Pacific Horticultural & Agricultural Market Access Plus Program

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Semi-commercial scale evaluation of the taro high pressure washer and hot water treatment efficacy for the treatment of Phytophthora colocasiae

Scientific Research Organisation of Samoa

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Semi-commercial scale evaluation of the taro high pressure washer and hot water treatment efficacy for the treatment of Phytophthora colocasiae

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Contents

Acronym List

Acronym	Description
CASPP	Corn meal agar modified with carbendazim, ampicillin, streptomycin, pimaricin and PCNB
DAWE	Department of Agriculture, Water and Environment (Australia)
DMV	Dasheen Mosaic Virus
dpi	Days post inoculation
EPA	Environmental Protection Agency (New Zealand)
HPW	High Pressure Washer
HTFA	High Temperature Forced Air
HWT	Hot Water Treatment
MAF	Ministry of Agriculture & Fisheries (Samoa)
MeBr	Methyl Bromide
MPI	Ministry of Primary Industries (New Zealand)
Pc	Phytophthora colocasiae
PFR	Plant & Food Research (New Zealand)
PHAMA Plus	Pacific Horticultural & Agricultural Market Access Plus
spp	species
SROS	Scientific Research Organisation of Samoa
TLB	Taro Leaf Blight

1. Introduction

Taro (Colocasiae esculenta) L. Schott is the fourteenth ranked staple food crop by production (Pacific Trade Invest, 2012), and fifth most important root crop grown in the world, and is widely distributed throughout Asia, tropical Africa and the Pacific (Paull & Chen, 2015). It is an herbaceous plant that can grow up to 2m in height depending on cultivar. The plant comprises leaves that are connected to petioles, which are connected to a cylindrical shaped corm from which roots and cormels (stem tubers) extend. Both the taro corm and leaves are edible and are extensively used in Pacific traditional cooking.

Taro is grown as a household crop and commercially for local and export markets. Suckers from harvested taro are usually grown 1m apart. Where taro do not need the petiole attached, the petiole is also replanted. Less commonly, taro can also be propagated using tissue culture. Taro grows optimally in well hydrated soil with pH 5.5-6.5, at temperatures above 21°C (Onwueme, 1999). Taro grown under sub-optimal conditions may have lower yields and odd dumb-bell shaped corms.

In the Pacific, taro forms a major part of the Pacific diet, so much so that even when Pacific Islanders emigrate to other countries, they continue to seek out and consume taro. This creates a market for the export of fresh or frozen taro to cater for the significant Pacific Islander expatriate populations overseas, particularly in New Zealand, Australia and the United States of America. Indeed, Pacific Islands are the major consumers of taro imported into NZ (Pacific Trade Invest, 2012). Truly, it is within the Pacific region where taro bears the utmost importance, not only for food security and cultural importance, but also as a key driver of Pacific economies. This also holds true for the Samoan taro industry.

In Samoa, taro was a major contributor to the economy pre-1994 (Fig. 1.1; Onwueme, 1999). In the early 1980's, taro was just starting to develop as an export product and by 1986, the value of taro exports was estimated to be SAT 21.7 million. This value gradually increased and peaked at SAT 37.3 million by 1992. However, the value of taro exports decreased to SAT 27.5 million by 1993 when the taro leaf blight (TLB) affected taro in Samoa, and exports of taro thereafter were negligible.

