Lethal taro viruses: Still unresolved

Grahame Jackson & David Gollifer

Sydney, Australia
& Great Bentley, United Kingdom

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Abbreviations

ACIAR	Australian Centre for International Agricultural Research
ACP	Anti-coated plate (ELISA)
AusAID	Australian Agency for International Development
CBDV	Colocasia Bobone-disease virus
CePaCT	Centre for Pacific Agricultural Crops and Trees
CIRAD	International Research for Development (France)
DAS	Double antibody sandwich (ELISA)
DsMV	Dasheen Mosaic virus
DSMZ	German Collection of Microorganisms and Cell Cultures
dsRNA	Double-stranded ribonucleic acid
ELISA	Enzyme-linked imunosorbent assay
EM	Electron microscope
EPPO	European Plant Protection Organisation
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FSM	Federated States of Micronesia
IND	Indonesia
INEA	International Network for Edible Aroids
LegCo	Legislative Council
LPS	Large particle symptom
MAFF	Ministry of Agricultural and Fisheries (Samoa)
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
PNG P	Papua New Guinea
PSB	Philippines Seed Board
RNA	CBDaV
RT-PCR	Reverse transcription-PCR
SPC	South Pacific Commission
SRNA	Small ribonucleic acid
TaBCHV	Taro bacilliform CH virus
TaBV	Taro badnavirus
TANSAO	Taro Network for Southeast Asia and Oceania
TaroGen	Taro Genetic Resources: Conservation and Utilisation
TaRV	Taro reovirus
TaVCV	Taro vein chlorosis virus
UK	United Kingdom
UNDP	United National Development Programme
USDA	United States Department of Agriculture
WWII	World War II

Summary

Research into the virus diseases of taro in Solomon Islands began in the late 1960s at Dala Experimental Station on the west coast of Malaita.

The diseases were not at first obvious as station workers removed infected plants from variety trials as they knew the risks from leaving the diseased plants. Malaita has two kinds of taro: “male” and “female”. This has nothing to do with their sex; “male” taro are generally larger, but die from a lethal disease locally known as *alomae*, whereas “female”, are resistant to *alomae*, but instead are susceptible to another disease called *bobone*. Most taro in Solomon Islands are “male”.

Plants with *alomae* often show yellow twisted young leaves, stop growing and succumb to a rapid necrosis; those with *bobone* develop several stunted thickened leaves, frequently with galls, and then the leaves recover and plants appear healthy. Early tests suggested that males were triploid and females diploid, but that was wrong: they are all diploid.

Diseased leaves was sent to Rothamsted, UK, in 1971, where three virus particles were found: a flexuous rod, and two bacilliform particles (one large and one small). Both the bacilliform viruses were new to science.

Transmission tests followed at both Dala and Rothamsted: the aphid, *Aphis gossypi*i, transmitted the flexuous rod; the taro planthopper, *Tarophagus proserpina,* transmitted the large bacilliform particle; and the mealybug, *Planococcus minor*, transmitted the smaller bacilliform particle*.* Our initial thoughts were that a*lomae* was caused by both large and small bacilliform particles, and *bobone* by the presence of the large bacilliform particle alone.

Unfortunately, we could not reproduce *alomae* by taking planthoppers fed on *bobone* (large particle), and mealybugs fed on *alomae* (small particle). However, we could produce *alomae* if planthoppers were fed on *alomae* plants. To us, at the time, either planthoppers transmitted both particles, or they transmitted the large particle and the small particle was latent in the test plants. But before we could find out by using tissue culture pathogen-indexed plants, Dala Experimental Station was closed at the end of 1975.

In 1998, research resumed associated with a sub-regional project known as TaroGen, funded by AusAID and ACIAR. The main aim of the project was to breed taro tolerant to taro leaf blight, following an outbreak of the disease in Samoa in 1993. If countries were to share the results from the breeding program, the new taro lines had to be free from virus. Hence, the need to know more about *alomae* and *bobone*.

Queensland University of Technology, Brisbane, took the lead and by 2003, five viruses had been identified and diagnostic tests developed. No longer were tests reliant on electron microscopy, but based on serological and molecular methods. Under the project, two new virus particles were found - another bacilliform virus (Taro vein chlorosis virus) plus a spherical one (Taro reovirus) – making the cause of *alomae* even more unclear. However, funding ceased in 2005, and the research stopped again.

Another 5 years passed until 2010 when an EU-funded global taro project created an international network for edible aroids as a model to improve clonally propagated root and tuber crops in tropical countries. This time, DSMZ, Braunschweig, Germany, took the lead, using even more sophisticated methods to check previous protocols for international germplasm movement. Again, there were new discoveries, the most important of which was the presence of a tenuivirus not previously reported.

This was an interesting find. Some tenuiviruses are transmitted by planthoppers. Could it be that the taro planthopper spread the tenuivirus as well as the large bacilliform virus (now named Colocasia Bobone disease virus)? This might mean that the small bacilliform virus (Taro badnavirus) was not involved in *alomae*; instead, it was caused by CBDV and the tenuivirus. But funding ceased at the end of 2016, and the question went unanswered.

Since 2016, there has been no research on *alomae* and *bobone*, or any of the other taro viruses. However, concern about these viruses still exists as there is evidence that they are being moved around the South Pacific as people take planting material from one country to another. For instance, Taro vein chlorosis virus from Vanuatu turned up in Samoa in April 2017.

The taro virus situation in Solomon Islands is complex, and answers have been slow to come by. Part of that has been stop-go funding, and part due to the need for the development of appropriate technologies. We can see parallels with viruses and phytoplasmas of other tropical root crops - sweet potato, cassava, yam, and also pineapples where there are multiple viruses, strains and insect vectors. Taro, though, is unfortunate in that it's an 'orphan' crop, not supported by international agricultural research centres; neither is it a major cash crop so getting funds for research is a problem, and it's inevitable that questions arise over whether it's deserving of the limited funds that exist for such crops.

Sometimes, the question is even wider: should we be bothering about taro at all! It's being overtaken by crops that originated from other parts of the world: African yam, cassava, sweet potato and *Xanthosoma*, so why bother? The short answer is that we should be paying more attention to all these crops: there are very good reasons for Pacific island countries to have a diverse range of food crop staples to protect nutritional and cultural sustainability.

In support of taro and its viruses, below are five reasons why research needs to continue:

1. Taro is a traditional crop in the Pacific and represents an expression of people's culture. Eating and exchanging it is a way of preserving their attachment to their communities. It makes nutritional sense too: taro is one of the few crops where the entire plant is consumed in Pacific island countries, with the leaves a nutritious vegetable.
2. Farmers are asking for solutions. Locally, the crop is popular and in many places in Solomon Islands enters domestic markers as a high-priced luxury food earning growers considerable sums. Having gardens destroyed by *alomae* causes a great deal of concern.
3. To provide solutions to farmers, we need to know how *alomae* is spread and what viruses are involved. Our latest results suggest that only planthoppers are involved, but we need to do the research to prove it. And from experiences in the UK and Germany, transmission tests are best done where the diseases occur.
4. Importantly, biosecurity of all Pacific island countries is compromised by not knowing the aetiology of *alomae*. Countries beyond Papua New Guinea and Solomon Islands cannot make informed decisions on importing valuable accessions from these countries, as they don't know which viruses are involved. If alomae or bobone spreads containing them will be difficult.
5. Finally, there is interesting science to be resolved. Donors should stay the course, given the complexity of the problems, not give up when things get difficult. What signal does that give to young scientists of the region? Also, support requires reasonable timeframes and an understanding of the limitations faced by Pacific islands countries, such as in staff numbers, training and facilities. Further assistance is required to build the capacity in relevant government agencies and university faculties to bring about sustainable change, impossible to achieve with present short-term, time-bound, donor assistance.

PART 1: Dala days, Solomon Islands

How it all began

"Why are there gaps in the rows? When we planted this plot there were no gaps, no missing plants. Where have they gone?" David asked Untulau, Classified Worker, in-charge of field trials at Dala Experimental Station in 1968.

"If I don't remove the sick plants, all the taro will die", replied Untulau.

"Sick plants?" Queried David, unconvinced.

"Anyway, please stop pulling them out. I want to see these sick plants!".

So begins our story on virus diseases of taro in Solomon Islands and our 50-year failure to find the cause.

Work on taro at Dala Experimental Station on Malaita, Solomon Islands, started with the arrival of David Gollifer. In those days the country was the British Solomon Islands Protectorate. David had spent the previous three years working as head of the cocoa development program for the Ministry of Agriculture and Lands in the west, on the islands near Papua New Guinea. But in 1965, Father Peter Thompson, the Legislative Council (LegCo) Member for West Kwara'ae, Malaita, had raised the concern of many about falling yields of food crop staples – sweet potato, yam, cassava and, above all, taro. The Director of Agriculture, Jack Spencer, had to respond. David was moved from Gizo to Dala as General Crops Agronomist to begin research on subsistence crops and spices.

Dala had been established in the 1950s by Ollie Torling and Dick Keevil, both MAL employees, to carry out cocoa trials. At the time of David's arrival in 1967, Andy Van der Loos, a citrus expert, was station manager, and David Friend, cocoa agronomist. There was plenty of land, about 250 acres, enough for all the trials that were needed. It was under a long-term lease held by Malaita Province; it was near two villages (Dala North and Dala South, Anglican and Catholic, respectively) where staff were recruited; had a good water supply from streams in the bush; and four hours electricity a day from its two large diesel generators. Communications with Honiara the capital were good too: the station had a short-wave radio and was not far from Auki, Malaita's one town, or from Gwaunaru'u airfield with flights to Honiara. More than anything else it was perched on a limestone terrace above the coastal plain with breath-taking views of Dala Bay and cool on-shore breezes at night. Living there was comfortable and focussed you on the work at hand.

But first we should put taro cultivation into the socio-political context of the times. In the 25 years prior to 1965, the time of Father Thompson's remark in LegCo, the Solomon Islands had been rocked by major events. In early 1942, Japan invaded and, later that year, American armies arrived to foil their advance, fearing it would isolate Australia, making it vulnerable to invasion. So began the battle for Solomon Islands during which some three thousand Solomon Islanders joined the Solomon Islands Labour Corps, many from Malaita, and assisted American troops on Guadalcanal, the Russell Islands

and Tulagi. Here new relationships developed exposing the feelings of inequality experienced under the colonial administration, which led to a post-war movement seeking self-determination, known as Ma'asina Rule. During this time, people came from the inland mountainous areas to live in large, fortified villages on the coastal plains, supported by vast communal gardens.

The movement was suppressed by the Government until 1951 when the leaders were released from jail. A little later, a final calamity occurred: taro leaf blight arrived on Malaita, which, as elsewhere in the country, made taro cultivation all but nigh impossible in coastal areas, with the loss of many precious and culturally important varieties. Sweet potato became the crop of choice in the lowlands, albeit taro continued to be grown on a much smaller scale in the cooler highlands.

After the turmoil of these events came the discovery of a new-to-science lethal disease of taro, likely to make the fulfilment of the Department's imperative to improve the crop a difficult challenge. And so it was to be.

Alomae: What do farmers believe?

David's first task was to make collections of all the root crops. For taro, varieties (called "cultivars" as they are cultivated) were taken from many parts of Malaita, especially from the central and northern districts, both from the coast and mountains. They also came from Guadalcanal, Isabel, Makira, from islands of the Western Province and the islands of Santa Cruz in the east. David even made trips to the outlying atolls of Sikiana and Ontong Java, populated by peoples of Polynesian descent. Hundreds of cultivars were assembled and scored for length to maturity, yield, taste, and resistance to taro leaf blight caused by the oomycete, *Phytophthora colocasiae*. Not only were they assessed in the field but also for post-harvest storage. Collections were still being made in early 1972 when Grahame Jackson arrived as plant pathologist.

It quickly became obvious to David from the early variety trials that Malaita was the home of a unique taro disease. Untalau was right. If the sick taro were not removed as soon as symptoms appeared then, whatever it was, quickly spread to adjacent plants and to all taro in the garden. Within a few weeks an entire planting of many hundreds of taro was likely to be wiped out. It was an exciting find, but the severity of the disease and its rate of spread were alarming.

The disease was called *alomae*, literally meaning "taro die" in the Kwara'ae language.

Talking to the farmers, especially the old men – taro is a man's crop - David found that the disease was well known on Malaita. Traditionally, there were many taboos associated with growing the crop, having sexual intercourse the night before entering a taro garden being one of them. Break the taboos and the spirits of past kin will punish you, perhaps sending a disease to infect your plants. Alternatively, an enemy might poison your taro or use sorcery to bring about their death. Today, farmers may not hold such beliefs: Christianity has trumped Animism, but superstitions are still held strongly; some have transitioned into cultivation practices.

It might be thought odd that the disease was unknown to agricultural officers on Malaita at the time. But the reason for this is straightforward. First, before Jack Spencer's direction to David, little attention was paid to food crops: they are subsistence foods, barely entering domestic trade and Government statistics. The focus of the time was on cash crops, cocoa and coconuts in particular; it is still the same today. Second, few agricultural officers would have known much about taro, especially as most came from the UK where the crop is unknown, and few people would have heard of it or understood its pathology. Third, when taro did get the Government's attention after WWII, it was because of taro leaf blight. Any other disease would have gone unnoticed or simply been diagnosed as blight. Of course, taro gardens may have looked healthy to Government officers because sick plants were pulled out as soon as *alomae* appeared[1].

Farmers on other islands either did not know about the disease, or it was less common and unimportant. No one had any theories why it should be so, why Malaita had the disease more than other islands.

Symptoms of *alomae* vary from plant to plant, but two things are common: the youngest leaf is first to show symptoms and the leaf is stunted with short leaf stalks. However, on some plants, the leaf blade remains partly or completely rolled, some vertical, others horizontal, and light green. On other plants, the leaf blade expands normally, although it is often smaller than normal, stays green, is distorted and points sharply towards the soil. In both cases, the following leaf or leaves are even more stunted, remain rolled and begin to rot beginning at the tip. Galls or outgrowths are often present on the leaf stalks, but this is variable. Plants stop producing leaves, older leaves collapse, and the plants die. From the first appearance of symptoms until death is four to six weeks.

