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TABLE OF CONTENTS

TABLES

FIGURES

APPENDICES

ABBREVIATIONS

ACIAR	Australian Centre for International Agricultural Research
DA	Australian Department of Agriculture
HTFA	High temperature forced air
HWT	Hot water treatment
ITS	Internal transcribed spacer
MAFFM	Ministry of Agriculture, Fisheries, Forests and Meteorology (Samoa)
MBIE	Ministry of Business, Innovation and Employment (New Zealand)
MPI	Ministry for Primary Industries (New Zealand)
PC2	Physical Containment (level 2)
PFR	The New Zealand Institute for Plant & Food Research Limited
PHAMA	Pacific Horticultural and Agricultural Market Access Program
RFLP	Restriction fragment length polymorphism
SNP	Single Nucleotide Polymorphism
TLB	Taro Leaf Blight
URS	URS Australia Pty Ltd
USD	United States dollar

EXECUTIVE SUMMARY

Phytophthora colocasiae (known as taro leaf blight, or TLB) was first reported in Samoa (then known as Western Samoa) in 1993. It spread rapidly throughout the country and affected all local cultivars of taro, particularly *Colocasia esculenta* 'Niue', which was the favoured cultivar for both local use and export. 'Niue' was, however, also highly susceptible to TLB. Within 6 months, there was little taro available to export and by 1994 exports had fallen from approximately USD3.5 million annually to less than USD60,000.

With the development of more tolerant cultivars, limited amounts of taro are now exported to New Zealand. Export of fresh taro to Australia remains a priority for the Samoan taro industry. However, *P. colocasiae* is a major biosecurity concern for Australia and Samoa will need to develop and demonstrate management measures that are acceptable to Biosecurity Australia before exports can commence.

In order to assess the feasibility of meeting those requirements, the New Zealand Institute for Plant & Food Research Limited was contracted by the Pacific Horticultural and Agricultural Market Access Program (PHAMA) to prepare a review of relevant literature and other technical information, to identify the key questions that need to be answered to meet Australian biosecurity requirements, and to prepare a research plan to address those questions.

The laboratory and field research needed to meet the requirements is expected to be substantial and there is no certainty that acceptable measures can be achieved. The research needed focuses on the recognition and quantification of corm rots caused by *P. colocasiae*, the likelihood of the new Samoan cultivars having corm rots, the likelihood of survival of external contaminant propagules, and post-harvest treatment of corms to eliminate external propagules.

1 INTRODUCTION

Taro leaf blight (TLB), caused by the oomycete *Phytophthora colocasiae* Racib, was first reported in Samoa (then known as Western Samoa), on the islands of Upolu and Savai'i, in 1993 (Anonymous 1993). It spread rapidly throughout the country and affected all local cultivars of taro (Fonoti et al. 2008). At that time, the cultivar *Colocasia esculenta* 'Niue' was favoured for both local use and for export because of its quality and taste. 'Niue' was, however, also highly susceptible to TLB (Brunt et al. 2001; Fonoti et al. 2008).

Before the incursion of TLB in 1993, taro comprised about 58% of Samoa's agricultural exports, with a value of USD3.5 million annually. Within six months there was little taro available to export and by 1994 exports had fallen to less than USD60,000 annually. (Singh et al. 2012). To avert serious food shortages, taro was replaced by the less palatable but TLB resistant root crop 'kapi' (*Alocasia macrorrhiza)*.

With the breeding and release of more tolerant cultivars, limited amounts of taro are now exported to New Zealand. Export of fresh taro to Australia remains a priority for the government and private sector in Samoa, but the taro industry faces significant issues, including supply, freight and other logistics, as well as biosecurity constraints. TLB is a major biosecurity concern for Australia, and Samoa will need to develop and demonstrate management measures that are acceptable to Biosecurity Australia before exports can commence.

The laboratory and field research needed to meet those requirements is expected to be substantial and there is no certainty that acceptable measures can be achieved. In order to assess the feasibility of meeting those requirements, the New Zealand Institute for Plant & Food Research Limited (PFR) was contracted by the Pacific Horticultural and Agricultural Market Access Program (PHAMA) to prepare a review of relevant literature and other technical information, to identify the key questions that need to be answered to meet Australian biosecurity requirements and to prepare a research plan to address those questions.

Figure 1-1 Taro cultivar 'Samoa 2' growing on Upolu, Samoa (2014)

2 LITERATURE REVIEW

This review aims to provide a summary of relevant information from recent comprehensive reviews and any available published or, as available, unpublished information on TLB and its symptoms, history and status in Samoa and the Pacific region; the biology and lifecycle of the causal agent; and the conclusions from the 2011 review by the Australian Department of Agriculture (DA) of import conditions for fresh taro.

2.1 Taro (*Colocasia esculenta*)

Taro (*Colocasia esculenta*) belongs to the monocotyledonous family Araceae and is primarily grown in humid, tropical regions (Singh et al. 2012). Two types are commonly cultivated:

1. *Colocasia esculenta* var. *esculenta* (taro, dasheen, 'large corm taro'), which forms a single, large edible corm, and
2. *Colocasia esculenta* var. *antiquorum* (eddoe, 'small corm taro'), which forms numerous cormels around a mother corm.