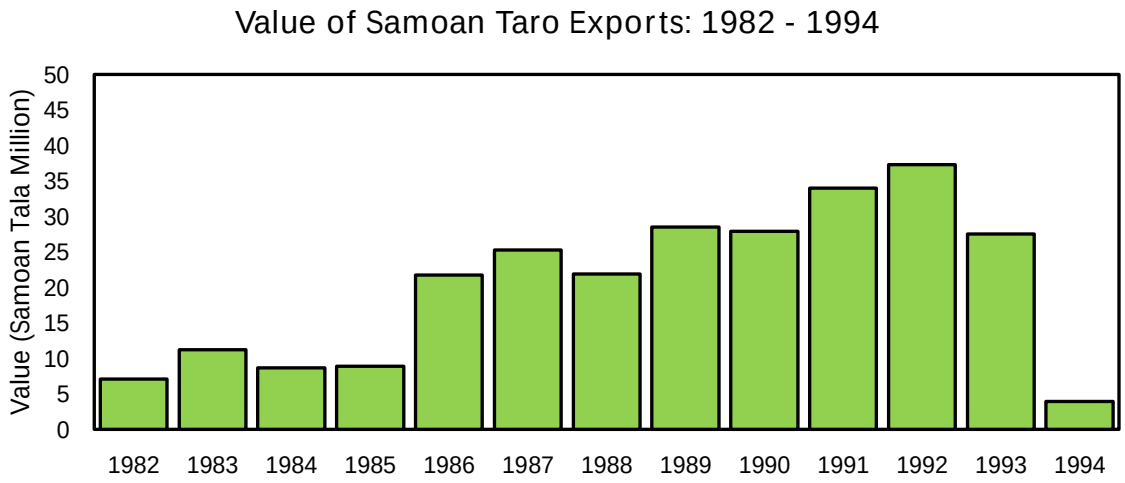

Figure 1.1: Taro export value in Samoa from 1982 to 1994

Taro formed a major part of the Samoan export economy from early 1980s to early 1990s, before the devastating effects of the taro leaf blight (TLB) reduced taro export values to negligible levels. (Adapted from Onwueme 1999)

1.1 Taro Leaf Blight/Phytophthora colocasiae

Globally, taro is affected by several pests and diseases, including the taro beetle (Papuana spp), taro leaf blight (TLB) caused by the oomycete Phytophthora colocasiae (Pc), the Dasheen Mosaic Virus (DMV), nematodes, various species of Pythium, armyworms, taro hornworm and the taro planthopper Tarophagus proserpina (Onwueme 1999; Revill et al., 2005; Yusop et al., 2019). Of all these pests and diseases, TLB bears the highest significance because it reduces leaf yield by 95%, and corm yield by 50% (Singh et al., 2012).

The environmental conditions in the tropics, particularly the temperature and humidity, promote TLB spread and infections. The humid wet conditions are ideal for sporangial dispersal, germination and blight development (Singh et al., 2012). When the reproductive Pc sporangia are generated under wet conditions, they are able to directly infect the leaf, or indirectly infect the leaf through the generation of zoospores that subsequently infect the leaf (Fig. 1.2) (Singh et al., 2012).

When affected with Pc, the most common and destructive symptom is observed on taro leaf lamina. Leaf infections occur under moist and wet conditions, either from rainfall, dew or guttation droplet accumulation, and initial leaf infection starts as small water-soaked lesions that develop as concentric margins, following a pattern of lesion expansion at night, and cessation of infection during daytime (Fullerton & Tyson, 2003). There have been some reports on Pc affecting taro petioles (Brooks, 2008), as well as corm rots (Jackson & Gollifer, 1975; Maslen-Miller et al., 2020).