David's enquiries found that *alomae* had been on Malaita for generations, so long that there were "*kastom*" or cultural cures. It wasn't a new disease like taro leaf blight.

And Untulae was right.

The way to stop *alomae* was to pull out plants immediately they developed symptoms. In the olden days, there may have been some utterances to the spirits before the taro was removed and then burned in the garden. Some more cautious growers would remove a ring of plants around the one with symptoms and burn these too, as they were bound to die - they had been polluted by the smell of the infected plant. And perhaps, finally, the stem of a strong smelling plant, such as *r'ii*, a plant "important for things to do with ghosts" [2], would be placed where taro had once grown to protect the remaining plants from unfriendly spirits.

These days, it was more likely the diseased plants would be left in the garden or thrown into the bush, and only plants with symptoms would be pulled out, not adjacent healthy ones. These days, farmers are less fearful of vindictive spirits and, with changing beliefs, *kastom* cures are discouraged.

[1] A report on a tour of Fataleka and Baegu areas, North Malaita (1949) by Tom Russell, District Officer Malu'u, states: "Taro is healthy and there is no sign of the disease which recently manifested itself in the Shortlands and Faisi." Miscellaneous Archival Notes, David Akin (pers. com.).

[2] Kwa'iola M, Burt B (2001) Our forest of Kwara'ae. The British Museum Press, 46 Bloomsberry Street, London.

Fig. 1 *Alomae*. Leaves short, green, fleshy, crinkled, bent and downward pointing. The leaves of all but the youngest leaves are wilting. The youngest leaf is rolled and distorted, and will not open. The plant will stop growing, rot and die. Photo: Gwaiau, Malaita, Solomon Islands.

Fig. 2 *Alomae*. A second common symptom. The leaves have wilted, but they are not fleshy and dark green, (as in Fig. 1), and the youngest leaf is rolled but not distorted. These leaves will not open further; they will rot and the plant will die. Photo: Gwaiau, Malaita, Solomon Islands, same garden as Fig. 1.

Fig. 3 *Alomae*. The disease has come late to this plant, when it was near maturity and had suckers. The main plant is without leaves and remains as a stump of rotting leaf stalks. Some suckers remain alive, and their leave are curled, crinkly and fleshy. These symptoms are typical of the way that plants with *alomae* die. (Photo: near the New Guinea Binatang Research Centre, Madang, Papua New Guinea, June 2015)

Bobone: Taro is both male and female!

It soon became evident from David's interviews that taro cultivation on Malaita was far more complex than he had imagined. Taro were classified into "male" and "female" kinds, *alowane* and *alokini* (or *alogeni*), respectively, and they had separate diseases. Maleness had nothing to do with flowers or sex. Some said that male taro were larger and produced larger corms, or their life cycles were longer; others said that male taro died from *alomae*, whereas female were resistant.

Most taro were male, many hundreds of different kinds, but there were only about five female varieties, with cultivars Akalomamale and Oga the most common. Akalomamale came in three colours, or sub-varieties. It was true that these female taro did not die from *alomae*, but they succumbed to *bobone* – "taro grows small" in the Kwara'ae language.

Plants with *bobone* are similar to *alomae* at first: plants are very short, leaves are green, thick, rolled or distinctly curled, twisted, and galls are invariably present on the leaf stalks. The difference is that the plants do not die; after producing one or two leaves with severe symptoms, there is a general recovery until the fourth, fifth or sixth leaf appears healthy. The number of leaves showing symptoms is variable between plants.

Fig. 4 *Bobone*. The first sign is a short leaf with stiff downward-pointing blade (left). Leaves remain green, but become extremely stunted, fleshy, and severely distorted. Galls may form on the leaf stalks. After five or more leaves with symptoms, plants begin to recover and, eventually, they appears healthy. Photo: Dala, Malaita, Solomon Islands.

In contrast to *alomae*, there is no *kastom* cure for *bobone*. It seems that female taro are not held in the same high esteem as male taro, perhaps because there are only a few of them, or they have appeared only relatively recently. But growers like them a lot, not only because they don't die, but *bobone* leaves are thick and tasty when cooked, and make an excellent cabbage! Whatever, the reason, the plants are not removed from plantings. They are just left to recover.

Reports from the early literature

The first report of serious taro disease in Solomon Islands comes from a memorandum written by Senior Agriculture Officer DJ Badcock after a visit to Shortland Islands in 1946[3]. At first, Badcock thought the disease was caused by a virus because of its severity, but later it was identified as *Phytophthora colocasiae* and confirmed in Port Moresby and also in Fiji[4]. The disease either came to Shortland Islands from Bougainville, Papua New Guinea, which is nearby, or was planted there by Japanese troops stationed pn the island during WWII.

The first account of a virus disease of taro is probably that provided by BA O'Connor, Entomologist, Australian New Guinea Administrative Unit, Papua New Guinea. It was reported in an unpublished document in 1945[5]. The report by O'Connor concerns two

[3] Badcock WJ (1947) Annual Report Department of Agriculture, British Solomon Islands 1946. 13 pp.
[4] Parham BEV (1947) Economic Botany Notes: 3. Disease of taro. Fiji Agricultural Journal 18: 80.
[5] O'Connor's find is cited in: Shaw DE, Plumb RT, Jackson GVH (1979) Diseases of taro (*Colocasia esculenta*) and *Xanthosoma* sp, In Papua New Guinea. Papua New Guinea Agricultural Journal 4: 71-97.

diseases in the Jacquinot Bay area of New Britain. One was widely distributed and in some gardens attacked many plants. The midribs and veins of leaves were distorted with thickening and crinkling of the leaf tissues. Yields were reduced but only a few plants died. The other was a wilt disease but this was not described.

O'Connor was first to identify *Phytophthora colocasiae* as the cause of taro leaf blight in Papua New Guinea[6], so it is unlikely that he was confusing the symptoms seen in Jacquinot Bay with those of that disease.

A little later, a survey of virus diseases was carried out in PNG by CJ Magee in 1954 which described a mosaic which had an acute and a chronic form[7]. David Gollifer's thesis on factors affecting taro production in Solomon Islands quotes Magee's description as follows[8]:

> *The acute form caused marked stunting of affected plants, with chlorosis, twisting and malformation of the central leaves. The symptoms of the chronic form were variable, ranging from a yellow mottling or streaking of the leaves without much malformation to the almost imperceptible minor streaking of the foliage.*

The description of the acute form is reminiscent of *alomae* with, perhaps, the chronic form describing DsMV.

Fig. 5 Dasheen mosaic virus. Often the symptom is along the veins (left), with a greyish-green, feather-like appearance (arrow), or along and also between the veins (right), where, and in this case it is much brighter. The wilting and major distortion of the leaves as occurs with *alomae* and *bobone* is not seen with DsMV. Photo: Unia, Yate, New Caledonia (left); Sarete, Santo, Vanuatu (right).

[6] Packard JC (1975), op.cit., p. 1.

[7] Magee CJ (1954) Report on survey of virus diseases of food crops in the territory of Papua New Guinea with special reference to plant quarantine, Part !!. Papua New Guinea Agricultural Journal 9: 17-26.

[8] Gollifer DE (1976) Factors affecting the production of taro, *Colocasia esculenta* in the Solomon Islands. Ph.D thesis, Reading University. 179 pp.

Reports of virus diseases of taro in Solomon Islands occurred later. In 1960, a plant disease survey was carried out by Anthony Johnson, Director, Commonwealth Mycological Institute, Kew, UK, on behalf of FAO[9]. In his report, he mentions a severe and a milder mosaic: plants with the severe form were stunted, leaves were badly deformed, wrinkled, cupped and downward pointing. The leaf blade displayed a mosaic pattern which was sometimes vivid and sometimes indistinct.

Fig. 6 Dasheen mosaic virus. The feathering patterns are throughout the leaves (left), whereas they are faint and more confined to veins (right). There is no leaf rolling or distortions as seen with *alomae* and *bobone*. Photo: Ponerihouen, New Caledonia (left); Sarete, Santo, Vanuatu (right).

In the most severely affected plants, the leaf blades were unable to open and rotted while still young and at this stage the affected plants died. Johnson suspected the plants were infected by a virus.

Some of the description by Johnston is very reminiscent of *alomae*, especially “leaf blades unable to open and rotting while still young”; the mention of mosaics is less so. But there seems no doubt that he saw a severe disease on Malaita, whereas on other islands symptoms of disease were mild.

It is also possible that the mosaic and milder symptoms seen by Johnson belonged to *Dasheen mosaic virus* (DsMV), common on taro worldwide. The feathery mosaic leaf patterns are quite distinct from *alomae*, and apart from a rare form of the disease in

[9] Johnston A (1960) A preliminary plant disease survey in the British Solomon Islands Protectorate. FAO, Rome.

French Polynesia causing yellow strap-like leaves, symptoms are mild[10]. However, DsMV had not been described at that time from taro, and it would be another decade before it was documented by Bill Zettler at the University of Florida in taro and other aroids[11].

Symptoms of *alomae* and *bobone* are quite different from the feathery mosaic patterns caused by *Dasheen mosaic virus* (DsMV), and DsMV does not kill taro, although a rare severe form occurs in French Polynesia that causes yellow strap-like leaves[12].

What scientists found

Types of taros, viruses and transmissions

David wrote up his observations at this time with John Brown, Professor of Plant Pathology, University of New England, Armidale, Australia, who was visiting regularly to advise on a variety of crop pests[13]. *Alomae* and *bobone* were described, and the severity of *alomae* detailed. In one trial, 100 cultivars were planted and by 150 days 91 had succumbed to the disease. By contrast, of 13 plants with symptoms of *bobone*, all recovered within six weeks and continued normal growth. In another trial,15% of the plants had *bobone* at 42 days and only 7% at 70 days due to recovery.

It was now time to find out what viruses were associated with the two diseases. David was in contact with Rothamsted Experimental Station, Harpenden, UK, which was assisting with statistical analyses of his trials, so arrangements were made to send leaf samples of *alomae* and *bobone* to Ray Kenten, the virologist. Ray was working on cocoa swollen shoot disease and other virus problems of tropical crops for the UK Overseas Development Administration.

However, it was not easy getting leaves to the UK in a fresh condition so that sap could be extracted for partial purification and electron microscopy by Rothamsted virologists. Not from the distance of Solomon Islands that is. Leaves had to be carefully cleaned to remove soil and insects to comply with quarantine regulations, wrapped in newspaper – not plastic as they would rot creating a foul smelling liquid by the time they reached their destination – and despatched in time to catch planes from Malaita and Honiara without delays. Consignments took seven to 10 days to arrive, and there were many mishaps, the most common of which was a hold up in quarantine in either Australia or the UK. As David says in his book on his time in Solomon Islands, he had to write “free from injurious pests and diseases” on the outside of consignments with the permit number so as to speed transit through quarantine[14]. Free from insect pests, yes, but diseases, not so sure!

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[10] Zettler W, Jackson GVH, Frison EA (eds.) (1989) FAO/IBPGR Technical guidelines for the safe movement of edible aroid germplasm. Food and Agriculture Organization of the United Nations, Rome/International Board for Plant Genetic Resources, Rome. 24 pp.

[11] Zettler FW, Foxe MJ, Hartman RD, Edwardson JR, Christie RG (1970) Filamentous viruses infecting dasheen and other araceous plants, Phytopathology 60: 983.

[12] Zettler W, Jackson GVH, Frison EA (eds.) (1989) FAO/IBPGR Technical guidelines for the safe movement of edible aroid germplasm. Food and Agriculture Organization of the United Nations, Rome/International Board for Plant Genetic Resources, Rome. 24 pp.

[13] Gollifer DE, Brown JF (I972) Virus diseases of *Colocasia esculenta* in the British Solomon Islands. Plant Disease Reporter 56: 597-599.

[14] David Gollifer (2018) A life to remember. Silverdart Publishing, UK.

Anyway, we were about to find out what viruses the leaves had inside them.

In 1973, Rothamsted published their findings[15,16]. There were three kinds of virus particles found in *alomae* samples: two were bullet or bacilliform-shaped, one was approximately 280 x 55 nm, the other 140 x 30 nm. The third particle had flexuous rod particles, 750 to 800 nm long. In leaves showing *bobone* symptoms, the larger of the bacilliform-shaped particles were commonly present, often with flexuous rods; the smaller bacilliform particles were absent. As the small bacilliform particle occurred only in male plants with *alomae*, and was never found in plants alone, it was speculated that it might multiply only in the presence of the large bacilliform particle.

In preliminary attempts to transmit the particles, mechanical transmission [to *Tetragonia expansa* (syn. *tetragonoides*) – family Aizoaceae] was successful with the flexuous rod virus, and it was also transmitted in a non-persistent manner using the aphid *Myzus persicae*, with taro as a test plant. The flexuous rod particles were similar to those reported previously from taro and were tentatively assigned to DsMV. It was thought that the bullet-shaped particles would be insect transmitted too, but tests were not done. Most likely the larger bullet-shaped particles would be transmitted by hoppers (jassids or delphacids) and the smaller particles by mealybugs by similarity to the cocoa swollen shoot group[17].

As much as the diseases were fascinating, so was the fact that over the years *alomae*-resistant plants had been selected by growers. Early thoughts were that males were triploids (2n=42) and females were diploid (2n=28); this followed from work in India by Abraham who had found that triploids could be identified by their larger leaves, inflorescences and corms, and fewer cormels[18]. Counts were first done at Dala during a visit by Franklyn Martin to collect yams as part of a mission for the USDA to establish a world collection in Puerto Rico. Unfortunately, either the male variety, Tabikakama, was unusual in being triploid, or we could not count! Later, we had to admit our mistake, and agree that all male taro were diploid, just like females. As far as we could tell, there was no simple distinction between *alomae*-resistant and *alomae*-susceptible plants.