In Samoa, as in many of the Pacific Island countries, taro (*Colocasia esculenta* var. *esculenta*) is a staple crop. Almost all parts of a taro plant are consumed. The corms are eaten for the high starch content and the leaves serve as a green vegetable (Miyasaka et al. 2013). Even the petioles and flowers are consumed in some regions of the world (Singh et al. 2012).

Worldwide, taro ranks fourteenth among staple vegetable crops with approximately 12 million tonnes produced annually. Most taro is grown in developing countries, and is characterised by smallholder production systems relying on minimal inputs (Singh et al. 2012).

2.2 Taro Leaf Blight – Geographical Range, Particularly in the Pacific

There are at least ten major diseases that affect taro in the Pacific Islands (Kohler et al. 1997). Of these, TLB, caused by the oomycete *Phytophthora colocasiae*, is one of the most destructive (Ooka 1990; Misra et al. 2008; Miyasaka et al. 2013). Several comprehensive reviews have been recently published on TLB; for example, Misra et al. (2008), Singh et al. (2012) and Miyasaka et al. (2013).

TLB has been known in the Pacific for more than a century. *Phytophthora colocasiae* was first recorded in Java (Indonesia) in 1900 by Raciborski (Packard 1975). In 1905, it was found in India. It was subsequently recorded in Formosa (Taiwan) in 1911 (Packard 1975), the Philippines in 1916 (Gomez 1925) and Guam in 1918 (Brunt et al. 2001).

Phytophthora colocasiae was found in Hawaii in 1920 (Packard 1975), where it is thought to have been largely responsible for the extinction of many traditional Hawaiian taro cultivars (Miyasaka et al. 2013), and later in China (Canton) in 1932. During World War II and its immediate aftermath, TLB was discovered in Bougainville in 1945, Solomon Islands in 1946 and Papua New Guinea (Finschhafen) in 1948 (Packard 1975). In 1993, the disease was discovered in Samoa (then known as Western Samoa) and American Samoa (Anonymous 1993). Within the Pacific, TLB has also been reported in the Federated States of Micronesia, Northern Mariana Islands and Palau (Carmichael et al. 2008).

Reports of *P. colocasiae* in Fiji (Viti Levu) were circulating as early as the 1940s, with an outbreak reported in Fiji in 1948 (Packard 1975). At that time, the infected taro plots were

sprayed with Bordeaux mixture and “good control was achieved”. No further outbreaks were recorded; in 1975, Firman noted that the report of *P. colocasiae* from Fiji needed confirmation as the disease had not been seen in the recent past (Firman 1975). Later reports (Brunt et al. 2001; Singh et al. 2012) accept that the Fiji report was a misidentification and it is now generally agreed that the disease is not present in Fiji (CABI/EPPO 2014).

Today, TLB is widely distributed throughout the tropical regions of the world (Fullerton & Tyson 2003) and is present throughout most of Asia and the Pacific region (CABI/EPPO 2014). Its geographical distribution continues to expand, with new outbreaks recently reported in Ghana (Omane et al. 2012), Nigeria (Bandyopadhyay et al. 2011) and Cameroon (Singh et al. 2012).

Phytophthora colocasiae is considered to have originated in South-East Asia (Zhang et al. 1994). It is heterothallic, requiring the presence of opposite mating types (A^1 and A^2) for the formation of oospores (Tyson & Fullerton 2007). Research on mating types suggests that Hainan Island, China, is within the centre of origin of the pathogen (Zhang et al. 1994).

2.3 *Phytophthora Colocasiae* Host Range

Phytophthora colocasiae has a very limited host range, most commonly affecting species of *Colocasia* (Fullerton & Tyson 2003).

Alocasia macrorrhiza has been infected in experimental pathogenicity tests by Gollifer et al. (1980), but these authors noted that lesion development ceased after a few days and considered that this host is unlikely to have a role in the perennation of the pathogen.

Xanthosoma taro is immune to TLB (Brunt et al. 2001). In pathogenicity tests, Gomez (1925) found that *Xanthosoma sagittifolia* (*Xanthosoma sagittifolium*) could not be infected by *P. colocasiae*.

Although infection of other host genera has been reported over the years, many are dubious records. For example, *P. colocasiae* was reported on elephant-foot yam (*Amorphophallus campanulatus*) in India in 1960 (Paharia & Mathur 1961). Gollifer et al. (1980) were unable to infect that species. Other dubious host records include a report on American ginseng (*Panax quinquefolius*) in samples received at the Plant Disease and Insect Clinic at North Carolina State University (Abad et al. 1994), and a report on *Piper betle* (Erwin & Ribeiro 1996). All of these records are unable to be substantiated and lack credence.