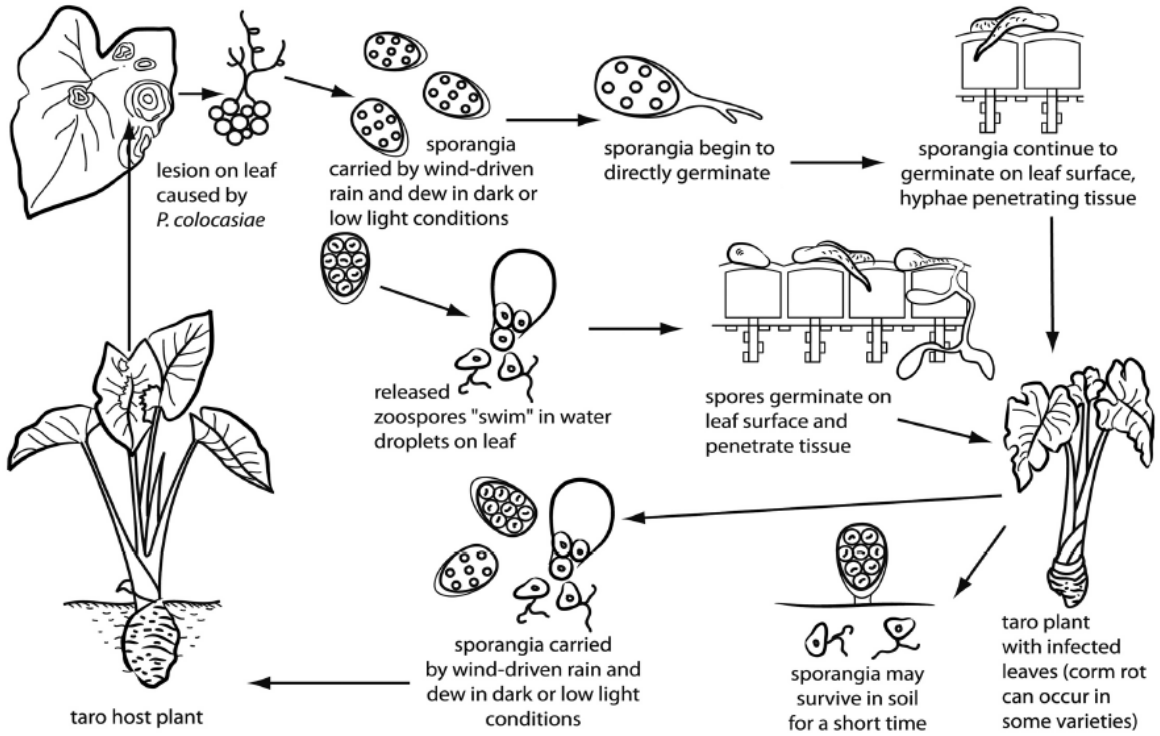

Figure 1.2: Disease cycle of Phytophthora colocasiae
Sporangia carried by rain and dew in dark/low light conditions can germinate directly and cause infection. Alternatively, zoospores can be released from sporangia. Zoospores can then germinate and cause new infection. (Source: Singh et al., 2012)

1.2 Resurrecting Taro Exports in Samoa

Circumventing Pc or TLB in Samoa meant undergoing a breeding program utilizing exotic and traditional cultivars as parents to produce TLB resistant/tolerant clones. Cycle 1 of the Samoan taro breeding program produced 30 clones that were tested, from which 10 were selected (Fonoti et al., 2008). To date, none of the original Cycle 1 clones are being exported and have been replaced with clones from Cycles 5, 7 and 10. Cycle 5 produced two clones that are currently approved for export, these being Samoa 1 and Samoa 2. Samoa 1 variety has since ceased being exported, while Samoa 2 is still being extensively exported to New Zealand, American Samoa and the United States of America as fresh commodities. Subsequent cycles have produced other varieties approved for export, including Talo Fusi, Talo Salani, and Talo Vave. The Samoan taro breeding program continues to produce potential new export varieties, and on-farm assessments of progenies are ongoing. Although the release of new TLB resistant taro varieties from cycles 1, 2 and 3 saw an increase in taro exports (Fig. 1.3), gaining market access back into New Zealand and Australia was not without challenges.