A more complicated picture emerges

At first, we thought we were dealing with two main types of taro, male and female, two diseases, *alomae* and *bobone*, two bacilliform particles of different size, and a flexuous rod virus, that was almost certainly DsMV. But in the next four years, to 1976, it became increasingly complicated. As more plants arrived as part of collecting expeditions, and more visits were made to taro gardens on Malaita and other islands, we began to see

[15] James M, Kenten RH, Woods RD (1973) Virus-like particles associated with two diseases of *Colocasia esculenta* (L.) Schott in the Solomon Islands. Journal of General Virology 21: 145-153.
[16] Kenten RH, Woods (1973) Viruses of *Colocasia esculenta* and *Xanthosoma saggitifolium*, Pest Articles & News Summaries19(1): 38-41,
[17] Later, the larger bacilliform particle was considered to be a rhabdovirus and the small bacilliform particle a badnavirus: Rodoni BC, Dale JL, Harding RM (1994) Review of alomae disease of taro. Papua New Guinea Journal of Agriculture, Forestry and Fisheries 37(2): 14-18.
[18] Abraham A (1970) Proceedings of the Second International Symposium of Tropical Root Crops (Honolulu) 1: 78 pp. Interestingly, Abraham was to visit Vanuatu in recent years to pollinate yams, crosses between Indian and local cultivars in the search for non-staking and anthracnose resistant lines.

symptoms in plants that were quite different from *alomae* and *bobone*. This is a summary[19].

We found male taro with thickened, distorted green patches on their leaves, often no more than 10 cm wide. Up to three leaves showed symptoms before apparently healthy leaves appeared. In these, Rothamsted found the large bacilliform particle. The symptoms on these plants was called LPS - large particle symptom.

Fig. 7 Large particle symptom (LPS) on male taro. Later, test would show that *Tarophagus* spreads the large bacilliform virus (renamed CBDV) to female taro causing *bobone*, and to male taro causing a mild patch-like distortion (as in the photo). Two or three leaves show symptoms before full recovery occurs. Photo: Malaita, Solomon Islands.

Contrary to initial observations, the small bacilliform particle was found by Rothamsted in female plants with *bobone* where the large bacilliform particles were present; they occurred either in the same or different leaves, but the plants always recovered.

Occasionally, and soon after planting, both male and female plants showed a yellowing of the minor veins, more pronounced near the margins, with areas between the veins remaining green. Often there was down-curling of the leaf blade. Plants were stunted at first but recovered. When symptoms developed on older plants, the first leaves were small, narrow, yellow, often with torn margins. On rare occasions, leaf stalks developed without leaf blades, but these plants also recovered. When samples from plants with symptoms were examined by Rothamsted small bacilliform particles were found.

[19] Gollifer DE, Jackson GVH, Dabek AJ, Plumb RT, May YY (1977) The occurrence and transmission of viruses of edible aroids in the Solomon Islands and the Southwest Pacific. PANS 23(2): 171-177.

Small particles were also found in taro examined by Rothamsted from Fiji, Papua New Guinea, Samoa, and Vanuatu, in *Xanthosoma* from Cook Islands, and from *Alocasia macrorrhizos* (previously *macrorrhiza*) in Samoa and Solomon Islands[20,21,22,23].

Fig. 8 Small particle symptom. Plants stunted, leaves with patches of vein-yellowing (e.g., arrow), crinkled, convex with margins turned under. One or two leaves usually show symptoms before recovery. Photo: Malaita, Solomon Islands.

Fig. 9 Joa, a condition on the island of Isabel. Plants were sent to Roger Plumb at Rothamsted and only the small bacilliform particle (TaBV) was found in them. Symptoms are similar to *alomae*. Unfortunately, there was no follow-up to check if other viruses were present.

[20] Gollifer DE*, et al.* (1977), op. cit., p. 172.
[21] Jackson GVH (1979) Taro virus diseases in western Samoa. Report of a visit 20 September to 6 October 1979. . South Pacific Commission, Noumea, New Caledonia. 20 pp.
[22] Jackson GVH (1980) Diseases and pests of taro. South Pacific Commission, Noumea, New Caledonia. 51 pp.
[23] Brunt AA (1987) Surveys for plant viruses and virus diseases in Solomon Islands. Strengthening Plant Protection and Root Crops Development in the South Pacific (RAS/83/001). Food and Agricultural Organisation of the United Nations: Rome, Italy. 15 pp.

Perhaps most surprising of all was the discovery that taro on the island of Isabel, Solomon Islands, died from a condition known locally as *joa*[24]. Plants showed severe stunting and yellowing but without the leaf distortions characteristic of *alomae* and *bobone*. Nevertheless, they died soon after symptoms appeared. Only the small bacilliform particle was found associated with these plants. We do not know if the plants are male or female as this distinction is not used on Isabel, and none were brought to Malaita to check. All we know is that the disease is long-known on Isabel.

Finally, the small particle was found in *Cyrtosperma johnstonii* sent from the botanic gardens in Honiara to Brisbane[25]. The plants showed no obvious symptoms.

A similar situation in Papua New Guinea

In 1973, taro gardens in lowland areas of Papua New Guinea were surveyed by Dala and DAL staff led by Dorothy Shaw[26]. Visits were made to Lae (Morobe Province), Keravat (East New Britain Province), and Buka, Buin and Kieta (now the Autonomous Region of Bougainville). Plants were seen showing typical symptoms of *alomae* and *bobone*; samples were collected and sent for examination by EM at Rothamsted.

The situation in PNG seemed identical to that in Solomon Islands, albeit growers did not distinguish between male and female taro, and it was difficult in the short time of the visits to know if plants with *bobone*-like symptoms would recover, or whether they were showing initial symptoms of *alomae*.

The large bacilliform was found in 33 samples, 22 times alone, eight times with the small bacilliform particle, and three times with flexuous rods. The small bacilliform particle was found twice alone, and flexuous rods were also confirmed twice alone in taro and three times alone in *Xanthosoma*.

Chasing the vectors

We followed Ray Kenten's advice about the insects likely to be spreading the large and small bacilliform particles, i.e., the vectors. The plant hopper, *Tarophagus proserpina*, was commonly present in taro gardens, and in dry times large infestations developed, irrespective of the presence of the egg-sucking bug, *Cyrtorhinus fulvus*, a natural biological control. Mealybugs were occasionally present, too. It seemed to be straightforward: *male taro were susceptible to both the large and small bacilliform particles, so they developed alomae; on the other hand, female taro were susceptible to the large bacilliform particle only, and they developed bobone.* We did not need to include DsMV as Rothamsted did not find the virus in all the plants with *alomae*, so we considered it was unlikely to be involved.

[24] Jackson GVH, Gollifer DE (1975) Disease and pest problems of taro (*Colocasia esculenta* L. Schott) in the British Solomon Islands. PANS 21 (1): 45-53.
[25] Jones DR, Shaw DE, Gowanlock H (1980) Australian Plant Pathology 9(3): 5-6.
[26] Shaw DE *et al*. (1979), op. cit., pp. 71-97.

Fig. 10 *Tarophagus* sp. the vector of the large bacilliform virus of *alomae* and *bobone* (CBDV). Different life stages are shown; most of the adults have wings. Winged forms appear when populations are high allowing them to disperse to nearby gardens, or further on the wind. Photo: Malaita, Solomon Islands.

Roger Plumb, assisted by Andy Dabek, took over from Ray Kenten, transmission tests were set up at Dala to show that planthoppers and mealybugs were the vectors of the large and small bacilliform particles respectively, and together the cause of the two diseases. When symptoms developed sample plants went to Rothamsted for analysis. In those days it was examination by an electron microscope only.

Test plants were screened for 6 months before they were used in transmission tests to ensure they were free from obvious virus, insects were collected from gardens free from symptoms, or from wild taro in isolated swamps on the Guadalcanal Plains along the road to Gold Ridge in the foothills of the island's mountains, given acquisition feeds on plants with *alomae* or *bobone* (for >2 days) and then placed on the test plants[27]. They were left on the plants for varying times.

Fig. 11 *Planococcus citri.* This is similar to the mealybugs collected at Dala Research Station, identified first as *P. citri*, then *P. pacificus*, and finally as *P. minor.* Photo: Citrus mealybug (*Planococcus citri)*. Jeffrey W. Lotz, Florida Department of Agriculture and Consumer Services, Bugwood.org.

Unfortunately, the results were not as we had hoped!

[27] Gollifer DE*, et al.* (1977), op. cit., p. 173.

With appropriate controls, and after innumerable repeats, the results could be summarised as follows (Table 1):

- *Tarophagus* planthoppers fed on alomae transmitted alomae to male plants and bobone to female plants.
- Tarophagus fed on bobone transmitted bobone to female plants, BUT they did not transmit bobone to male plants.
- These results held whether virus-free planthoppers were given acquisition feeds or they were taken from diseased plants in the field.

There were other discrepancies, and those which we considered to be of most interest are listed below; they mostly concerned the large bacilliform particle (Table 1):

- Planthoppers fed on *alomae* and transferred to male test plants produced LPS plants in three tests – in total, six of 47 plants developed the symptom.
- Planthoppers fed on *bobone* and transferred to male test plants also produced LPS plants in one test – in total, one of 29 plants developed the symptom.

Transmission tests were also done using plants with *joa*, in which Rothamsted found only the small bacilliform particle, to see if *Tarophagus* could transfer this virus, even though this was thought a remote possibility. If the small bacilliform particle was a badnavirus then mealybug species or aphids were likely to be involved.

In some tests, *Tarophagus* were fed on *joa* and *bobone* and then placed on male test plants to see if it was possible to create *alomae*. The results failed to show that *Tarophagus* transmitted the small bacilliform particle:

- Planthoppers fed on *joa* and transferred to male test plants produced 0 symptoms in 5 plants.
- Planthoppers fed separately on *joa* and *bobone,* then combined on male test plants, produced 0 symptoms in 5 plants in one test, and 1 in 5 plants with LPS in a second, where both large and small bacilliform particles were present.
- Planthoppers fed together on *joa* and *bobone*, and then on male test plants, produced 3 of 5 plants with LPS; only large bacilliform particles were present.
- Planthoppers fed on *joa* and then *bobone*, and then male test plants, or fed on *bobone* and then *joa* and then male test plants did not produce symptoms in four plants tested in each case.

This is not what we expected. We did not expect to see the LPS symptom, which we associated with the large bacilliform particle in male taro, when we took planthoppers from *alomae* and put them on male taro. We expected *alomae*.

What was the explanation?

Table 1. Transmission tests using planthoppers (*Tarophagus* sp.) from male plants with *alomae* and female plants with *bobone* to male and female test plants

		Plants with symptoms						Plants with viruses			
Acquisition feed	Test plants	No. trials	No. plants	No. *alomae*	No. *bobone*	No. LPS	No symptom	No. plants LP+SP	No. plants LP	No. plants SP	Healthy plants
Alomae	Male (Tabikakama)	11	47	15	0	6	26	11	3	5	26
Alomae	Male (Unknown)	2	10	8	0	0	2	0	10	0	2
Alomae	Female (Akalomamale)	3	12	0	10	0	2	0	10	0	2
Alomae	Female (Unknown)	2	9	0	4	0	5	0	4	0	5
Bobone	Female (Akalomamale)	6	24	0	11	0	13	3	5	0	13
Bobone	Male (Tabikakama)	7	29	0	0	1	28	1	0	0	28
Alomae + bobone	Male (Tabikakama)	4	18	0	0	3	15	0	2	0	15
From field - *alomae*	Male (Tabikakama)	2	23	13	0	0	10	2	10	0	10
From field - *bobone*	Male (Tabikakama)	2	10	0	0	0	10	0	0	0	10
From field - *alomae*	Female (Akalomamale)	1	5	0	0	0	5	0	0	0	5
From field - *bobone*	Female (Akalomamale)	2	11	0	3	0	8	0	3	0	8

LPS, large particle symptom; LP, large bacilliform particle; SP, small bacilliform particle; + means that plants were given acquisition feeds first on *alomae* and then *bobone*.

But first, what were our expectations?

1) The planthopper was not transferring the large and small bacilliform particles simultaneously, and there were no other reports of planthoppers transferring small bacilliform particles like that in taro, or simultaneously transmitting viruses that were so dissimilar, OR
2) We expected the large bacilliform particle to be transmitted by *Tarophagus* and the small bacilliform particle to be transmitted by mealybugs because of its similarity to *Cocoa swollen shoot virus*. But we also thought it was likely to be latent in most male plants.

When *Tarophagus* fed on *alomae*, and the LPS occurred on test plants, we assumed that the small bacilliform particle was absent. *Alomae* occurred only when *Tarophagus* was given acquisition feeds on *alomae* and the test plants had latent infections of the small bacilliform particle.

However, if that were the case, transferring the large bacilliform particle from *bobone* to male plants should have produced *alomae*, perhaps not every time - the small particle was not always latent - but at least occasionally. That was not our experience: it never happened. All we got was LPS.

These were not the only confusing results. One test is worth mentioning. Two lots of planthoppers were given feeds on *alomae* (for 14 days) and then on two groups of Hawaiian cultivars. In one, the result was *alomae* (12 of 12 plants), in the other the result was LPS, 7 of 7 plants. And in both only large bacilliform particles and flexuous rods of DsMV were found. Here again there seemed to be no role for the small bacilliform particle.

As regards the potential involvement of DsMV, which occasionally occurs in plants on Malaita either alone or with symptoms of *alomae* or *bobone* (as happened in the last test with plants from Hawaii), there was no consistent association of DsMV with *alomae*. It was unlikely to be involved in the aetiology of *alomae*.

We were left with only one explanations for *alomae*: the planthoppers were transmitting the large bacilliform virus and another, yet to be identified, virus or similar entity.

It was obvious to us at this point that unless we had better ways of testing for the viruses than EM, as well as access to virus-free plants we would not be able to solve the vector-virus conundrum.