2.4 TLB Symptoms

Leaf blight

This is the most common and destructive form of the disease. The first symptoms of the disease are small flecks on the leaves, brown on the upper surface and water-soaked below. These flecks rapidly enlarge over a few days to form irregular dark brown leaf spots, often with a yellow margin (Carmichael et al. 2008; Misra et al. 2008). The larger lesions have a zonate appearance, resulting from the fluctuating day/night growth pattern of the pathogen, with the lesion expanding at the margin during the night and the newly infected band of tissue drying out during the following day (Fullerton & Tyson 2003). This typically leads to a “bull’s-eye pattern” on older discrete lesions (Miyasaka et al. 2013). In susceptible cultivars, the leaf lesions frequently coalesce to destroy large areas of leaf (Fullerton & Tyson 2003).

Typically, each morning, the newly colonised tissues at the margins of the lesions are marked by a white powdery band of spores (sporangia), which were produced during the previous night. In addition, the expanding nocturnal margin is also characterised by droplets of orange or reddish exudate that subsequently dry to form dark, red-brown pellets (Fullerton & Tyson 2003; Carmichael et al. 2008).

TLB lesions often begin where rainfall or dew collects at the sides of the leaf and supports infection (Carmichael et al. 2008; Singh et al. 2012). The disease can cause rapid and complete defoliation of susceptible taro cultivars (Fullerton & Tyson 2003). The normal lifespan of a healthy taro leaf is about 40 days; TLB-affected leaves may die in 10–20 days, or even less for the very susceptible cultivars (Misra et al. 2008).

Corm rot

In addition to the destructive foliar form of the disease, invasion of corms by *P. colocasiae* may also occur. As the corms are the major commercial commodity, corm infections constitute the greatest biosecurity threat.

Most recent publications tend to focus on the 'leaf blight' symptoms of TLB, usually with a brief statement that it may also cause a corm rot. Many of these statements cite Jackson and Gollifer (1975), who reported corm rots in Solomon Islands. However, primary reports of corm rots of taro caused by *P. colocasiae* are also available from India (Butler & Kulkarni 1913; Kulkarni & Sharma 1975) and the Philippines (Gomez 1925). Uchida & Trujillo (n.d.) report corm rots in Hawaii. Corm rots were also noticed when TLB was first found in Bougainville in 1945 (Packard 1975).

Descriptions of corm rot symptoms tend to vary between authors, possibly the result of observations on different species and cultivars and the confounding presence of other organisms.

- Jackson and Gollifer (1975) described rots caused by *P. colocasiae* on *Colocasia esculenta* cv. 'Akalomamale initoa' as light brown and firm, often with a distinct margin.
- Butler and Kulkarni (1913) described the rots caused by *P. colocasiae* in India on *Colocasia antiquorum* as 'dry rots' and considered the corm rot stage to be probably more destructive than the leaf blight.
- Uchida and Trujillo (n.d.) state that, in the early stages, *P. colocasiae*-infected corm tissue is slightly discoloured and difficult to recognise externally. Internally, the infected area has a very faint tan colour (as opposed to the white colour of healthy corms) and the infected tissue is rubbery, not firm. As the corm rot progresses, the discoloured area becomes larger and more pronounced, although the edge of the infected area remains diffuse with no distinct border. In advanced stages, the rots become brown to purplish (Uchida and Trujillo n.d.).
- In the Philippines, a soft rot of 'gabi' (*Colocasia antiquorum*) corms has been recorded under favourable moisture conditions (Gomez 1925). This author stated that "although oospores were seldom produced in pure culture, they were able to be produced on Lima bean juice agar". As *P. colocasiae* is heterothallic, and needs two mating types to form oospores, this account of corm rot caused by *P. colocasiae* may have been a misidentification.

- *Phytophthora colocasiae* rots usually start from areas damaged at harvest, such as where the petiole bases and suckers are removed, especially during or after wet, warm conditions. In the advanced stages of corm rot, the decayed corm tissue may be invaded by *Lasiodiplodia theobromae* and turn black (Singh et al. 2012, cites Jackson & Gollifer 1975).

Corm rots caused by *P. colocasiae* occur in very susceptible cultivars, particularly during or after wet, warm conditions. These corm rots typically start from the stem end, although infection can occur on any part of the corm and develop rapidly after harvest.

Jackson and Gollifer (1975) found that in Solomon Islands the majority of fungi associated with corm rots, including *P. colocasiae*, gained entry to undamaged taro corms only after harvest, when cormels were detached and the surface of the corm was scraped to remove roots and leaf debris. Jackson (1999) stated that, at harvest, spores washed from leaf lesions into the soil invade the corms at the points of injury. The subsequent post-harvest corm rots were difficult to detect unless the corms were cut open. Storage rots can destroy the corms within 5–10 days from harvest (Jackson 1999).

Uchida and Trujillo (n.d.) noted a varietal difference in susceptibility to corm rots, and stated that they are common in very susceptible cultivars (i.e. 'Niue') and are less common on cultivars such as 'Lehua'. The corm rots are thought to occur at higher frequencies following environmental periods highly conducive to the disease.

The corm rot phase limits production in the Marianas and Caroline Islands and is probably the principal cause of storage rots in Solomon Islands and other islands in Melanesia and Micronesia. Up to 70 percent of the rots in Solomon Islands are attributed to *P. colocasiae*; however, corm rots are not thought to be a problem in Hawaii (Ooka 1990).