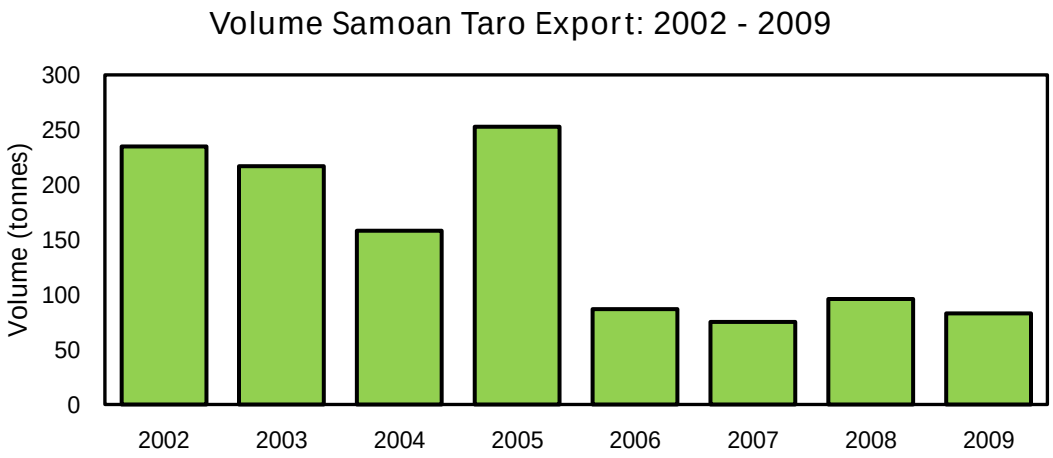

Figure 1.3: Samoa export volumes of taro (in tonnes) from 2002 to 2009.
The taro breeding program which commenced in 1996 in Samoa started producing taro varieties with some degree of TLB resistance. This led to the re-initiation of taro exports from Samoa in the years 2002 to 2009. Although export volumes were higher in the early 2000s, they started to wane again from mid to the late 2000s. (Adapted from Shepherd 2012)

1.3 Market Access Hindrances

When the new TLB resistant taro varieties were being produced by farmers across Samoa, exporters were eager to sell the new varieties overseas to start earning back a portion of the taro industry in New Zealand, Australia and the United States. Although New Zealand, Australia and the US all had requirements for taro entering their respective countries, the biggest challenge hindering market access was on the supply side of the value chain (Shepherd, 2012). In the early 2010s, when launching new taro varieties in New Zealand, farmers did not have access to sufficient planting materials. Additionally, farmers had limited understanding of export markets, and were mainly supplying the local market. As a result, exporters often struggled to source sufficient taro for export. On the importer side of the value chain, there was wariness of importers to engage with Samoan exporters due to inconsistent supply and quality of taro.

Taro imported into New Zealand needs to be free from biosecurity pests such as mites and nematodes. Consignments generally do not meet this requirement, and therefore need remedial treatment such as methyl bromide (MeBr) fumigation. Fumigation is an added expense and reduces the shelf-life of taro. On the other hand, a 2011 review of taro import conditions by Biosecurity Australia stipulated that taro could only be imported from areas/countries free of TLB or when an alternative measure was accepted (Biosecurity Australia, 2011). Specifically, the major concern was the high risk of importation of Pc sporangia or zoospores on corms particularly between petiole bases.

In light of the risk identified by Biosecurity Australia (2011), Samoa sought to identify the steps required to address this concern. The Pacific Horticultural and Agricultural Market Access Plus (PHAMA Plus) program, in collaboration with MAF, subsequently designed a systems approach, including application of the results of HPW-

HWT work by the Plant & Food Research (PFR) New Zealand, to assess the feasibility of meeting the requirements for gaining market access into Australia. This study identified the need to characterise Pc pathogenicity in corm rots if Pc can induce corm rots (Fullerton & Tyson, 2014). This research was subsequently funded by the Australian Centre for International Agricultural Research (ACIAR). It was found that the overall risk of Pc infection in fresh taro corms from Samoa was very low and there was potential for hot water treatment (HWT) to be used to remove any remaining inoculum of Pc on taro corm surfaces (Fullerton et al., 2019). The Department of Agriculture, Water and Environment (DAWE) Australia, upon reviewing this report and receiving an official request for reconsidering Samoa's fresh taro export from the Ministry of Agriculture and Fisheries (MAF), considered the application, and put forth requirements for Samoa to meet, one of which included the establishment of a satisfactory hot water treatment.