If the small bacilliform particle was latent it would be hard to pin down the cause of *alomae*. We needed virus-free plants. Seed was our best hope. Fortunately, fertile seed heads were found in Sasamungga, Choiseul, from a taro called Mesara, a favoured taro in the area, and one that was said to die from a disease reminiscent of *alomae*. In Sasamungga it was called *zuiki*.

David used the seed in tests with mealybugs and *Tarophagus* in an attempt to create *alomae*. The results of these tests were not convincing, however, but they are summarised here:

- *Alomae* occurred only in one instance - when *Tarophagus* and *Planococcus longispinus* were fed on *alomae* and then placed on Mesara seedlings. The only virus found by Rothamsted was the small bacilliform particle.
- On three other occasions, LPS occurred when *Tarophagus* and mealybugs fed on *alomae* (either *P. longispinus* or *P. citri*) were placed on Mesara seedlings.
- On one occasion, LPS occurred when *Tarophagus* and *Aphis gossypii* were place on Mesara seedlings, and the small bacilliform particle was found.
- On two occasions no symptoms occurred when *Tarophagus* was used alone or with *P. citri* after feeds on *alomae*.

Although seedlings might be free from virus (although there was a suggestion from the third test above they weren't), they were small and fragile, and often arrived at Rothamsted in a poor condition, so much so that it was difficult to examine and find viruses in them by EM.

David left Solomon Islands in 1975 to take up the position of Chief Research Officer in Botswana and, to the surprise of everyone, Dala was closed a year later. Attempts were made to carry on the work at Dodo Creek Research Station on Guadalcanal, but it was difficult because taro were not grown commonly on the Guadalcanal Plains where the research station was situated, the rainfall was much less than on Malaita and taro did not grow well; furthermore, there was no land for trials initially, and the virus diseases, *alomae* and *bobone*, were unknown in the area.

However, tests did continue at Rothamsted using seed. In 1974, seed was obtained from wild taro growing beside canals in Bangkok, and given to Rothamsted. Seedlings grown from the Bangkok variety showed no symptoms of virus, and no virus particles were seen when they were examined by EM.

The results of the transmission tests done later at Rothamsted are summarised as follows[28]:

> *The appearance of* alomae *symptoms on apparently healthy field grown plants following transmission by* T. proserpina *suggests that both bacilliform particles were transmitted. However, when plants raised from seed and, therefore, unlikely to contain virus were used, transmission of the large but not the small particle by* T. proserpina *was confirmed and no* alomae *developed.*

A clearer pictured emerged from tests with mealybugs which showed that the small particle was transmitted to seedlings on seven occasions out of 50 after acquisition feeds of 6-72 hours, once by *Planococcus longispinus* in Solomon Islands (the test included *Tarophagus*), and six times by *P. citri* alone at Rothamsted.

Transmission tests at Rothamsted also used the aphid *Aphis gossypii* and showed that it transmitted DsMV using a Kenyan cultivar, Nduma. This aphid is common on taro in Solomon Islands and other Pacific island countries.

Perhaps it was just as well that we put a stop to the investigation at that point. Our use of seedlings was not the answer. We were under the impression that the small

[28] Gollifer DE, *et al*. (1977), *op. cit*., p. 174.

bacilliform virus was unlikely to be seedborne, but later this was shown to be incorrect. The view at the time was that none of the small bacilliform-like viruses, similar to the one in taro, was seedborne. This was not the case.

Seedborne transmission was first reported by *Kalanchoe top-spotting virus*[29] and, later, *Cocoa swollen-shoot virus*[30]*,* and *Piper yellow mottle virus*[31]. In 2005, clear evidence was presented that *Taro bacilliform virus* was also seedborne[32]*.*

Another problem concerned the identification of both the planthoppers, and the mealybugs used in the tests. We will have more to say about this later when we look at changes to the taxonomy of *Tarophagus* and *P. citri*.

Impact of the diseases

While efforts were being made to uncover the aetiology of the taro virus diseases, trials were being carried out at Dala on their impact. We have already mentioned that preliminary observations showed that *alomae* was capable of complete destruction of taro plantings, whereas *bobone* caused a severe deformation of leaves, but plants eventually recovered and appeared healthy once more.

Further studies were made on collections of taro held at Dala to confirm the impact of the diseases[33].

Between 1971-1974, 5200 plants of 297 cultivars were screened for resistance to *alomae*. Of these, 169 male cultivars from Malaita, and 115 cultivars from the Eastern District, Guadalcanal, Santa Isabel and the Western District in Solomon Islands, as well as introductions from Hawaii, New Zealand and Vanuatu, were found to be susceptible to the disease. Plants that showed *alomae* symptoms early in the trials soon died, and even those that showed symptoms later and survived to harvest gave no useful yield; the decrease in yield was roughly proportional to the percentage of plants infected. However, 13 cultivars from Malaita survived; they displayed symptoms of *bobone* and were considered to be female.

Interestingly, weekly sprays of plants with malathion beginning 15 weeks after planting "did little to restrict virus spread" as the experimental plots were close to established taro where *alomae* was present[34].

Findings on the impact of *bobone* on yield reported in the same paper are more equivocal. Two trials investigated incidence of disease, the effect on yield, time taken to recover, and the number of plants where symptoms reoccurred. In the two trials, there

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[29] Hearon SS, Locke JC (1984) Graft, pollen, and seed transmission of an agent associated with top spotting in *Kalanchoë blossfeldiana*. Plant Disease 68: 347–350.

[30] Quainoo AK, Wetten AC, Allainguillaume J (2008) Transmission of cocoa swollen shoot virus by seeds. Journal of Virology Methods 150(1-2): 45-9.

[31] Deeshma KP, Bhat AI (2014) Further evidence of true seed transmission of *Piper yellow mottle virus* in black pepper (*Piper nigrum* L.) Journal of Plantation Crops 42: 289–293

[32] Devitt L, Ebenebe A, Gregory H, Harding R, Hunter D, Macanawai A (2005) Investigations into the seed and mealybug transmission of Taro bacilliform virus. Australian Plant Pathology 34: 73–76.

[33] Gollifer DE, Jackson GVH, Dabek AJ, Plumb RT (1978) Incidence, and effects on yield, of virus diseases of taro (*Colocasia esculenta*) in the Solomon Islands. Annals of Applied Biology 88, 131-135.

[34] Ibid., p. 134.

were 9 and 16 plots of 48 plants. Insecticide was used to control *Papuana* beetle corm damage in the second trial.

In the first trial more than 80% of the plants showed symptoms, and in the second it was 32%. However, in each case, there were three to five leaves with symptoms and most plants had recovered completely before harvest. Where *Papuana* beetles were controlled, recovery took two to eight weeks. *Bobone* symptoms reoccurred on about 12% of plants between 6 and 22 weeks after their first appearance. There were significant differences between yields in terms of when symptoms occurred, but only where *Papuana* was controlled. Where symptoms occurred 6, 8 and 10 weeks after planting, the mean weight of corms was nearly 26% less than from symptomless plants.

Interestingly, our trials showed that culling plants when they showed symptoms was effective in reducing the incidence of *bobone* from initial levels of 30% to less than 1% in three successive plantings[35].

Breeding for resistance

We started breeding taro for tolerance to several diseases – taro leaf blight *(Phytophthora colocasiae)*; *mitimiti*, caused by the nematode, *Hirschmanniella miticausa*; and *alomae* - after the move to Dodo Creek Research Station at the end of 1976[36]. In the year before, UNDP/FAO and SPC had sent a mission around the region to look into production and development needs of nine Pacific island countries. As the mission was concerned particularly with constraints to improving productivity which might lead to commercialisation of root crops, potential limitations from pests and diseases were of interest.

Following the country visits, a regional meeting was held in Fiji where current research was presented, and needs discussed. In a paper outlining their recommendations, Keith Templeton and Michel Lambert from FAO and SPC, respectively, gave special mention of the work done on viruses of taro in Solomon Islands. They maintained that the diseases held a risk for the region because of the possibility of their transfer in infected plants. Under their proposed project, a virologist was needed to continue the work, and there was also a need to develop tissue culture to provide virus-indexed plants which could be transferred safely into and around the region, possibly a lab outside the region because of the inherent quarantine concerns, and the need for sophisticated equipment.

In 1978, or thereabouts, UNDP financed an FAO-implemented project, *Root Crops Development in the Pacific* (RAS/74/017) in association with SPC, which was later incorporated as part of *Strengthening Plant Protection* and *Root Crops Development in the South Pacific* (RAS/83/001) in 1983. Under those projects, UNDP volunteers and Associate Experts were employed to assist participating countries.

Zaheer Patel took up his position as plant breeder at Dodo Creek Research Station in 1980 to breed taro with resistance to taro leaf blight, and other diseases. He continued the hybridisations between male and female taro that had been started by Moses

[35] Jackson GVH, Gollifer DE (1975), op.cit., p. 48.

[36] Jackson GVH, Pelomo PM (1980) Breeding for resistance to diseases of taro, *Colocasia esculenta* in Solomon Islands. International Symposium on Taro and Cocoyam. Visayas State College of Agriculture, Baybay, Leyte. International Foundation for Science. Provisional Report 5: 287-298.

Pelomo, Research Assistant and Grahame[37]. The rationale behind this was to combine the different tolerances of male and female taro to *alomae* and *bobone*: male taro had tolerance to the large bacilliform taro, they did not develop *bobone* - infection from this particle caused a very mild disease, a slight puckering and thickening of veins and leaf tissue, which we called LPS. On the other hand, female taro were tolerant to *alomae* – they succumbed to *bobone*, but recovered. We did not know what caused *alomae*, but we did know that female taro were resistant. Crossing the two types, male and female, might produce hybrids tolerant to both diseases, but with the characteristics of male taro: larger corms with diverse eating and other sort after characteristics.

Several hundred seedlings, crosses between Akalomamale (female) and Luma'abu (male) where planted out at Dala in 1982 and monitored[38]. The result was disappointing: all plants died. However, symptoms and death took longer than expected during epidemics of *alomae*. However, without plots of the parents for comparison, it was impossible to determine if the differences were meaningful. This experiment needs to be repeated with appropriate controls.

Taxonomic problems of putative vectors

David sent *Tarophagus* planthoppers to the Commonwealth Institute of Entomology, UK, and these were identified as *Tarophagus proserpina* by a leading expert on the group at that time. However, in 1989 the genus was revised and, "Surprisingly, *T. proserpina* could not be found from the Solomon Is."[39]. The species present were *T. persephone* and *T. colocasiae.* Consequently, it is unknown which *Tarophagus* species was used in the transmission tests. This is of concern as some were collected from Dala and others from the Guadalcanal Plains. Collections from these localities need to be made and identified.

A similar problem exists for the mealybugs used in the transmission tests. David remembers taking mealybugs used in the transmission tests from cocoa leaves at Dala. They are common on leaves and on pods, and frequently tended by ants 'milking' them for their honeydew. Samples for identification were carried by David to the British Museum and they were passed to Douglas Williams then at the Commonwealth Institute of Entomology. They were identified as *Planococcus citi.*

The identification of *P. citri* in Solomon Islands is complex[40]. In 1981, Jennifer Cox of the British Museum had found that environment had an impact on morphological characteristics of *Planococcus* species[41]. From her work, *P. citri* was considered the same as *P. citricus*; the latter being a high-temperature form. Additionally, and more importantly, the paper described a new species, *P. pacificus*, first intercepted in New

[37] Jackson GVH, Pelomo PM (1980) Breeding for resistance to diseases of taro, *Colocasia esculenta* in Solomon Islands. International Symposium on Taro and Cocoyam. Visayas State College of Agriculture, Baybay, Leyte. International Foundation for Science. Provisional Report 5: 287-298.

[38] Lab notebooks with details of the research were destroyed by the destruction of Dodo Creek Research Station during the Ethnic Tension in 2000.

[39] Åsche M, Wilson M (1989) The three taro planthoppers: species recognition in *Tarophagus* (Hemiptera: Delphacidae). Bulletin of Entomological Research 79: 285-298.

[40] Macanawai AR, Ebenebe AA, Hunter D, Devitt LC, Hafner GJ, Harding RM (2005) Investigations into the seed and mealybug transmission of the Taro bacilliform virus. Australasian Plant Pathology 34: 73-76.

[41] Cox JM (1981) Identification of Planococcus citri (Homoptera: Pseudococcidae) and the description of a new species. Systematic Entomology 6: 47-53.

Zealand on croton from Samoa; it was also identified from Fiji, Papua New Guinea and other Pacific island countries, as well in countries of Southeast Asia.

The following year, Dr Williams now at the British Museum (Natural History) produced a paper that mentioned the confusion over the correct identification of *P. citri* and agreed with Cox about *P. pacificus*. He states:

"Although *P. citri* is present in some islands, it is now certain from the work of Cox (1981) that *P. pacificus* Cox is far the most widespread species of *Planococcus* and is probably present in most islands"[42]. He goes on to say: "It now seems certain that only *P. pacificus* is present in Papua New Guinea and Solomon Islands".

Williams concluded that all the records of *P. citri* by various authors including himself from these countries should refer to *P. pacificus*.

Most important for our story is this quote from William's paper[43]:

> *Records of* P. citri *in the Solomon Islands on taro and other edible Araceae by Gollifer et al. (1977) should refer to* P. pacificus*, but it is not certain which species was used on these plants for successful transmission of smaller particles of Dasheen mosaic virus (DMV) in England although the article mentions* P. citri[44].

Williams mentions *P. pacificus* collected from cocoa on Malaita, but not from taro. He does mention that specimens were sent from taro on Guadalcanal in 1977, however.

But the story of mealybug identification does not end there. In 1989, Cox reported *P. pacificus* as synonymous with *P. minor*, and lists *Colocasia antiquorum* as a host[45].