2.5 TLB Effects on Yield

Brooks (2008) found a high correlation between taro corm weight, leaf number, and the number of healthy leaves per plant. As TLB significantly reduces leaf area of infected plants, so too the corm yield is reduced (Singh et al. 2012). Under severe TLB conditions, yield losses may reach 50–60%, and the more susceptible cultivars can be completely destroyed (Brooks 2008).

In addition to corm yield losses resulting from reduced leaf area, losses from subsequent corm rot can be significant. As noted above, Jackson and Gollifer (1975) concluded that *P. colocasiae* was probably the principal cause of storage rots in Solomon Islands and other islands in Melanesia and Micronesia and that up to 70% of the rots in Solomon Islands were attributable to that pathogen.

2.6 TLB Epidemiology

Rainfall, humidity and temperature are the key factors controlling the disease cycle and epidemiology of *P. colocasiae* (Fullerton & Tyson 2003), with free water being the most important factor. However, TLB lesions can also be found during dry seasons in moist niches, e.g. the lower leaves (Miyasaka et al. 2013).

The **sporangia** are the primary reproductive unit of *Phytophthora colocasiae*. The sporangia are caduceus (deciduous), readily shed in water but not in wind (Erwin & Ribeiro 1996; Misra

et al. 2008), and are spread within and between plants and nearby fields by dew and wind-driven rain (Putter 1976; Anonymous 1993; Jackson 1999).

Sporangial germination requires free water. Sporangia germinate either directly to form a germ tube, or indirectly by releasing zoospores. Direct germination, where the sporangium forms a germ tube, occurs in 5–6 hours at 20–35°C. Indirect germination occurs at 12–25°C and can result in the release of up to 12 motile **zoospores**. The zoospores encyst within 20 minutes of release, and the cysts then germinate to form a germ tube within 30 min after encysting (Misra et al. 2008).

Maheshwari et al. (2011) found that direct sporangial germination was favoured between 25 and 35°C and that zoospore formation was favoured between 12 and 25°C.

Phytophthora colocasiae is a heterothallic species of *Phytophthora*, requiring the presence of opposite mating types (A^1 and A^2) for the formation oospores. Heterothallic *Phytophthora* readily produce oospores in intra- or interspecific pairings of two compatible mating types (Tyson & Fullerton 2007). An outline of the mating types present in the Pacific and other areas is given in Appendix A.

Chlamydospores (thick-walled, spherical resting spores) are occasionally seen in pure culture (Misra et al. 2008), and are rare in some cultures and common in others (Erwin & Ribeiro 1996), but have not been found in field conditions (Quitugua & Trujillo 1998). The importance of chlamydospores in the epidemiology of TLB is unknown, but it is feasible that they could allow survival of the pathogen in the soil between crops (Singh et al. 2012).

Movement of infected planting material for longer distances can hasten the spread of the disease between plantations, islands and countries (Anonymous 1993; Misra et al. 2008). *Phytophthora colocasiae* has been found to survive on experimentally infested taro planting material for up to 3 weeks (Gollifer et al. 1980).

2.7 Breeding and the New Cultivars

At the time of the TLB incursion into Samoa, the crop was genetically almost uniform, with the cultivar ‘Niue’ predominating. ‘Niue’ subsequently proved to be highly susceptible to the disease and was therefore vulnerable to a major disease epidemic. In addition, the practices of continuous planting and interplanting of new taro between near-mature taro for staggered harvesting, in combination with ideal weather conditions for spread and infection, meant that the industry was vulnerable to complete destruction by the disease (Brunt et al. 2001).

Attempts to control the disease by fungicides and management activities such as removal of infected leaves were ineffective because of financial considerations and labour demands. Resistant varieties were seen as the only sustainable solution to the problem (Brunt et al. 2001; Fonoti et al. 2008).

Varieties with durable resistance to *P. colocasiae* were known from the Philippines, the Federated States of Micronesia, and Palau. Breeding programs for TLB resistance had previously been carried out in Hawaii and Papua New Guinea. Some of the releases from these breeding programs have been pathogen-indexed and conserved at the Secretariat of the Pacific Community Regional Germplasm Centre, Fiji (Carmichael et al. 2008).

In Samoa, a breeding program based on recurrent selection was initiated in 1996 using exotic and traditional cultivars as parents. Breeding cycle 1 was screened for resistance to leaf blight during 1998 and 1999. Thirty clones were identified with good resistance to leaf blight and vigour. Following taste test evaluations, ten clones were selected for further multiplication and evaluation. The Samoa Ministry of Agriculture, Fisheries, Forests and Meteorology (MAFFM) then officially released six of the clones to farmers (Fonoti et al. 2008).

Two cultivars, now known as Samoa 1 and Samoa 2, are currently exported from Samoa to New Zealand and the USA. Samoa 1 has longer corms, with slightly pink/purple flesh. Samoa 2 has rounder corms with white flesh. Both varieties were developed at the University of the South Pacific in the breeding program led by Tolo Iosefa, and then tested at MAFFM, Nu'u, for tolerance to TLB. Initially, five varieties were identified as acceptable in terms of taste and TLB tolerance; for export, this was reduced to the current two based on consumer preferences (Tyson pers. comm., based on discussions with MAFFM staff in November 2014).