1.4 Existing Taro Treatments

While research and negotiations were underway for Samoa's fresh taro market access to the Australian market, research was also being conducted to improve the existing market access pathway for fresh taro to New Zealand. Although fresh taro from Samoa was being exported to New Zealand, with remedial MeBr fumigation commonly required at the border to remove pests and diseases, the Environmental Protection Agency (EPA), with the support of the Ministry of Primary Industries (MPI) in New Zealand, had put in place a goal for full recapture of MeBr following fumigation (EPA, 2021). There was strong encouragement for research to explore alternative processes and subsequently, PFR-NZ undertook research into high pressure washing (HPW) and the hot water treatment (HWT) of fresh taro to manage the biosecurity risks of mites and nematodes (Jamieson et al., 2018). Through a combination of funding from the Australian and New Zealand governments through the PHAMA Plus programme, PFR-NZ was able to establish that a HPW of 50 psi for 15 seconds followed by HWT at 48.0°C for 25 minutes was proven effective for the treatment of mites and nematodes (Wallace et al., 2021).

1.5 Preliminary SROS Research

Following this initial work on New Zealand market access, SROS, together with PFR-NZ, prepared a comprehensive research approach to investigate HWT for the treatment of Pc on fresh taro corms intended for the Australian market. This approach included an investigation to establish the temperature-time response of Pc in both mycelial and sporangial forms, mapping the heat transfer profile of taro, laboratory-based assessment of the efficacy of treatment on external inoculum (sporangia on the surface of the corm), laboratory-based assessment of the efficacy of treatment on internal infection, assessment of the effect of treatment on taro quality and a simulation trial treatment. However, due to limited funding available, this research approach could not be undertaken. Nevertheless, having noted the importance of this research to meet Australia's requirement to effectively manage TLB, SROS decided to adopt the New Zealand treatment at 48.0°C for 25 minutes and assess its efficacy to treat Pc at the laboratory scale.

In 2020, SROS conducted internally funded research to assess the efficacy of the 48.5°C treatment against Pc (Molimau-Samasoni et al., 2021). Samoa 2 corms from a commercial farmer were collected, , washed and bleached to remove surface contaminants. Corms were then divided into two experimental groups of 90 corms each. The first group comprised a positive control inoculated with Pc mycelial plugs (corms inoculated with Pc but not HWT), a negative control (corms not inoculated, and not HWT), and HWT treatment (corms inoculated with Pc mycelial plugs then HWT at 48.5°C for 25 mins) The second group comprised a positive control inoculated using sporangial suspension (corms inoculated with Pc but not HWT), a negative control (corms not inoculated, and not HWT), and a HWT treatment (corms inoculated using sporangial suspension, then HWT at 48.5°C for 25 mins) (Table 1.1). Inoculated corms were given two days to allow for Pc inoculum to infect corms. Three replicates comprising 10 corms per replicate were assessed. At 2 days post inoculation (dpi), treatment corms were submerged in hot water at 48.5°C for 25 mins, then allowed to cool. All three treatments were then divided into two groups: corms from which isolations were taken at 2dpi, and corms from which isolations were taken at 4dpi (two days after HWT).

Table 1.1: Summary of preliminary hot water treatment research

Corms were harvested then taken through the following steps, depending on the treatment group. Each treatment group had three replicates, with each replicate comprising 10 corms. For inoculated corms, one positive control batch (3 x 10 corms) were inoculated with Pc mycelial plugs, and the second positive control batch (3 x 10 corms) was inoculated in a Pc sporangial suspension. One of the treated batches (3 x 10 corms) was inoculated with mycelial plugs, and the second batch was inoculated in a Pc sporangial suspension.

	Wash	Bleach	Inoculate (0 dpi)	HWT (2 dpi)	Isolations (2 & 4 dpi)
Negative Control	✓	✓			✓
Positive Control	✓	✓	✓		✓
Hot Water Treatment	✓	✓	✓	✓	✓

When analyses were conducted, Pc was isolated from positive control corms (not HWT) indicating that the introduction of Pc to the corms was successful. An average 50% infection rate was detected at 2 dpi, and an average 100% infection rate was detected at 4 dpi. Conversely, Pc was not isolated from corms that were inoculated then HWT at 48.5°C for 25 mins (Fig 1.4A). When the treatment time was reduced and corms were treated at 48.5C for 15 mins, Pc was isolated from both positive control corms, and treatment corms. This suggested that the treatment time of 25 mins was necessary to eliminate Pc, and supported the adoption of the HWT at 48.5°C for 25 mins, for the treatment of Pc.