Quoting from the description of *P. minor*:

> P. minor *is very similar to* P. citri*, and the existence of the second species was not established until the variation of individual populations was studied using rearing experiments …*

Further, Cox refers to Williams concerning the misidentification of *P. minor*:

> *Although* P. citri *has frequently been recorded from the South Pacific Islands, Williams (1982) comments that most of these records are misidentifications of* P. minor*. His records show* P. minor *to be much more common than* P. citri *in this area, and to have been substantially longer established, the earliest record given of* P. citri *from the area being 1975 and that of P. minor, 1922*[46]*.*

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[42] Williams DJ (1982) The distribution of the mealybug genus *Planococcus* (Hemiptera: Pseudo- coccidae) in Melanesia, Polynesia and Kiribati. Bulletin of Entomological Research72: 441-455.
[43] Ibid., p. 442.
[44] The mention of *Dasheen mosaic virus* here is an error; the sentence should have read: "… for *successful transmission of the small bacilliform virus in England* … ".
[45] Cox JM (1989) The mealybug genus Planococcus (Homoptera: Pseudococcidae). Bulletin British Museum (Natural History) (Entomology Series) 58: 1-78.
[46] Cox JM (1989), op. Cit., p. 55.

Finally, Cox states in the same paper that *P. minor* is a common species on many economically important plants, particularly cocoa, throughout its geographical range. That and the fact that Williams records *P. pacificus* from cocoa at Dala, and David took mealybugs from that crop, we are confident that the mealybugs used in the Dala small bacilliform particle transmissions were *P. minor*, a conclusion supported by Apaitia Macanawai and others[47].

Summary of Part 1

Diseases of taro caused by *alomae* and *bobone* are common on Malaita, Solomon Islands, where they have long been known. One of them is *alomae* which kills varieties known as “male”, and is such a serious disease, that farmers use traditional “*kastom*” practices to stop its spread. Commonly, growers pull out infected plants and burn them. There are also a few resistant varieties, called “female” taro, which are smaller but not as popular. The female taro succumb to a disease related to *alomae*, which is called *bobone*. Plants recover from this disease and appear healthy, although occasionally the disease reappears later in the crop.

Male plants with *alomae* show stunted, yellow, rolled young leaves, which rapidly rot from the leaf tip and die. There is some variation in initial symptoms with first leaves staying green, downward pointing, with rolled-under margins, but ultimately they die. Female plants with *bobone* develop thick, twisted stunted leaves that remain green, but the leaves gradually recover. The disease occurs most commonly soon after planting.

Virus particles were first reported by Ray Kenten at Rothamsted Experimental Station in 1972, in sap examined by electron microscopy sent from Dala Experimental Station, Malaita, by David Gollifer. Two kinds of bullet-shaped (or bacilliform) virus particles of different sizes were found. In plants with *alomae*, the large bacilliform particle (later named *Colocasia bobone disease virus, a rhabdovirus*), and a small bacilliform particle (later named *Taro badnavirus*), occur together, whereas in plants with *bobone* the large bacilliform particle occurs on its own. Only once have the large and small bacilliform particles been found in the same leaf.

Similar diseases and virus particles were found in a survey in PNG in 1974.

Later, it was realised that symptoms of the large bacilliform particle occur in male taro causing a patch of green thickened tissue on the leaf, and the small bacilliform particle occurs in both male and female taro causing yellow, stunted, leaves with torn margins, or leaves with yellow marginal veins and downward curling of the leaf, depending on age of plants.

There was a direct correlation between incidence of *alomae* and yield; the effect of bobone was to cause a 25% loss of yield of individual plants. Incidence varied from 30-80% of plants. Some plants developed *bobone* twice in a crop.

[47] Macanawai et al. (2005), op. cit., p. 75.

Tests showed that *Tarophagus* planthoppers (possibly *T. colocasiae*) fed on *alomae* and then male test plants produced *alomae*, and *Tarophagus* fed on *bobone* and then female plants produced *bobone*. In tests with mealybugs (possibly *P. minor* and *P. longispinus)*, they were shown to transmit the small bacilliform particle to seedlings. (Taxonomic revision of both groups of insects have occurred since the transmission tests began.)

However, our tests also showed that sometimes the small bacilliform particle is latent in test plants. *Tarophagus* fed on *alomae* produced *alomae*, but sometimes LPS (iarge particle symptom). By contrast, when *Tarophagus* fed on *bobone* and then male test plants they produced LPS, but never *alomae*.

In 1976, when the virus transmission tests were concluded, the hypothesis was that *Tarophagus* transmitted the large bacilliform particle, and the small bacilliform particle was transmitted by mealybugs. It was also latent. But, importantly, the small particle was unlikely to be involved in *alomae*.

Another virus particle or virus-like entity, together with the large bacilliform particle, both transmitted by *Tarophagus*, was needed to cause *alomae*, and explain our results.

PART 2: Intermission: Surveys and outbreaks

After the closure of Dala Research Station in 1976, research on taro viruses in Solomon Islands ceased. It was logistically too difficult to do transmission tests at the research station on Guadalcanal. Taro is not grown commonly on the Guadalcanal Plains: it is too dry for part of the year for the crop to flourish, and as the diseases were not nearby - only on the isolated southern weathercoast - it meant going to Malaita for source plants. Also, David left Solomon Islands for Botswana in 1975, and Grahame for Fiji in 1983.

For about the next 20 years until the start of TaroGen in 1998 our knowledge of taro viruses came from *ad hoc* pest surveys in the region and investigations of outbreaks of taro diseases.

Regional surveys

In 1982, while still based in Solomon Islands, Grahame visited Vanuatu for the UNDP/FAO *Root Crops Development in the South Pacific* project to look at pests of root crops[48].

Alomae and *bobone* disease had not been reported from Vanuatu, but during the survey several virus particles similar to those found in Solomon Islands were recorded. Plants with DsMV were also seen on Santo, showing the typical feathering patterns along veins, but were not common.

Of greater interest, were taro with localised yellowing of the minor veins especially at the margins of the leaf blade, seen first at Serete, South Santo. A characteristic of the

[48] Jackson GVH (1982) Pests of root crops in Vanuatu. Report of a visit, 22-30 August 1982. UNDP/FAO Root Crops Development in the South Pacific (RAS/74/017). Rome, Italy. 31 pp.

disease was young leaves rather more upright than usual with edges curled under, giving an umbrella look On some plants, the yellowing was more extensive and accompanied by puckering and twisting. Three plants in one garden were seen with more severe symptoms: the leaf stalks were short, the youngest leaf still rolled and, on one, the leaf was rotting.

These plants were similar to *alomae* in Solomon Islands, but they also had similarity to plants infected with the small bacilliform virus. However, examination by Roger Plumb at Rothamsted found only large bacilliform particles. This was curious because the small bacilliform particle had already been found in plants from Vanuatu, in quarantine in Solomon Islands, not the large particle[49]. On the plants sent to Roger, leaves were smaller than usual and veins at the margins were yellow and prominent, just like plants with this virus in Solomon Islands and in other Pacific island countries.

Our thinking was still that *alomae* was a combination of large and the small bacilliform viruses. The plants in Vanuatu had severe symptoms, and might die, but the symptoms were quite different from *alomae*. Surely, if the viruses had been present in the country for a long time, it was likely the particles would have come together on one of the islands. There was no knowledge of any lethal disease, apart from the opinion of one farmer in South Santo. We needed to check the fate of the plants in the garden at Serete, but this was impossible during the survey.

In 1986, taro were again surveyed in Vanuatu as part of a general virus survey of seed sown and perennial crops, ornamentals and weeds by Alan Brunt, Glasshouse Crops Research Institute, Littlehampton, UK[50]. Taro in Serete, South Santo were sampled once more, and again the large bacilliform virus was identified; on this occasion, it was listed in the survey report as *Dasheen bobone rhabdovirus*.

What was going on?

The answer was quite simple really. The large bacilliform virus (i.e., *Dasheen bobone rhabdovirus* of Alan Brunt) associated with vein yellowing in Vanuatu and the large bacilliform virus found in taro with *alomae* and *bobone* in Solomon Islands and Papua New Guinea, were different viruses. Identification of the particles at Rothamsted had been done using electron microscopy and this was not sufficient to differentiate between them.

[49] In 1978, several cultivars from Vanuatu were sent to Solomon Islands to check for resistance to both taro leaf blight and alomae. While in quarantine, three of the plants showed symptoms of small bacilliform virus, and leaves from one (cv. Anleyau, Lumawa village, Epi) were sent to Rothamsted where the small bacilliform virus was identified.

[50] Brunt AA (1989) Survey for plants viruses and virus diseases in Vanuatu. UNDP/FAO Strengthening Plant Protection and Root Crops Development in the South Pacific (RAS/83/001). Food and Agriculture Organization of the United Nations in association with the South Pacific Commission. Suva, Fiji. 14 pp.

Fig. 12 TaVCV – *Taro vein chlorosis virus*. Another rhabdovirus found first in surveys in Vanuatu. Symptoms are most obvious at the margins of the leaves where the bright yellow feathering borders the veins. Sometimes leaves also show mild distortions, but nothing like those of *alomae* and *bobone*. Photo: Sarete, Santo, Vanuatu.

Fig. 13 TaVCV – *Taro vein chlorosis virus.* On this leaf the feathering symptom is alongside and also between major veins, but again most concentrated at the margins. There is some minor distortion. Photo: Tanafoli, Santo, Vanuatu.

Further surveys between 1987 and 1991 in the Federated States of Micronesia (States of Chuuk, Kosrae, Pohnpei, Yap States), Fiji, the Republic of Palau, Tuvalu and Mindanao, Philippines, found taro with symptoms of vein yellowing similar to those in Vanuatu, and associated with large bacilliform particles[51]. These plants were examined together with those showing *alomae* and *bobone* from Solomon Islands by Mike Pearson at the University of Auckland, using electron microscopy and two ELISA tests (ACP and DAS). The polyclonal antisera was supplied by Alan Brunt, prepared from taro with vein yellowing symptoms from Fiji and Vanuatu[52].

[51] Jackson GVH (1986) Preliminary results from surveys of plant diseases in the Federated States of Micronesia and Palau. UNDP/FAO/GTZ/IRETA Regional Crop Protection Workshop, Apia, Western Samoa, 8-12 September 1986. pp. 106-113.
[52] Pearson MN, Jackson GVH, Saelea J, Morar SG (1999) Evidence for two rhabdoviruses in taro (*Colocasia esculenta*) in the Pacific region. Australasian Plant Pathology 28: 248-253.

Fig. 14 TaVCV – *Taro vein chlorosis virus* Fiji. In general, the diseases in Fiji and Vanuatu are similar except that the yellowing of the veins is not as bright in leaves from Fiji compared to those from Vanuatu. Whether that is due to differences in the virus or the varieties between the two countries is not known. Photos: Varieties Vutokoto (left) and Naloaloa (right), Vanua Levu, Fiji.

The conclusion from differences in serological reaction, particle size and disease symptoms was that the vein-yellowing and *bobone*-associated viruses are not identical, although they are serologically related. Whether they should be called different species or different strains of the same virus was left undecided.

Interestingly, variable results from the serological tests, particularly those of *alomae*, indicated that both rhabdoviruses could be present in the same leaf. This is supported by the fact that symptoms of both viruses have been seen occasionally in the same leaves in Papua New Guinea and Solomon Islands. Additionally, whereas the vein yellowing virus could be purified by standard techniques both Mike Pearson and Mari James failed to purify rhabdovirus particles from either *bobone* or *alomae*.
As part of the national surveys of Pacific island countries, Alan Brunt, visited Solomon Islands in 1984 where he confirmed the presence of small and large bacilliform particles in *alomae* and large alone in *bobone*[53].

The name *Taro vein chlorosis virus* (TaVCV) was suggested for rhabdovirus causing the yellow vein symptoms to differentiate it from the large bacilliform virus associated with *bobone*, which Alan Brunt first named *Dasheen (or Taro) bobone rhabdovirus*[54],

[53] Brunt AA (1987), op. cit., p. 6.
[54] Brunt A, Cabtree K, Gibbs A (1990) Viruses of tropical plants . Descriptions and lists from the VIDE database. CAB International , Walling ford, UK. This was the name first given to TaVCV, but transferred to the taro large bacilliform virus (associated with *alomae* and *bobone*) when it was realised that TaVCV was a different virus.

and then *Colocasia bobone disease virus* (CBDV)[55]. He named the small bacilliform virus *Dasheen (or Taro) 'badnavirus'* (later becoming TaBV) [56].

Virus disease outbreaks

Samoa

Surveys in Samoa in 1977 found DsMV was common in taro and also ta'amu *(Alocasia)*, but some plants also showed moderate leaf distortions. In 1978, the small bacilliform particle - previously only reported from Solomon Islands - was found by Rothamsted in taro and ta'amu from Samoa quarantined before release in Solomon Islands. As there was concern that other viruses might be present, further surveys were arranged that year. The result confirmed that DsMV was widely distributed in both taro and ta'amu, and a dieback disease of taro was seen on Savaii and this was attributed to an unknown potyvirus (a virus similar to DsMV). However, no bacilliform viruses were found. In 1979, Grahame was asked to visit and check[57].

The result of the 1979 survey was DsMV was confirmed to be widely distributed in both Upolu and Savaii. Both taro and ta'amu showed the usual feathering characteristic of the disease. There was considerable variation of symptoms on taro leaves, and the incidence of infection was very high. Infections of DsMV were seen commonly after planting but, later, during the period of rapid growth, plants appeared to be healthy until near the time of harvest when the disease appeared again on either the mother plants or suckers. No symptoms were seen similar to the serious diseases of Solomon Islands. The situation in ta'amu was similar, except that the small bacilliform particle was found at Tanumalata.

The taro dieback on Savaii was caused by Pythium root rot, not a new virus disease.

French Polynesia

In 1979, a severe disease of taro was discovered by Leon Mu, Plant Pathologist, Service de l'Economie Rurale, Station de Recherche Gerdat-Irat de Papara. Leaf samples were sent to the Laboratoire Central de Patholie-Vegetale Gerdat-Irat, Montpellier, France, and the sap tested using antiserum prepared by Bill Zettler, University of Florida. DsMV was confirmed, but it was thought that other viruses might be present too as the symptoms were far more severe than usually occurs in taro infected by DsMV.