The use of resistant varieties has been shown elsewhere to have a significant impact on yield, and commercial production in Samoa is recovering. To date, selection for resistance to TLB has been based solely on tolerance to leaf infection. It is not known whether tolerance in the leaves is linked with similar tolerance to corm rots. Nevertheless, there is limited evidence that there is a relationship. Uchida and Trujillo (n.d.) noted that while corm rots were very common in susceptible cultivars such as 'Niue', they were less common in cultivars such as 'Lehua', although they could occur at higher frequencies following weather conditions conducive to disease. This suggests that the incidence of corm rots can be influenced both by genotype and environment. Thus the potential risk of spread of the pathogen on infected corms could differ significantly between genotypes.

3 CONCLUSIONS FROM THE AUSTRALIAN DEPARTMENT OF AGRICULTURE REVIEW OF IMPORT CONDITIONS FOR FRESH TARO

3.1 Overview

In November 2011, DA released a review of the quarantine risks associated with the import of fresh taro corms from all countries (Biosecurity Australia 2011). The report recommended that fresh taro corms be a permitted import into Australia, and set out specific pest risk management measures.

Six quarantine pests were identified as requiring additional quarantine measures to manage risks to a very low level in order to achieve Australia's appropriate level of protection. These pests included TLB.

In 2006, the import of small corm taro to Australia was prohibited because of concerns that these could be used for propagation. That decision did not affect Samoa, as only 'large corm' taro is grown for export from Samoa.

The quarantine measures relevant to Samoa and TLB are:

- Inspection of taro corms on arrival in Australia to ensure that quarantine pests and other regulated articles are detected and consignments are subjected to appropriate remedial action
- Removal of all petiole material and the apical growing points from corms of large corm taro (*Colocasia esculenta* var. *esculenta*)
- Only importing taro corms sourced from areas declared free of TLB (country freedom).

The review adds that alternative measures to area freedom will be considered on a case-by-case basis, and that if the quarantine risks can be effectively mitigated by other measures, then alternative import conditions will be proposed (Biosecurity Australia 2011).

3.2 Import Risk Analysis

Existing import conditions for large corm taro in Australia require topping to remove all petiole bases and the apical growing points to prevent the corms being propagated. If the petiole bases and the apical growing points are excised, the remaining lateral buds on the corm will usually not sprout. This topping is an additional quarantine measure, and is not considered when assessing the unrestricted risk (Biosecurity Australia 2011).

The import pest risk analysis for *Phytophthora colocasiae* on fresh taro corms is summarised in Table 3-1.

Table 3-1 Summary of the Australian Department of Agriculture's import pest risk analysis for *Phytophthora colocasiae* on fresh taro corms (Biosecurity Australia 2011)

	Risk estimate
Probability of importation	High
Probability of distribution	Moderate
Probability of entry (importation x distribution)	**MODERATE**
Probability of establishment	High
Probability of spread	High
Probability of entry, establishment and spread	**MODERATE**
Consequences	Moderate
Unrestricted risk estimate	**MODERATE**

The review states that "the major risk from *Phytophthora colocasiae* is the importation of corms bearing viable sporangia or zoospores (particularly between petiole bases) that are subsequently diverted from their intended use for human consumption and used as planting material" (Biosecurity Australia 2011).

3.3 Pest Risk Management Measures

The specific pest risk management measure proposed for fresh taro corms from all countries to reduce the restricted risk of *Phytophthora colocasiae* to a level that achieves Australia's appropriate level of protection is:

- Area (country) freedom from TLB.

3.4 Response to the Import Risk Analysis

PHAMA (2011) formulated a comprehensive response to the *Draft Review of Import Conditions for Fresh Taro Corms*. This response argues that due to the ephemeral nature of sporangia, the risk of entry by external contaminant sporangia or zoospores of *P. colocasiae* is **very low**, but that the separate risk of corm rots raises the probability of importation to **moderate**.

4 QUESTIONS REQUIRING INVESTIGATION

This section seeks to develop a list and description of the questions that require investigation in order to develop risk management measures for TLB that would be both acceptable to DA and feasible to implement, including decision points on whether it is feasible to pursue these measures.

4.1 The Problem

Currently, the only measure for *Phytophthora colocasiae* is country freedom. There is a need to investigate other options that would satisfy the import health requirements. Biosecurity Australia (2011) currently considers that "the major risk from *Phytophthora colocasiae* is the importation of corms bearing viable sporangia or zoospores (particularly between petiole bases) that are subsequently diverted from their intended use for human consumption and used as planting material".

There is also the risk that the corms may be infected internally, which is also discussed in the risk assessment. The only propagules on the surface or between petioles would be sporangia or encysted zoospores, both of which are fragile, short-lived and killed by very short periods of dehydration. The more resistant oospores will not be present, as only one mating type (A^2) is known to be present in Samoa and thus oospores cannot form. Infected corms, on the other hand, may carry significant volumes of viable vegetative mycelium in an environment in which they are protected from dehydration and may therefore survive for extended periods of time. In the development of treatment methods to minimise risk, both sources of contamination need to be addressed.