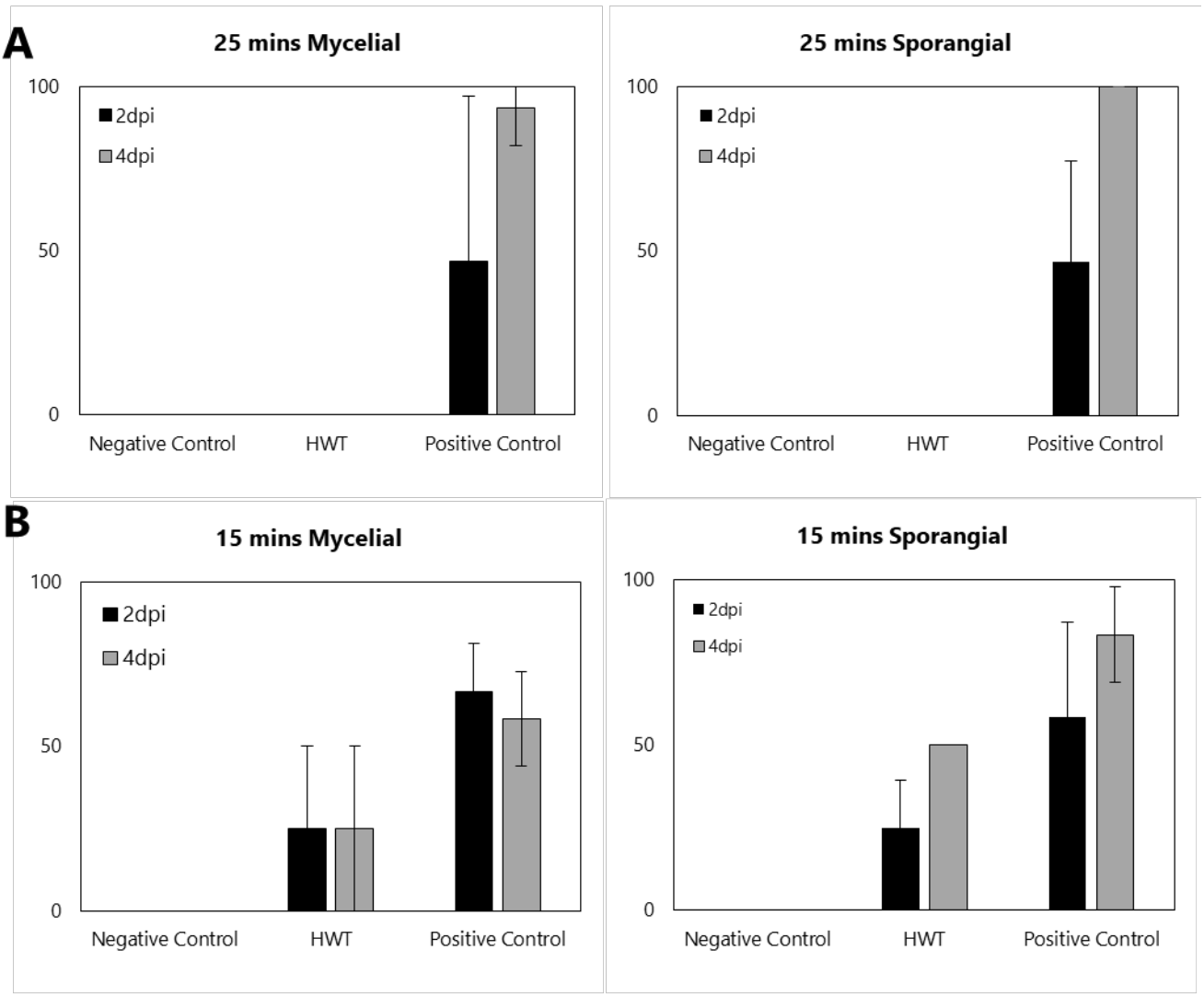

Figure 1.4: Preliminary research on hot water treatment against Phytophthora colocasiae.
A: Experiment 1. Corms were harvested, washed and bleached. Negative control corms were then kept at room temperature. HWT corms were firstly inoculated with Pc (as mycelial plugs or through sporangial suspension) then HWT at 48.5C for 25 mins. Positive control corms were inoculated with Pc (as mycelial plugs or through sporangial suspension) but were not HWT. B: Same as Experiment 1, except taro treatment was at 48.5C for 15 mins. (Adapted from Molimau-Samasoni et al., 2021)

1.6 Next Steps & Purpose of this Research Activity

Given the promising results obtained from SROS' preliminary research, testing the treatment at the semi-commercial/pilot scale facility became of utmost importance and the obvious next step to open the market for fresh taro corms from Samoa into Australia. This led to the instigation of this project, with the Scientific Research Organisation of Samoa (SROS) working together with the private sector, particularly the Ah Liki Group who had HWT experience and is the largest taro exporter in Samoa, to assess the efficacy of the HPW+HWT treatment.

The Ah Liki Group was responsible for the pack-house operations, including the collection of taro as they would normally do with their taro export operations, and operation of the HPW and HWT equipment. MAF was

responsible for all quality control assessments at the pack-house through treatment processes up to when corms were placed in sacks. SROS was responsible for keeping the corms in cool store and conducting laboratory assessments to determine if Pc was present on the corms.

The specific research objectives for SROS were:

- To determine the efficacy of HPW+HWT treatment (previously tested for the New Zealand market) against Pc, and
- To determine the shelf life of HPW+HWT treated taro.

2. Assessing the Efficacy of High Pressure Washing and Hot Water Treatment against Phytophthora colocasiae on Fresh Taro

2.1 Background

