was made, and identified as *T. colocasiae*, a species previous reported in Queensland.

5 References

Biosecurity Australia 2011, *Review of import conditions for fresh taro corms*, Biosecurity Australia, Canberra.

Matthews, PJ 2003, 'Taro planthoppers (*Tarophagus* spp.) in Australia and the origins of taro (*Colocasia esculenta*) in Oceania', *Archaeology in Oceania,* no. 38, pp. 192–202.

Revill, PA, Jackson, GVH, Hafner, GJ, Yang, I, Maino, MK, Dowling, ML, Devitt, LC, Dale, JL & Harding, RM 2005, *Australasian Plant Pathology,* no. 34, pp. 327–331.

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Appendix A Representative Symptoms Observed During the Survey

Plate A-1 Plant sample 1

Plate A-2 Plant sample 2

Appendix A

Plate A-3 Plant sample 5

Plate A-4 Plant sample 6

Appendix A

Plate A-5 Plant sample 10

Plate A-6 Plant sample 12

Appendix A

Plate A-7 Plant sample 17

Plate A-8 Plant sample 19

Appendix A

Plate A-9 Plant sample 20

Plate A-10 Plant sample 24

Appendix B Sequence of Potyvirus RT-PCR Fragment (Sample 2)

Length = 716 bp (including forward primer) with 95% identity to Dasheen mosaic virus isolate from New Zealand (GenBank accession no. gb|AY994105.2|).

ATGATGTGGTGTATGGAGAACGGAACATCACCTGATATTAACGGGGCTTGGGTGATGATGGACG
GAAATGATCAAATTGAATACCCGTTAAAACCAATTGTGGAAAATGCCAAACCAACCTTGCGTCAGA
TTATGCATCACTTTTCCGACGCAGCAGAGGCATACATTGAACTGAGAAATGCGGAAAAACCATAT
ATGCCTAGATATGGTTTAATCCGCAATCTTCGTGATGCAAGTCTCGCCCGGTATGCTTTTGACTTC
TATGAAGTAAATTCAAAGGCACCGGTTCGAGCTAGGGAAGCAGTAGCACAGATGAAGGCTGCCG
CCCTATCTAAGTTACCACTAGGTTATTTGGATTGGATGGTAACGTTTCTACATCAAGTGAGAACAC
TGAGAGGCATACTGCGAAGGACGTGACACCAAATATGCACACTCTGCTTGGTGTTTCACCTCCAC
AGTGAAGGGCTGGTCAACAGACCACAGTTATAGTCTCACTATCCGTATCTTTATTTTGCATTTAGT
ACTGTTGGTGTGGATATAGTTTACTCTATTTACTGCACTTACTACAGAGTGGTCTGCCACCGGTAT
GGAGTGGTGCTGTGCACCCTATATTTCATATCCTTTATGCTTTCTAAAGTTACTGAGCTACTGCAC
TTATGTCGAGCCGTAAGTGCGCCAATGGCGTGGTAGGTAAGACACTGAGTGCACGGTGCTC

Appendix C Sequence of Potyvirus RT-PCR Fragment (Sample 19)

Length = 710 bp (including forward primer) with 93% identity to Dasheen mosaic virus isolate from China (GenBank accession no. emb|AJ298036.1).

ATGGTGTGGTGTATGGAGAATGGAACATCACCCGATATCAACGGGGCTTGGGTGATGATGGACG
GAAATGATCAAATTGAATACCCGTTGAAGCCAATTGTAGAAAATGCAAAACCCACCTTGCGTCAGA
TAATGCATCACTTTTCTGACGCTGCAGAGGCGTACATTGAACTTAGAAATGCGGAGAAACCGTAC
ATGCCTAGGTACGGTCTTATTCGCAACTTACGTGATGCAAGTCTCGCCCGGTATGCTTTCGATTT
CTATGAAGTCAATTCTAAGACGCCGGTGCGAGCAAGAGAGGCAGTTGCGCAGATGAAGGCCGCT
GCACTCTCCAACGTTACTACTAGGTTGTTTGGTTTGGATGGTAACGTTTCAACTTCAAGCGAGAAC
ACTGAAAGGCACACTGCGAAGGACGTCACACCCAACATGCATACATTGCTTGGTGTGGCACCTC
CGCAGTAAAGGTCTGGTAAACAGACCATAGTTATTGTCTCGCTATCTGTAGTTTTATATATTTAAAG
TACTGTTTGTATTCGAATAGTGTTATTTGATTATAAACTACAGAGTGGTTTTCCACCGATGTAGAGA
GGTGCTGTGCATCCTATTATCTACGTCCTTTAAATATAAGAAAACTGCTGAACTACTGCACCTACA
TCAGACCGTAAGTGCGCCATGGGCGCGGTAGGCGAGATGCTTCGTGCACGGTGTTC