The most promising options to achieve minimum risk are the use of TLB-resistant taro varieties that would pose a low risk of surface contamination and corm infection, in combination with appropriate postharvest treatments. Options for treatments are sanitisers, high pressure water washing, and heat treatments. These could eliminate viable propagules from the surface of the corm and, in the case of heat treatments, kill the pathogen if it had established internally.

While the newly released TLB-resistant Samoan varieties have a degree of foliar tolerance to the pathogen (not complete resistance), their susceptibility to corm infection and the subsequent rate of spread within the corms is not known.

Heat treatments to eliminate mites and nematodes from export taro and thus avoid methyl bromide fumigation at the New Zealand border are being investigated as part of a project funded by the New Zealand Ministry of Business, Innovation and Employment (MBIE) (Development of risk management treatments for root crops from the Pacific Islands). The potential of those treatments to also eliminate *P. colocasiae* either externally or internally from corms is not known.

4.2 The Crop

- Which cultivars would be exported to Australia?

Two cultivars, now known as Samoa 1 and Samoa 2, are currently exported from Samoa to New Zealand and the USA. Samoa 1 is longer, with slightly pink/purple flesh. Samoa 2 is rounder, with white flesh. Both varieties were developed at the University of the South Pacific in the breeding program led by Tolo Iosefa, and then tested at MAFFM, Nu'u, for tolerance to

TLB. Initially, five varieties were identified as acceptable in terms of taste and TLB tolerance; for export, this was reduced to the current two due to consumer preferences (Tyson pers. comm., based on discussions with MAFFM staff in November 2014).

- Would the taro corms be devitalised if they were to be exported to Australia from Samoa?

Devitalisation is a specific pest risk management measure required for other pests such as viruses (e.g. in Fijian taro exported to Australia). This involves the removal of all petiole material and the apical growing points from corms. If corms cannot be re-planted in Australia, this significantly affects the likelihood of establishment in Australia.

- Which cultivars are grown in Australia?

4.3 The Pathogen

Disease-free place of production will not be an acceptable measure for taro from any part of Samoa as the disease is widespread on both main islands. Questions that need to be answered before risk management measures can be developed include:

- Do the new, tolerant Samoan taro cultivars develop *Phytophthora colocasiae* corm rot? The new cultivars develop leaf lesions at a significantly lower rate; therefore it is likely that the inoculum load on wounds caused during harvest will also be significantly lower.
- Are corm rots initiated at or prior to harvest? Previous research has shown that *P. colocasiae* gained entry to undamaged taro corms only after harvest. This would need to be confirmed for each of the new cultivars.
- How rapidly and to what depth in the corms do the rots progress?
- What is the incidence of rotting – for example, how many are likely to be in each consignment? Is this incidence different between cultivars?
- Are there any external symptoms of TLB corm rot? If so, how effective is visual culling of rotted corms likely to be?
- How long do the external 'contaminant' propagules of TLB survive in typical transport conditions? The spores are very delicate and die within 2–3 hours on sunny days as the humidity falls (Jackson 1999).
- What is the tolerance of external 'contaminant' propagules to hot water treatment (HWT)?
- Are there other post-harvest treatments that may be suitable for the Samoan situation?
- Regarding Biosecurity Australia's risk analysis: Is the likelihood of establishment reasonable? Would a climate analysis show the risk of establishment? Where are taro grown in Australia and what is the likelihood of spores from infested corms coming into contact with other taro plants?

4.4 Potential Management Measures

To develop acceptable measures, it is likely that it would need to be shown that:

- Pest-free place of production can be proven

OR

- The varieties being exported do not develop corm rots **OR** rots are able to be detected and reliably culled prior to shipment **OR** that corm rots are only initiated by external propagules at harvest ***AND***
- External propagules are killed by HWT or other measures **OR** external propagules do not survive the transit conditions.

Devitalisation of the corms could be considered as an additional measure to further assure that establishment in Australia will not occur.

5 RESEARCH AND DEVELOPMENT PROGRAM

This section seeks to provide a basic description of the research that could be done to close the knowledge gaps around *Phytophthora colocasiae*. The major components would be around corm rots, external contaminant spores, and potential treatment options. This includes consideration of current capability and resources (in Samoa and within PFR) to do the required work and prepare suitable technical information for consideration by DA.

The research needed to close the knowledge gaps around *Phytophthora colocasiae* and potentially to develop risk management measures for TLB focuses on:

- The likelihood of the new Samoan cultivars having corm rots;
- The recognition and quantification of corm rots caused by *P. colocasiae* in those cultivars;
- The likelihood of survival of external contaminant propagules; and
- Post-harvest treatment of corms with external propagules.

All research would need to be able to be published and thus would also need to be designed in concert with statisticians. The initial development of methodology could be done in New Zealand on commercially imported corms, as can much of the laboratory-based work.

5.1 Recognition and Quantification of Corm Rots Caused by *Phytophthora Colocasiae*

The currently available literature on corm rots is scarce, and descriptions of the rots vary greatly. It is likely that corm rots will present differently in tolerant cultivars than in susceptible cultivars.