Jamieson et al., (2018) assessed the efficacy of HPW+HWT against mites (Rhizoglyphus sp.) and nematodes (Meloidogyne sp.). In their study, varying temperatures and time combinations were tested against various forms of mites (mite eggs, nymphs and adults) and nematodes (juvenile and eggs). While mites and juvenile nematodes were treated effectively at 47.0°C at less than 5 mins, nematode eggs were more tolerant of HWT and required treatment at higher temperatures for longer. At the conclusion of their study, HWT at 50.0°C for 12.5 mins, or HWT at 48.0°C for 30 mins in combination with HPW at 50 psi for 15 s was recommended.

Wallace et al. (2021) translated these findings to the semi-commercial/pilot scale HPW and HWT equipment. A total of ten shipments comprising 600 corms each that had been HPW treated at 50 psi for 15 s and HWT at 48.0°C for 25 mins followed by water cooling at ambient temperature for 25 mins and air drying overnight. Although only nine of the ten shipments were passed by MPI, the one that failed was due to termites that should have been removed during the grading step at pack house operations. It is therefore fair to extrapolate that all ten out of ten shipments were successful in demonstrating the HPW + HWT efficacy in removing mites and nematodes.

SROS had previously determined the efficacy of hot water treatment at 48.5°C for 25 mins against Pc under laboratory conditions. This was a SROS funded research conducted at the laboratory scale, using bench-top water baths heated to 48.5°C, and assessing three replicates of 10 corms each. This was small-scale preliminary research given limited funding available. At the time, a miscommunication between the PFR-NZ and SROS led to assessment of the HWT at 48.5°C instead of the reported 48.0°C (Wallace et al., 2021). To ensure that minimal diversions occur should this protocol be approved for the Australian market, SROS opted to run this current research for semi-commercial/pilot scale equipment following protocols that were already in place at the Ah Liki Group Packhouse, where the PFR semi-commercial/pilot scale HPW and HWT equipment were located.