In September 1980, samples were sent to Roger Plumb at Rothamsted to see if other viruses were present. Particles of DsMV were present in large numbers, but no other viruses were seen.

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[55] Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L (Eds.) (1996) Viruses of Plants. pp. 437- 438. CAB International, Wallingford, UK.

[56] Brunt AA (1990), op. cit., p. 242.

[57] Jackson GVH (Taro virus diseases in Western Samoa. Report of a visit, 20 September-6 October 1979. South Pacific Commission, Noumea, New Caledonia. 20 pp.

Fig. 15 S-DsMV. Severe-*Dasheen Mosaic Virus* in French Polynesia. There is severe distortion of the leaves, and symptoms of DsMV occurs on most of them in contrast to symptoms in other Pacific island countries. The other contrast with 'normal' DsMV is that the plants do not recover from the symptoms. It is seen most often on variety Mana Ura. Photo: Tahiti, French Polynesia.

The problem persisted and, in August 1982, Grahame visited French Polynesia for SPC[58]. Several thousand taro on Tahiti and Moorea were inspected. DsMV symptoms were common in the plantings, but there were no symptoms from infections by large or small bacilliform viruses. In a majority of plants, symptoms were similar to those reported in many countries where DsMV occurs: leaves showed pale, green-to-yellow "feathering" patterns along the main veins, but they were seldom distorted, or reduced in size. Mostly, symptoms were present on young plants and those close to harvest.

The severe symptoms that had given cause for concern were seen only occasionally. The main differences between these plants and "normal" DsMV was that they were shorter, symptoms were present on all leaves, and there was no recovery. Feathering patterns were present throughout the leaf, leaves were small, often misshapen, and in some cases leaf blades were absent or transformed into short, thin, slightly thickened, strap-like structures without lobes. There was also a loss of colour in normally red-pigmented varieties, such as Mana Ura. However, incidence of severe-DsMV was low, about 5% up to harvest, and perhaps twice that number on suckers left to grow after harvest; the incidence of symptoms in other cultivars, Veo, Ere Ere and Poitere, was much lower.

Observations found planthoppers and aphids common on severe-DsMV plants, and it was notable that in some fields the disease appeared to be spreading from the borders into the plantings in the direction of the prevailing wind.

Leaves were also examined by Peter Fry, Department of Scientific and Industrial Research, Auckland. Particles of DsMV were in abundance, but also present at a low concentration were spherical particles, about 30 nm diameter. These were not identified, as they failed to infect any of several indicator plants (unspecified) or react with antisera of two New Zealand strains of *Cucumber mosaic virus.*

[58] Jackson GVH (1982) A virus disease of taro in French Polynesia. Report of a visit, 16-21 August 1981. South Pacific Commission, Noumea, New Caledonia. 26 pp.

The aetiology of severe-DsMV remains unresolved, as does the presence of the small spherical particle, although we will say more about both later.

Summary of Part 2

In the 1970s and 1980s regional pest surveys associated with UNDP/FAO projects to check the status of taro and other root crops became quite popular. Most of the islands of Vanuatu were visited by Grahame in 1982. The small bacilliform particle had been found previously in plants quarantined in Solomon Island so it was thought likely this would be common in the country. Sure enough, plants were found in South Santo with marginal vein yellowing and floppy umbrella-like young leaves. They were sent to Rothamsted, but only large bacilliform particles were present. In 1984 and 1986, Alan Brunt visited Solomon Islands and Vanuatu, respectively, for general plant virus surveys and he found that a large bacilliform virus was present in all these countries. He called the particle *Colocasia bobone disease virus.*

Plant disease surveys by Grahame took place in Micronesia for the first time in 1987 and later in 1991, and once more the large bacilliform particle was found. By this time Alan Brunt had prepared antisera to the large bacilliform particles and Mike Pearson was able to use it in ELISA to differentiate between the large bacilliform particle of Fiji, Vanuatu and Micronesia with that in Solomon Islands. In this way, he discovered a new virus, *Taro vein chlorosis virus* (TaVCV).

Interestingly, when plants in Solomon Islands were checked with serological methods, TaVCV and the large bacilliform virus (CBDV) were found in leaves with *alomae*.

During these years there were also disease outbreaks on taro thought to be caused by viruses in other parts of the region. In Samoa, the small particle had been recorded in taro quarantined in Solomon Islands and, later, during surveys, plants were reported to show DsMV, but also some unusual symptoms. Visits by Grahame in 1979 found that near the time of harvest all plants inspected should signs of DsMV, but infections from small bacilliform particles was present only on ta'amu (*Alocasia*), but could not be found on taro.

Later, a severe outbreak of DsMV was reported from French Polynesia causing the production of yellow, stunted, under-sized leaves on about 5% of the plants in some plantings. As this was the first occurrence of such symptoms farmers were concerned. Grahame visited in 1982 and found that symptoms were mostly on the variety Mana Ura: leaves were reduced to strap-like structures or were without leaf blades. Bill Zettler, University of Florida examined samples and only DsMV was identified. Peter Fry in DSIR, New Zealand found DsMV and a 30 nm diameter spherical particle.

The discovery of TaVCV and severe DsMV was of interest, but did not compensate for a lack of research into the cause of *alomae*!

PART 3: TaroGen: Indexing, surveys, seed transmission

Sensitive methods

TaroGen was about taro leaf blight; it was a response to the disaster that overtook Samoa when taro was annihilated by the disease in 1993. Under TaroGen, breeding programs to produce blight-tolerant plants were set up in Samoa and Papua New Guinea in 1998. To support the work, ACIAR financed the *Virus Indexing and DNA Fingerprinting for the International Movement and Conservation of Taro Germplasm* project.

DNA fingerprinting was led by Ian Godwin, Professor, School of Land and Food Sciences, University of Queensland, with the aim of providing a core of Pacific island countries based on the genetic diversity of the region. Accessions selected for virus indexing were established as meristems in tissue culture in the country of origin, at UQ or at the RGC, Fiji, and once regrown into plants of sufficient size sent to the AQIS PEQ, Brisbane, and subsequently DNA fingerprinted at UQ and indexed at QUT. Indexing was carried out first on tissue cultured plants and then when these plants were 3 and then 6 months old. Suckers of each accessions were retained at the Regional Germplam Centree (RGC), Fiji, set up by TaroGen to hold a core collection of taro from the region that could be safely shared.

Testing for viruses (indexing) was led by Rob Harding, Professor, Plant Biotechnology Program, Science Research Centre, Queensland University of Technology. The team's aim was to determine what viruses were present in the region, and to develop sensitive methods to test for them. The methods had to be such that they could be transferred to the RGC.

Prior to the start of the project, four viruses were known from Pacific island countries: DsMV, two viruses thought to be rhabdoviruses - *Taro vein chlorosis virus* (TaVCV) and *Colocasia bobone disease virus* (CBDV) - and *Taro bacilliform virus* (TaBV) – thought to be a badnavirus. Of these only DsMV had been characterised.

At the first TaroGen Project Planning Workshop, 3-4 September 1998, at the Horticulture and Food Research Institute of New Zealand Ltd., Auckland, Pacific Island countries as well as regional and international organisations and research institutes associated with the project, formulated a Code of Conduct for the sharing of taro germplasm. Under this, TaroGen partners agreed that: i) exchanges were for research purposes, and remain the property of the original source country; ii) germplasm would be freely exchanged between the participants of the project; and iii) any material acquired will not be transferred beyond the participants without prior consent of the original source country, and then only under an MTA[59].

[59] Anon (2005) Development and application of virus indexing protocols for the international movement of taro germplasm. Plant Biotechnology Program Science Research Centre Queensland University of Technology Australia and The Regional Germplasm Centre Secretariat of the Pacific Community Suva, Fiji. QUT, Brisbane. 27 pp.

The project was successful and, by 2003, the following had been achieved to characterise the taro viruses of Pacific island countries[60,61,62,63]:

DsMV (Dasheen mosaic virus)

- Sequence variability investigated and PCR and serological-based diagnostic methods developed.
- The methods had proven to be both sensitive and robust.
- PCR degenerate primers were designed based on the sequences of numerous virus isolates collected throughout the Pacific, to ensure they amplified virus sequences from all the countries surveyed.

TaBV (Taro badnavirus)

- An isolate from PNG was characterised and shown to be a definitive badnavirus; sequence variability was investigated throughout the Pacific and a PCR-based diagnostic test developed.
- Considerable variation was found in isolates from Solomon Islands suggesting that TaBV either originated in that country or that the virus first arrived there before transfer elsewhere.
- A PCR test was available for integrated TaBV-like sequences (used on both plants and seeds).

TaVCV (Taro vein chlorosis vírus)

- Partial characterisation of the genome was carried out.
- PCR-based diagnostic test developed (subsequently published).

CBDV (Colocasia bobone disease virus)

- Partial characterisation of the genome.
- PCR-based diagnostic test developed.
- Southern hybridisation tests were done to check PCR because of the importance of this virus.

TaRV (Taro reovirus)

- A reovirus found in several countries for the first time.
- The reovirus was partially characterised.
- PCR-based diagnostic test developed based on variability of numerous isolates throughout the Pacific.

As of 10 December 2003, 159 plants – traditional cultivars from the Pacific and the TANSAO collection from the Pacific and Southeast Asia (initiated into tissue culture at LIPI, Indonesia, and regrown from meristems at the RGC), and breeders' lines from breeding programs in Papua New Guinea and Samoa – had been tested according to agreed protocols. Of the 159 plants, 110 tested negatively for five viruses[64]. Five of the 49 that were positive for DsMV, the remainder for TaBV.

[60]Harding RM, Revill PA, Hafner GJ, Yang I, Maino MK , Devitt LC, Dowling M, Dale JL (2005) Characterisation of taro viruses and the development of diagnostic tests. Third taro symposium. Edited by Guarino L, Taylor M, Osborn T (2003) Report of a meeting (technical). Secretary of the Pacific Community. 242 pp.

[61] Yang IC, Hafner GJ, Dale JL, Harding RM (2003a) Genomic characterisation of taro bacilliform virus. Archives of Virology 148: 937-949.

[62] Yang IC, Hafner GJ, Dale JL, Harding RM (2003b) Sequence diversity of South Pacific isolates for Taro bacilliform virus and the development of a PCR-based diagnostic test. Archives of Virology 148: 1957-1968.

[63] Revill P, Trinh X, Dale J, Harding R (2005) Taro vein chlorosis virus: characterization and variability of a new nucleorhabdovirus. Journal of General Virology 86: 491–499

[64] Anon (2005) op. cit., p. 2.

Surveys - Pacific island countries

Using these diagnostic tests, the distribution of taro viruses in 11 Pacific Island countries was determined, to allow them to make informed decisions regarding the risks associated with the importation of taro germplasm (Table 2)[65].

Table 2. Virus incidence in Pacific Island countries

Country	Samples tested	DsMV*	CBDV	TaBV	TaRV	TaVCV	Mixed infections
American Samoa	16	9	0	3	0	0	3
Cook Islands	4	4	0	3	0	0	3
Fiji	23	10	0	18	0	11	16
FSM	5	3	0	0	0	3	3
Marshall Islands	3	1	0	1	0	0	1
New Caledonia	36	15	0	24	0	5	13
PNG	62	16	13	14	2	15	15
Samoa	29	18	0	18	0	0	9
Solomon Islands	31	4	17	31	15	9	24
Tonga	16	7	0	11	0	0	2
Vanuatu	38	27	0	36	14	15	35

*Number of samples testing positive for virus.

Apart from further work required to develop molecular-based tests for CBDV, the project completed its objectives.

In addition to the indexing strategies developed and the survey of taro viruses in the region, the results at QUT produced a number of other interesting results:

- DsMV showed a wide range of symptoms as had been noted in other countries, Samoa in particular.
- A TaBV-like sequence was found in most taro plants tested, including those that were symptomless and those indexed as TaBV-free; this sequence may be a second taro badnavirus or it may be an integrated sequence. Integration was favoured because of “the ubiquitous nature of the sequence”[66]. Only in Samoa were vein-clearing symptoms consistently associated with infection, especially in the variety PSB-G2 from the Philippines.
- FSM was the only country where TaBV was not detected in taro; it was detected in *Alocasia*, however.
- DsMV and TaBV are widely distributed in the region, whereas TaVCV and TaRV were more restricted (but see later for TaVCV).
- TaVCV was found in Papua New Guinea and Solomon Islands, sometimes associated with *alomae* and *bobone*.
- TaVCV in Vanuatu showed a more intense vein yellowing than elsewhere.
- TaRV was detected only in association with other viruses, and no symptoms could be attributed to it. Its impact on taro is unknown.
- CBDV was found only in Papua New Guinea and Solomon Islands.

[65] Revill RA, Jackson GVH, Hafner GJ, Yang I, Maino MK, Dowling ML, Devitt LC, Dale JL, Harding RM (2005) Incidence and distribution of viruses of Taro *(Colocasia esculenta)* in Pacific island countries. Australasian Plant Pathology 34: 327-331.
[66] Yang IC *et al.* (2003b), op. cit., p. 1966.

- Plants with *alomae* were found infected with TaBV, but in some cases it occurred other viruses - DsMV, TaRV, or TaVCV, either singly or in combinations. Plants with *bobone* were also found infected with TaBV.

Some comments on these results.

TaRV is a spherical particle, approximately 70 nm diameter, twice the size of the virus particles in taro from French Polynesia with symptoms of severe DsMV examined by Peter Fry in 1981. The French Polynesia particles were the size of CMV, about 30 nm diameter. A similar particle was also recorded from Solomon Islands, twice in a female taro cultivar with TaBV, and once in a male taro with *alomae*[67].

Symptoms of TaBV, similar to those described previously[68], were only seen in Samoa in the variety Talo Fili (PSB-G2); this variety was introduced from the Philippines in 1993 and used in the TaroGen taro leaf blight breeding program. Talo Fili was introduced into Samoa in tissue culture, but whether indexed for TaBV (and other viruses) using sensitive methods, is unknown. (This variety was used by Apaitia Macanawai in mealybug transmission tests in Samoa.)