Appendix D Sequence of Potyvirus RT-PCR Fragment (Sample 22)

Length = 716 bp (including forward primer) with 92% identity to Dasheen mosaic virus isolate from New Zealand (GenBank accession no. gb|AY994105.2|).

ATGGTGTGGTGTATGGAGAATGGAACATCACCCGATATCAACGGGGCTTGGGTGATGATGGATG
GAAACGATCAAATTGAATACCCGTTGAAACCAATTGTGGAAAACGCAAAACCAACCTTGCGTCAG
ATTATGCATCACTTTTCAGACGCAGCAGAGGCATATATTGAACTGAGAAACGCGGAGAAACCGTA
CATGCCTAGGTACGGTTTGATTCGCAATCTTCGTGATGCAAGTCTCGCCCGGTATGCTTTTGACT
TTTATGAAGTTAATTCAAAGACACCGGTTCGAGCTAGGGAAGCAGTAGCGCAGATGAAGGCTGCT
GCCCTATCTAACGTTACCACTAGGTTGTTTGGATTGGATGGTAACGTTTCTACATCAAGTGAGAAC
ACCGAGAGGCACACTGCAAAAGACGTGACACCAACTATGCATACTCTGCTTGGTGTGTCGCCTC
CACAGTAAAGGTTTGGTAAACAGACCACAGTTATAGTCTCACTATCTGTATCTTTGTTATGCATTTA
GTATTGTATGTGTGAGTATAGTTTGCTCTATATGATGCACTTACTACAGAGTGGCTTGCCACCGAT
ATGGAGTGGTGCTGTGCACCCTACATTGCATATCCTTTATGCTTTCTAAAGTTACTGAGCCACTGC
ACTTATGTCGAACCGCAAGTGCGCCAATGGCGCGGTAGGTAAGACACTGAGTGCACGGTGCTC

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Appendix E Sequence of TaBV PCR Fragment (Sample 8)

Length = 603 bp (including primers) with 92% identity to positions 2838–3440 of the published TaBV full length genome (GenBank accession no. gb|AF357836.1) [partial coat protein gene sequence]

TCCGAATATGATGCTTTGGTCCAACAGAGTGATGAAACCCAGAACCTGCTCTCTCAGGTTCGAAG
AATTTTTCTTTTACAAGACCCCTATCAGGGATCAACAGCGGAACAAGATCAAGCCTATAATGATCT
TGAAAGAATTTCATGTGATAATATCAAGGACTTAATTCCTTATCTGATTCAGTTCCGCAATTTGGCT
GCAAAATCTGGACGTTTATTTCTTGGTCCAGAATTATCTGAAAAATTGTTCAGGAAAATGCCGCCT
CTCATAGGCAAAGAAATTGAAGCAGCATTCATAGCCAAGCACGGTAATGCAAACATCACTGTTAT
GCCCAGAATTCATTTTGCTTACCATTATTTGGCTGAATTATGCAAAAAGGCAGCATTACAGAGGTC
TTTGAAAGATCTCAGCTTCTGCAACCAGATTCCTCTTCCAGGAATCTATTCAAAAGGTAACAAGAA
ATTCGGCCTCAGAAAGGCCAGAACATACAAAGGTAAACCACATCCAACTCATGTACGGGTATTCA
AAAAGGCAAAATACCAGCGTACAAAGAAATGCAAATGCTTTATCTGTGGTGAACCAGGACATTTTG
CTCGAGAATGC

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