In order to assess the base level of rots in the field and after harvest, assess the effect of any treatments, and quantify the likelihood of infected corms entering the supply chain, corm rots caused by *P. colocasiae* first need to be able to be recognised and distinguished from other common corm rots such as those caused by *Pythium*, *Fusarium* and *Botryodiplodia* species.

Key components:

A. Progression of rots and symptoms in different cultivars (laboratory work)

Questions addressed:

- *Do the new, TLB-tolerant Samoan taro cultivars develop* Phytophthora colocasiae *corm rot?*
- *How rapidly and to what depth do the rots progress?*
- *What are the external symptoms of TLB corm rot?*

Research elements:

- Develop a reliable laboratory-based corm inoculation method (this may be partially achieved by a current MSc student at PFR).
- Inoculate corms or slices of corms with a range of isolates of *P. colocasiae* in a Physical Containment Level 2 (PC2) laboratory.
- Document the progression and appearance of rots.

- Use Samoa 1, Samoa 2, and also Niue, if available, as a susceptible control.

B. Laboratory study of survival and rate of progression of rots in corms

Questions addressed:

- *How rapidly do rots develop?*

Research elements:

- Inoculate whole corms or sections of corms.
- Hold at different temperature/time combinations, including cold treatment and in standard transport conditions (8°C).
- Document the progression and appearance of rots.
- Attempt to re-isolate the pathogen.

C. Field survey of incidence of corm rots caused by *Phytophthora colocasiae*. Progression of rots and symptoms in different cultivars (Field or Samoa-only work)

Questions addressed:

- *What is the incidence of rotting – for example, how many are likely to be in each consignment?*
- *Is the incidence of field rots different between cultivars?*
- *Are there any external symptoms of TLB corm rot?*
- *Are corm rots initiated at or prior to harvest?*

Research elements:

- Field survey to determine incidence of pre-harvest rots in different cultivars, heavily infected field v. low prevalence.
- Potentially do the survey during the rainy season to ensure good conditions for potential rots (worst case scenario).
- Incidence of post-harvest rots in different cultivars (as above).
- Visualisation of rots at harvest, tracking rots over the following week (expected timeframe from harvest to Australian marketplace).
- Surface-sterilise corms of both cultivars at harvest and store to determine whether corms are infected prior to harvest (as per Jackson & Gollifer 1975).
- Inoculate wounds at harvest on both cultivars and a susceptible control cultivar and monitor development of rots at normal transport temperature.

5.2 Survival of External Contaminant Propagules

Biosecurity Australia (2011) considers that a major risk from *Phytophthora colocasiae* is the importation of corms bearing viable sporangia or zoospores that could establish in Australia. This section details research to determine the likelihood of survival of external contaminant propagules on taro corms.

Key components:

A. Survival of TLB spores that are likely to be external contaminants under different moisture and temperature conditions (laboratory work)

Questions addressed:

- *What is the tolerance of external 'contaminant' propagules to HWT?*
- *How long do the external 'contaminant' propagules of TLB survive in typical transport conditions?*

Research elements:

- Establish survival capacity of sporangia and zoospores under dehydrating conditions *in vitro.*
- Establish temperature/time response of sporangia and zoospores under wet conditions *in vitro.*
- Apply propagules (sporangia/ zoospores) to corms and hold at different temperature/time combinations, including cold treatment and standard transport temperature (8°C).
- Attempt to re-isolate the pathogen.

5.3 Postharvest Treatments/Decontamination of Corms

Key components:

A. Trials of HWT for external propagules of TLB (temperature/time matrix) (laboratory work)

Question addressed:

- *What is the tolerance of external 'contaminant' propagules to hot water and other disinfestation treatments?*

Research elements:

- Test the tolerance of different forms of propagules of the pathogen to different treatments (e.g. sporangia, contaminated soil – chlamydospores, encysted zoospores).

B. Post-harvest survival of external contaminant propagules under normal conditions of the export pathway (Field or Samoa-only work)

Question addressed:

- *How long do the external 'contaminant' propagules of TLB survive in typical transport conditions?*

Research elements:

- Inoculate/contaminate corms with *P. colocasiae.*
- Hold in typical transport conditions.
- Re-isolate *P. colocasiae* over time to check viability.

C. Post-harvest disinfestation of corms with hot water and sanitisers (Field or Samoa-only work)

Questions addressed:

- *What is the tolerance of external 'contaminant' propagules to HWT?*
- *Are there other post-harvest treatments that may be suitable for the Samoan situation?*

Research elements:

- Test HWT as a post-harvest control method for external propagules under standard commercial handling conditions.
- Test surface-sterilisation (NaOCl) as a post-harvest control method. Jackson (1999) suggested that to combat post-harvest decay caused by *P. colocasiae*, corms can be treated with a dilute solution of bleach (1% sodium hypochlorite) for 2 minutes and then dried before being placed in polythene bags.
- Test modified high temperature forced air (HTFA) treatment as a post-harvest control method for external propagules.

5.4 Critical Work

In order to undertake this work, both in New Zealand and in Samoa, researchers would need to be in possession of at least 20 different strains of *P. colocasiae* from Samoa. At present, there are only two strains in the PFR collection and they have been in culture for more than 15 years. Cultures often 'stale' and lose pathogenicity with repeated sub-culturing and long-term storage.