In Peter Revill's 2005 paper, plants from PNG are listed with *bobone*, not *alomae*. However, it was probably premature to make this diagnosis, which could only have been made by waiting to see if the plants recover or die. In the short visits to gardens during the survey this was not possible. Also, farmers met during the survey in PNG did not distinguish taro as male or female as they do in Solomon Islands, so that the survey team could tell if plants with symptoms had *alomae* or *bobone*.

And as to the sensitivity of the methods, a quote from the report written at the end of the project summarising the results and commenting on this matter[69]:

> *The indexing methods (PCR and Southern hybridization) are the most sensitive available. Great effort has been taken to ensure that the primers used in the PCR tests detect as many different isolates as possible, hence many of the primer pairs used in this study are degenerate primers, based on more than one viral genome. It has not been possible to design degenerate primers as yet for TaVCV and CBDV, as the viral sequence has only been obtained for one isolate and variability studies are yet to be completed. However, the primers used, do detect virus isolates from a range of countries, although it is impossible to say that any primer set, be they degenerate or specific, will detect all virus sequences*.

But did the results tell us anything new about the aetiology of *alomae*?

If we leave out the PNG results in the paper because we are not sure if the plants were *alomae* or *bobone*, and concentrate on the Solomon Islands, we can say that all the plants tested contained CBDV and TaBV. Further than that there was nothing consistent about the other virus infections in these plants that would suggest they were involved in either disease (Table 3). And what about elsewhere? Were severe symptoms seen?

[67] Gollifer DE (1976), op. cit., p.47.
[68] Jackson GVH (1980), op. cit., p. 13.
[69] Anon (2005) op. cit., p. 20.

Rarely, but occasionally severe symptoms associated with TaVCV were seen in both New Caledonia and Vanuatu. For example, variety IND 409 an accession from the TANSAO collection from Indonesia, and the cultivar Bourbon from New Caledonia, a triploid taro. These two symptoms were not investigated, unfortunately.

Fig. 16 Severe infections with similarities to *alomae* and *bobone.* Left: TANSAO accession (IND409) growing in Vanuatu; right: Variety Bourbon, New Caledonia. Both have symptoms of TaVCV infection. Photos: Saratou, Santo, Vanuatu (left); Ponerihouen, New Caledonia (right).

Table 3. Viruses associated with *alomae* and *bobone* disease of taro in Solomon Islands (Revill *et al*., 2005, modified).

Symptoms	Virus status
Bobone	CBDV, DsMV, TaBV, TaRV, TaVCV
Alomae, vein chlorosis	CBDV, DsMV, TaBV, TaRV, TaVCV
Alomae	CBDV, TaBV, TaRV, TaVCV
Bobone	CBDV, TaBV
Alomae	CBDV, TaBV, TaRV
Alomae	CBDV, TaBV, TaRV
Bobone	CBDV, TaBV
Bobone	CBDV, TaBV
Alomae	CBDV, TaBV, TaRV
Alomae	CBDV, TaBV
Bobone	CBDV, TaBV
Bobone	CBDV, TaBV, TaRV

The sensitive methods developed by the project were extremely valuable. For the first time, they provided degrees of accuracy greater than searching for particles under the EM. It was unfortunate, however, that the project ended before they could be applied to the diseases of Solomon Islands. Had transmission tests been done, the new technologies could have been put to work uncovering the aetiology of *almoae*.

Transmission of TaBV

Seed in general is considered an effective method of transferring plant varieties: it's easy to distribute and often acts as a filter for viruses. With the establishment of a breeding program under TaroGen to produce lines tolerant to taro leaf blight in Samoa there was the need to test that seed was free of virus and could be sent safely to other countries. However, taro in Samoa was known to be infected by a badnavirus and there were reports of seed transmission of other badnaviruses, e.g., *Banana streak virus*, *Kalanchoe top-spotting virus, Mimosa bacilliform virus* and *Commelina yellow mottle virus*. Therefore, research was done at the University of the South Pacific, Alafua Campus, Samoa, by Apaitia Macanawai, to determine the risk associated with seed transfer. Rob Harding supervised the work.

The study at Alafua confirmed that mealybugs transmitted TaBV. Mealybugs were collected from the field from symptomless plants (PSB-G2) and placed for 3 months on a PCR-tested, mealybug-free, PSB-G2 plant maintained in a screenhouse. The host plant remained symptomless and tests should that it was negative for TaBV at the beginning of the trial. Mealybug transmission was investigated by exposing 51 PCR-tested, 1-2-month-old symptomless PSB-G2 suckers to mealybugs reared on TaBV-infected plants. Typical virus symptoms developed on 17 plants between 24 and 36 days. These and 13 symptomless plants tested positive for TaBV by PCR.

Fig. 17 TaBV – *Taro badnavirus*. In this leaf the feathering is very obvious, and similar to that which occurs with TaVCV infection. In this instance, the feathering is between the veins. Often, it is difficult to differentiate between the two infections on symptoms alone, especially when only part of the leaf it showing vein-yellowing. Photo: Safaatoa, Upolu, Samoa.

The mealybugs used in these tests were again identified by Landcare Research, Auckland, New Zealand as *Planococcus solomonensis*, which is present in many Pacific island countries.

Summary of Part 3

The outbreak of taro leaf blight in Samoa in 1993 required the safe movement of taro accessions to be used in breeding programs and for conservation. This meant indexing for viruses. A project to support the work of TaroGen began at QUT in 1998 and quickly developed sensitive methods to detect all viruses known in Pacific island countries.

Predictably, as more surveys were done, leaves examined, and techniques evolved, other viruses were found. A reovirus was found for the first time in PNG, Solomon Islands and Vanuatu. It always occurred with other viruses, and its impact remains unknown. PCR tests were produced for DsMV, TaBV, TaVCV, and TRV; and CBDV was partially characterised. Importantly, some TaBV sequences were found in the DNA of plants, which meant that tests for TaBV often resulted in false positives. However, it was thought unlikely that these sequences could produce symptoms.

Tests based on sensitive molecular or immunological methods were made available for all viruses. The tests involve analysis for virus nucleic acids using PCR and virus-specific primers and, as such, are many times more sensitive than direct observations by EM. The methods represent an important advance. Unfortunately, there was insufficient time before the project ended to use the methods to uncover the aetiology of *alomae* and *bobone*, and severe virus-like infection in other countries.

Surveys showed that DsMV and TaBV are widely distributed in the region, whereas CBDV, TaVCV and TaRV were restricted. However, the find that TaVCV is in the Samoan islands (see Part 4) may change that. TaRV was detected only in association with other viruses, and no symptoms could be attributed to it. Its impact on taro is unknown.

Plants with *alomae* were all found infected with TaBV, but in some cases it occurred with combinations of DsMV, TaRV and TaVCV. In most plants with *bobone*, TaBV was also present.

Research at USP Alafua provided proof that TaBV was seedborne and seed-transmissible, and also spread by mealybugs, in this case, *Planococcus solomonensis.*

PART 4: INEA: Revisiting *alomae* and *bobone*

DSMZ and the International Network for Edible Aroids

After the end of TaroGen in 2004, there was another intermission in the taro virus saga until 2011 with the start of a global taro project, *Adapting clonally propagated crops to climatic and commercial changes*, a five-year initiative funded by the EU. The aim was to use edible aroids as a model to improve clonally propagated root and tuber crops of tropical countries. There were 16 countries worldwide, supported by four European research institutions, and an international research-for-development organisation. It was led by the Pacific Community, with technical support from CIRAD.

Under the project, INEA, the *International Network for Edible Aroids* was established. This Network was set up with three main aims: i) to help countries access plants of varied genetic backgrounds; ii) to assist with breeding strategies; and iii) to demonstrate the effective use of modern technologies. The first aim required sound indexing protocols, and the development of these was the task of Stephan Winter, Head, Plant Virus Department, German Collection of Microorganisms and Cell Cultures, Braunschweig (DSMZ), Germany.

As with the TaroGen project, movement of germplasm globally required indexing plants for viruses and removing the infections if they are found. While DSMZ dealt with testing germplasm for viruses, removing infections was the work of SPC CePaCT, the Centre of Pacific Crops and Trees, the newly expanded Regional Germplasm Centre, established previously by TaroGen.

Apart from the development of indexing procedures for all known taro viruses, and their application to germplasm moving between countries, DSMZ targeted the viruses of PNG and Solomon Islands, and the question: What is *alomae*?

The strategy at DSMZ was as follows:

- Using standard virological methods and NGS – Next-Generation Sequencing:
    - Research the rhabdoviruses, TaVCV and CBDV: carry out sequence analysis and develop primers.
    - Undertake transmission tests using *Tarophagus* planthoppers.
    - Analyse plants from Solomon Islands and PNG for viruses.

Robust diagnostic tests for routine indexing were developed based on ELISA or PCR. For PCR the molecular assays had to be constantly adjusted because of the diversity of the virus isolates. ELISA tests were successfully developed for DsMV and TaBV, but not for all other viruses of taro.

DSMZ also investigated seed transmission of TaBV and concluded that it was very unlikely that seeds contributed to virus spread.

Results at DSMZ (Stephan Winter and Marion Liebrecht) 2011-2016:

- ***DsMV:***
    - Numerous DsMV isolates were analysed and specific primers designed for virus detection by RT-PCR (previously published RT-PCR tests failed in DSMZ lab evaluations).
    - Antisera from recombinant coat proteins were developed into ELISA tests and used in taro, *Amorphophallus* and other aroids.
    - ELISA tests were validated for sensitivity, specificity, repeatability and reproducibility following EPPO guidelines. ELISA can be used for routine detection.
- ***TaVCV:***
    - *Tarophagus* sp. from Solomon Islands were capable of transmitting TaVCV from Vanuatu.
    - Using published primers, RT-PCR was not able to detect TaVCV sequences in all samples from Vanuatu.

    - TaVCV in Fiji and Solomon Islands differs from that found in Vanuatu.
    - TaVCV published sequence from USA (Hawaii) is different from that found in Vanuatu.
    - An improved RT-PCR test has been developed.
    - TaVCV is a nucleorhabdovirus.
- ***TaBV:***
    - RT-PCR tests were unreliable because of integrated sequences.
    - Complete genome of "real" or episomal virus (checked by EM) reconstructed from *alomae* in Solomon Islands.
    - ELISA test developed based on a recombinant coat protein gene. This is the preferred test for the episomal virus as serological tests can discriminate between the episomal and integrated sequences.
    - There was no evidence that the integrated sequences can become episomal viruses.
    - Immunocapture PCR test developed.
- ***Tenuivirus:***
    - A tenuivirus has been found in *Tarophagus* sp. and in plants from Solomon Islands. CBDV was also present in the plants.
    - The tenuivirus has been found in Solomon Islands, PNG, and Vanuatu.
    - The tenuivirus has been transmitted from *bobone*-like plants in PNG to *Nicotiana benthamiana*, causing curling of leaf margin, chlorotic spots, mottling and malformed leaves. However, virus-like structures were not seen by EM, but dsRNA fragments showed presence of a putative tenuivirus.
    - A RT-PCR protocol for detection of the tenuivirus has been developed. The test needs to be validated on further samples.
    - Antisera-based methods for the detection of the tenuivirus needs further work.
- ***CBDV***:
    - CBDV is always present in plants with *alomae* or *bobone*.
    - When the complete genome of 10 isolates of CBDV from PNG and Solomon islands were compared there was considerable diversity, indicating geographical isolation of CBDV isolates.
    - Previously, primers for CBDV gave false positives. An improved RT-PCR protocol has been developed. The protocol, primers and reference virus is ready for field testing.
    - Using deep sequencing techniques (Illumna HiSeq2000), the full genome of CBDV from PNG has been constructed.
    - CBDV has been detected from haemolymph in the hind legs of *Tarophagus colocasiae*.
    - Rolling circle amplification is being developed for the CBDV (and also the tenuivirus) so that there are accurate ELISA and PCR methods available for their detection.
    - CBDV is a cytorhabdovirus.

The discovery that a tenuivirus is present in taro with *alomae*, together with CBDV, is of considerable interest. Is this the breakthrough we have been waiting for?!

What is a tenuivirus?

Tenuiviruses cause important plant diseases; they are especially important in rice and maize. The virus in taro is related to *Rice stripe virus*, the type member of the group. It is a single stranded RNA virus. Other members include *Maize stripe virus, Rice grassy stunt* and *Rice hoja blanca virus*. In nature, the viruses are transmitted by insects belonging to the Delphacidae (family of planthoppers), in a persistent manner. The virus particles are thin (3-10 nm diameter), and 500-2100 nm long.

These viruses circulate through the insect (stylet, gut, haemocoel, salivary glands) and multiply in the process, i.e., they are circulative and propagative; they can be acquired after 15 mins to 4 hours of feeding by the planthopper host, and they are not limited to the phloem.

After acquiring the virus, and before being able to transmit it, there is period of up to 30 days when transmission by the planthopper vector is not possible. But after that time, the insect is able to transmit the virus until it dies, i.e., its persistent. Inoculation of the plant host takes from a few minutes to several hours.

Most tenuiviruses are transmitted through the egg (transovarially) at rates up to 20%, and through sperm. Viruses in this group are not known to be seed or pollen transmitted.

What is alomae?

Over the years, since samples were first sent to Rothamsted, it has been assumed that CBDV and TaBV were the cause of *alomae*. But with the discovery of a tenuivirus another possibility arises. *Alomae* might be caused by dual infections of CBDV and the tenuivirus, both transmitted by *Tarophagus* spp. Does it fit the facts?

The short answer is “Yes”. There are two provisos: i) the tenuivirus is either absent in plants with *bobone* or in very low concentration, and ii) other viruses, DsMV, TaVCV and TaBV are present in the plants, but are not involved in *alomae* and *bobone*.

There is also a question of whether the two viruses are transmitted simultaneously or separately. That is of interest but not fundamental to the hypothesis that they come together in taro plants and cause *alomae*. The answer will be found by analysis of *Tarophagus* individuals and transmission tests.

What of *joa*, the disease on Isabel, that was supposed to be lethal. This is more difficult to explain. The only particle seen by EM was TaBV. If that is the correct situation, it does not fit. However, only a few necrotic plants were examined. More observations using up-to-date methods are needed before anything can be concluded about the cause of this disease.

But let’s not forget DsMV, TaVCV and TaBV. What’s to say they are not involved instead of the tenuivirus?

Fig. 18 Plants with *alomae*, but also showing symptoms of TaVCV. Left: plant from the same garden as Figs. 1&2. It is showing TaVCV on the two expanded leaves, and initial signs of *alomae* on the still rolled leaf. Above: TaVCV on the expanded leaf, which is also distorted; the rolled leaf is showing typical early *alomae*. Photos: Fote FES, Malaita, Solomon Islands (right)

Fig. 19 Plant showing both LPS (CBDV) puckering symptom (arrows), and TaVCV (lower front), but without symptoms of *alomae*. Photos: Fote FES, Malaita, Solomon Islands.

Neither David's tests at Dala using *Aphis gossypii* or Kenten and Woods tests using *Myzus persicae* at Rothamsted showed that DsMV was involved in either *alomae* or *bobone*. We think we can safely exclude that virus.

As for TaVCV, we are not so sure that we can rule it out. In Papua New Guinea and Solomon Islands, plants with *alomae* often show symptoms of TaVCV. However, occasionally male plants are seen with both LPS (i.e., CBDV infection) as well as TaVCV. As the plants do not shown signs of *alomae* it suggests that the two particles can co-exist without causing that disease. Nevertheless, the virus will have to be included in future work to check.

It cannot be argued that the tenuivirus alone causes *alomae*, because it is found widely distributed outside Solomon Islands and PNG, in countries where *alomae* does not exist. This means the presence of CBDV is likely to be essential for *alomae* as it appears to be for *bobone*.

We know from transmission tests that planthoppers can transmit CBDV to both female and male plants after acquisition feeds on *bobone*. From these tests, female plants develop *bobone*, and male plants develop a very mild *bobone*-like symptom (we called it LPS – large particle symptom). When *alomae* did not result in the male test plants, or only LPS occurred, we assumed it was because the planthoppers did not pick up both rhabdoviruses or that there were no latent infections of TaBV in the test plants. We have doubts about the involvement of TaBV as a component of *alomae*. A more plausible alternative possibility is that the planthoppers failed to acquire and transfer CBDV together with the tenuivirus.

If we are right about the tenuivirus, it means that there is only one vector for *alomae*, and that is *Tarophagus.* Our hypothesis does not deny that mealybugs spread TaBV, or aphids, DsMV, only they do not play a part in *alomae*. We will, however, keep an open mind about TaVCV as that has been shown to be transmitted by *Tarophagus*!

Finally, the experience of DSMZ over the period of INEA is that studies on viruses, vector transmission and epidemiology, need to be done where the viruses and vectors are endemic and environmental conditions support the growth of taro. It is difficult to do the transmission tests in Germany, as it was difficult to do them in England 35 years before.

Structure of CBDV

Although Stephan Winter worked out the genome sequence and structure of CBDV from PNG during INEA, and realised that it was a cytorhabdovirus, the work remains unpublished. Instead, it was left to a collaboration between teams at universities in Australia and New Zealand using *bobone*-affected taro from Solomon Islands that had been obtained 11 years previously, and maintained in a glasshouse at the University of Auckland, New Zealand[70], to publish it in 2016. Both Rob Harding and Mike Pearson were members of the teams.

[70] Higgins CM, Bejerman N, Ming Li M, James AP, Dietzgen RG, Pearson MN, Revill PA, Harding RM (2016). Complete genome sequence of *Colocasia bobone* disease-associated virus, a putative cytorhabdovirus infecting taro. Archives of Virology 161: 745-748.

The virus was named *Colocasia bobone disease-associated virus*, CBDaV. The group was not able to establish that the virus was the same as CBDV, as that virus had not been sequenced, or that their CBDaV caused *bobone* disease, hence use of the word "associated" in its name.

Detection of TaBV

In 2015, a badnavirus was characterised from Chinese taro obtained in two fields in Hubei Province, central China, showing "a mild feathery mosaic symptom on young leaves and brown spots on matured leaves"[71]. The newly named TaBCHV was identified from the virus infected plants by sRNA sequencing and RT-PCR amplification, showing a genetic structure similar to other badnaviruses. A year later, "leaves of a taro plant showing feather-like chlorosis and mosaic symptoms" were collected from the University of Hawaii, Oahu, Hawaii, and subject to PCR using primer sets for TaBCHV[72]. The products were sequenced and subject to other assays, confirming the presence of the virus. But whether the virus is integrated into the taro genome was not determined.

Outbreaks of TaVCV in Samoa

In April 2017, Grahame was in Samoa for SPC, documenting the use of CePaCT germplasm and visiting the backup tissue culture collection at USP.

Fig. 20 Plant showing distinctive symptoms of TaVCV. Note the umbrella-shaped leaves typical of the infection by the strain in Vanuatu (see Fig. 14, left). The leaf at top left shows the feathered vein-yellowing at it appears from the underside of the leaf. Photos: west coast Upolo, Samoa.

[71] Kazmi SA, Yang Z, Hong N, Wang G, Wang Y (2015) Characterization by Small RNA Sequencing of Taro Bacilliform CH Virus (TaBCHV), a Novel Badnavirus. PLoS ONE 10(7): e0134147. doi:10.1371/journal.pone.0134147.

[72] Wang YN, Hu JS, Borth WB, Hamim I, Green JC, Melzer MJ (2016) First Report of Taro bacilliform CH virus (TaBCHV) on Taro (*Colocasia esculenta*) in Hawaii, U.S.A. APS Publications. https://doi.org/10.1094/PDIS-02-17-0172-PDN

The previous week, members of SPC Plant Health had been asked to look at a disease on the south coast of Upolu that was of concern to farmers. The disastrous taro leaf blight epidemic was still remembered acutely, even though it occurred 30 years previously, and farmers were ever on the lookout for new pest and disease problems. SPC thought the symptoms were caused by TaBV, a reasonable diagnosis under the circumstances as the virus is common in Samoa.

However, the possibility that a new disease had arrived caused considerable alarm, and a directive was given to MAFF staff, to uproot infected plants, spray those remaining, and ban sending taro - corms and planting material - to Savaii to prevent further spread of the problem.

On 18 April 2017, Grahame visited the same taro fields at Falealili and Safata when it became obvious that it was not TaBV, the small bacilliform virus, but TaVCV, one of the two rhabdoviruses, that was causing the new disease.

As we have seen, there are two strains of TaVCV, one is in Vanuatu and the other is present in most other Pacific island countries. These two strains are difficult to tell apart based on symptoms: both produce a vein yellowing at the leaf margins. However, in Vanuatu, infection by TaVCV produces a floppy umbrella-like symptom of young leaves. Taro in Samoa were clearly showing that.

Samples were sent to Stephan Winter to check using RT-PCR, but they were delayed in transit and did not arrive until 11 May. Unfortunately, the first lot were rotten upon arrival and no viruses were detected. A second batch was sent to Germany in June, followed soon after by leaves dried over calcium chloride. Finally, on 29 June, Stephan could say definitively that the only virus present was TaVCV. The full genome sequences of TaVCV from Samoa were 100% identical to those from Vanuatu, whereas they were only 84% identical to those from Fiji (Stephan Winter; pers. comm).

How the virus got to Samoa is not known. It is unlikely that it was introduced from SPC as all plants distributed from CePaCT are sent as virus-tested tissue cultures. The virus was reported from Hawaii in 2014[73], and is known to be present in American Samoa[74]. Stephan Winter's considers that TaVCV from Vanuatu is not the same as the TaVCV reported from Hawaii, but is similar to that of American Samoa (Stephan Winter (pers. comm). But we won't know any more about these introductions until detailed comparisons are made between TaVCV in all four locations.

Fortunately, there have been no reports of any impacts on the yield of taro or any other damaging symptoms from America Samoa, Hawaii, Samoa or Vanuatu. Nevertheless, it is of concern that taro with its viruses are being moved about the region. The fear remains that the viruses involved in *alomae*, the lethal disease, could just as easily be transferred unintentionally in planting material.

[73] Long MH, Ayin C, Li R, Hu JS, Melzer MJ (2014) First Report of *Taro vein chlorosis virus* Infecting Taro (*Colocasia esculenta*) in the United States. Plant Disease 98(8): 1160.

[74] Atibalentia N, Fiafia ST, Gosai RC, Melzer MJ (2018) First report of *Taro vein chlorosis virus* on Taro (*Colocasia esculenta*) in the U.S. Territory of American Samoa. Plant Disease 102(4): 828.

Summary of Part 4

The work on taro viruses resumed in 2011 with the start of an EU-financed project *Adapting clonally propagated crops to climatic and commercial changes*. Under this 5-year project INEA, the International Network for Edible Aroids was established. There were 16 country partners and five regional and international development assistance or European institutes involved. It was led by SPC with technical advice from CIRAD. DSMZ, Germany provided expertise on viruses to ensure safe movement of germplasm between partners. The institute's remit was to ensure that indexing methods were sound and transferable to partners and to investigate those viruses of uncertain aetiology.

DSMZ produced ELISA tests for DsMV and episomal TaBV; found that TaVCV in Vanuatu and Fiji were different (which is supported by symptom differences); produced an RT-PCR test for CBDV that is ready for field testing; and, most importantly, identified a tenuivirus in *Tarophagus* sp., and in plants from Solomon Islands, PNG and Vanuatu. It has been transmitted to *Nicotiana benthamiana*. Virus-like structures were not seen by EM, but dsRNA fragments of the putative tenuivirus were detected. A RT-PCR protocol for the detection of the tenuivirus has been developed, but needs validation.

Additionally, the full genome of CBDV from PNG has been constructed, and CBDV has been detected from haemolymph in the hind legs of *T. colocasiae*.

If *alomae* is caused by CBDV and the tenuivirus acting together, and they are both spread by *Tarophagus* does that agree with transmission tests? The answer is "yes". Innumerable transmission tests carried out at Dala in the 1970s showed that *Tarophagus* planthoppers fed on *alomae* for a few days and then transferred to healthy test plants produced *alomae*. Symptoms of the disease occurred in about four weeks. In our transmission tests at Dala it was never possible to reproduce *alomae* when *Tarophagus* were fed on *bobone* plants and then to test plants. If a tenuivirus is involved in *alomae* it may mean that either the tenuivirus is absent in female taro or at a very low concentration, so low that it was not transmitted by *Tarophagus*.

As a group, plant tenuiviruses are thin flexuous rods, so possibly confused in EM for DsMV; they are transmitted in a circulative and propagative fashion by delphacid planthoppers in a persistent manner, and most are transmitted through the egg (transovarially) at rates up to 20%, and through sperm, but are not known to be seed or pollen transmitted.

TaVCV, Vanuatu strain, was isolated from taro in Samoa. It was previously reported in Hawaii in 2014, and later in American Samoa in 2018.

PART 5: WHERE TO FROM HERE?

Why bother about taro viruses?

It is a simple question: should we be bothered about these complex difficult-to-research diseases when there are so many other pressing problems that might demand our

attention? Sometimes the question is even wider than that, and it is whether we should be bothering about taro at all: it's being overtaken by sweet potato, cassava and African yams in many countries, but particularly in Papua New Guinea and Solomon Islands where taro has these peculiar diseases, so why bother?

The short answer is that we should be paying attention to ALL these crops, they all need support; there are very good reasons for Pacific island countries to have a diverse range of food crop staples to protect food and nutritional sustainability. And remember, countries interested in taro can't rely on the international agricultural research centres for assistance as none of them deal with aroids. Taro is a so-called 'orphan crop', mainly a crop of smallholders in West Africa, South and Southeast Asia and the Pacific. In many of these places it is the food crop of the poorest people.

However, we don't want to get into arguments of these kinds, but just to say why we think there should be a continuation of research into *alomae* and *bobone*. Here is our take.

First, there is a real satisfaction from growing the crop among the people who eat it. It's not just for the calories and other nutritional values - they can be got from any of the roots crops – they are all about the same. It is for something different. Taro is a traditional crop and represents an expression of people's culture, their belonging to their district, island or country. Growing and consuming it is a way of preserving that attachment. Proof of this can be seen among people in urban areas, who have moved away from their roots (literally and metaphorically): they still want to eat taro, even if only occasionally because prices are so very high. And we must not forget that taro is one of the few crops where the entire plant is eaten, with the leaves making nutritious vegetable dishes.

Secondly, and following from the above, growing a garden of taro and having *alomae* come and scythe down the plants in a matter of a few weeks causes a lot of anguish.

Thirdly, if we knew what was spreading *alomae* we would have more to say about management. At present, we talk about *Tarophagus*, planthoppers, and mealybugs, and that both are involved, but we are not sure. Our latest results suggest that it might only be planthoppers, but we need to do the work to prove that. There can be no guessing, no speculation. And from experiences in the UK and Germany, transmission tests are best done where the diseases occur.

Fourthly, biosecurity of countries is compromised by not knowing the aetiology of *alomae*. Countries cannot make informed decisions on importing valuable accessions from Papua New Guinea, not when they don't know which viruses are involved. We might assume from the latest research that CBDV and the tenuivirus are the cause, but this is only a hypothesis. And, even if correct, the testing protocols are not assured for either virus: they need field testing. This means that countries may take the view that the taro leaf blight-resistant breeders' lines from Papua New Guinea cannot yet be imported to protect farmers against this deadly disease.

Lastly, we should finish what we start. Donors should not give up too soon. They should stay the course, especially in difficult to investigate problems, such as taro virus aetiology. This means stopping demanding success within unnecessary, unreasonable

time frames. We should all understand the limitations faced by Pacific islands countries in numbers of staff, training and facilities, and try to build capacities in government agencies and universities that bring real sustainable change, not a temporary 3 to 5-year surge of activity only to be abandoned when the projects ends, leaving little gain.

END