Fresh cultures isolated from disease in the field are needed to ensure the validity of the work. This will also ensure that any diversity in the population is accounted for. The first step in implementation therefore would be a short-term visit to Samoa to collect fresh strains of *P. colocasiae*. Permits are in hand to import the cultures to New Zealand, and PFR has New Zealand Ministry of Primary Industries (MPI) approval to work with the pathogen under PC2 laboratory containment conditions at PFR.

6 POTENTIAL SCIENTIFIC RESEARCHERS AND CURRENT RESEARCH CAPABILITY

6.1 New Zealand Researchers

Plant pathologists: Joy Tyson and Bob Fullerton from PFR have significant experience working with TLB (Tyson & Fullerton 2007; Singh et al. 2012). See Appendix B for a full list of this experience.

6.2 New Zealand Resources

A PC2 laboratory is required for work in New Zealand on 'unwanted organisms' such as *Phytophthora colocasiae*. In addition, permission must be received from MPI to work on any 'unwanted organism'. PFR, Mt Albert, has applied for, and received, Chief Technical Officer approval to work on *Phytophthora colocasiae* in a PC2 laboratory (MPI permission 20141201) at PFR in Auckland.

A collection of *Phytophthora colocasiae* isolates from the Pacific is already held by this laboratory; however, a more extensive set from Samoa needs to be collected in order to ensure that any diversity in the population is accounted for.

Much of the laboratory work could be done in New Zealand in controlled conditions, particularly developing the methodology.

Specialist equipment available in New Zealand includes the fully resourced PC2 laboratory facility, media-making facilities, incubators and trained staff.

6.3 Samoan Researchers/Staff

- Asuao Kirifi Pouono (Samoa National Market Access Coordinator)
- Pathologist: Dr. Seuseu Joseph Tauati (MAFFM Pathologist)
- Nematologist/pathologist: Angelika Tugaga (Angelika.Matafeo@maf.gov.ws)
- Pathologists currently working on other long-term projects: Parate Matalavea (MAFFM Principal Research Officer), Tauelii Mauga
- Taro cultivars and export questions: Toilolo Pueata Tanielu (Principal Development Officer)
- Taro breeding: Tolo Iosefa.

6.4 Samoan Resources

It would not be difficult to fit out / gather together the equipment needed to do some of the laboratory work at Nu'u, Samoa.

Basic pathology equipment is available, or could be easily acquired from New Zealand. Media for isolation and growth of *P. colocasiae* can be made on site.

Several hot water baths that could be used in the HWT trials are currently situated in the Nu'u entomology laboratory. These baths were left temporarily in Samoa by Allan Woolf (PFR, Mt Albert) for use with the ongoing MBIE-funded project 'Development of risk management treatments for root crops from the Pacific Islands'.

Specialist equipment available in Samoa includes the HTFA machine sited at Atele; however, this may be in some disrepair.

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APPENDIX A RECORDS OF MATING TYPES OF *PHYTOPHTHORA COLOCASIAE*

Country of origin	A^1 mating type	A^2 mating type	A^0 mating type*	No. tested	Reference
ASIA					
Asia (unspecified)	0	4	0	4	Ho et al. 1983
China	0	0	2	2	Ho et al. 1983
China, Hainan Is.	136	102	42	280	Zhang et al. 1994
China, Hainan Is.	18	47	0	65	Shrestha et al. 2014
China, Guangxi	0	12	0	12	Lu et al. 2013
Philippines	0	2	0	2	Tyson & Fullerton 2007
Taiwan	0	799	0	799	Ann et al. 1986
Thailand	0	0	2	2	Tyson & Fullerton 2007
Vietnam	0	2	0	2	Tyson & Fullerton 2007
Vietnam	2	95	0	97	Shrestha et al. 2014
INDIA					
North India	75	0	0	75	Narula & Mehrotra 1980
Northern India	0	5	0	5	Tyson & Fullerton 2007
Eastern India	0	2	0	2	Roy et al. 2009
India	12	2	0	14	Misra et al. 2011
India, Kerala	41	0	0	41	Nath et al. 2013
PACIFIC					
Guam	0	3	0	3	Tyson & Fullerton 2007
Hawaii	0	16	0	16	Tyson & Fullerton 2007
Hawaii	114	0	0	114	Ko 1979
Hawaii	2	212	0	214	Shrestha et al. 2014
Hawaii	0	218	0	218	Lin et al. 2014
Indonesia	0	1	1	2	Tyson & Fullerton 2007
Papua New Guinea	0	5	11	16	Tyson & Fullerton 2007
Pohnpei	0	1	0	1	Tyson & Fullerton 2007
Samoa	0	5	0	5	Tyson & Fullerton 2007
UNKNOWN					
uncertain origin	0	5	0	5	Savage et al. 1968
uncertain origin	0	5	0	5	Brenneman & Gallegly 1967

*A^0 mating type refers to isolates that do not form oospores when paired with either mating type. These isolates are also referred to as neuter.

APPENDIX B TYSON & FULLERTON *PHYTOPHTHORA COLOCASIAE* OUTPUTS

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