2.2 Methodology

To assess the efficacy of the HPW+HWT treatment already in place for NZ against Pc, five batches of 200 corms each were processed through the biosecurity protocol that is already approved in principle for taro export to Australia. The process included using only export approved varieties with demonstrated high degree of tolerance to TLB and topping of taro to remove the petiole part from the corm.

Packhouse Handling & Treatment: Corms were subsequently HPW'ed at 50 psi for 15 seconds, then inspected by quarantine officers. Corms were then HWT'ed at 48.0°C for 25 mins using the pilot scale HWT equipment. Corms were cooled in an ambient temperature water bath for 25 minutes, then air-dried. After a final inspection by quarantine officers, 200 corms were packed in groups of 10 corms per sack (20 sacks). All sacks were transported to SROS facilities, where they were kept at 10.0°C. A total of 1000 corms (5 batches comprising 20 sacks holding 10 corms each) were HPW+HWT treated for subsequent laboratory assessments to determine the efficacy of the treatment against Pc. Table 2.1 below summarizes dates at which these activities were undertaken. An additional 20 corms per batch were treated as above, but kept in cool store for shelf-life assessments (Section 3). It is also important to note here that during the time of this study, there was a taro shortage in Samoa. It was difficult for the Ah Liki packhouse to source sufficient quality corms, so a compromise was made regarding the size of the corms, to ensure we had sufficient corms to conduct the study with.

Laboratory Assessments: Sacks from the 10.0°C cool store were removed for assessment at two time points - ten sacks were removed at 2 weeks and ten sacks at 4 weeks. At each time point for each batch, all 100 corms were lightly peeled to expose the corm surface (approximately 0.5 – 1.0 mm removed). Ten skin slices were excised from each corm covering both cut ends and body of the corm, and plated onto two CASPP (corn meal agar modified with carbendazim, ampicillin, streptomycin, pimaricin and PCNB) agar plates (Fullerton et al., 2019). Due to limited availability of some antibiotics, the latter batches of taro were plated on CASP (without pimaricin) and CSP (without ampicillin and pimaricin) plates. Particular attention was paid to areas of the corm that showed rot and/or brown discolouration. CASPP plates were incubated at 25.0°C and assessed after 5 days and 7 days. CASPP plates showing mycelial growth were used to inoculate V8 plates. Plugs of mycelial growth from CASSP were plated onto V8 media, which were then incubated at 25.0°C. Visual assessment for growth on V8 plates was conducted after 2 days, and microscope confirmation for Pc was conducted at 4 days when fungal growth was producing sporangia. Slides for microscope confirmation were prepared by taking a mixture of hyphae and sporangia from V8 plates and placing them onto a drop of iodine that had been previously spotted onto the middle of the slide then covered with a coverslip. Microscopic assessments to identify Pc were based on whether the unique sporangial morphology of Pc was present in any of the growths on V8 media.

Table 2.1: Summary of the dates of when key work was undertaken.

Corms were harvested a day before treatment. Corms were placed into the cool store on the same day of treatment (HPW+HWT) after air-drying. Corms were removed after two and four weeks of cool store. Subsequent laboratory assessments (isolating skin slices from corms and plating onto CASPP, plate assessments, V8 plating, plate assessments and microscopic confirmations) are not shown in the table.

Batch Number	Harvest	Variety Used	Treatment & Cool Store	Week 2 Assessment Commencement	Week 4 Assessment Commencement
1	1st Feb 2022	Talo Salani Talo Vave	2nd Feb 2022	16th Feb 2022	2nd Mar 2022
2	3rd Feb 2022	Samoa 2	4th Feb 2022	18th Feb 2022	4th Mar 2022
3	7th Feb 2022	Talo Fusi Samoa 2	8th Feb 2022	22nd Feb 2022	8th Mar 2022
4	8th Feb 2022	Samoa 2	9th Feb 2022	23rd Feb 2022	9th Mar 2022
5	9th Feb 2022	Talo Fusi Talo Vave	10th Feb 2022	24th Feb 2022	10th Mar 2022

2.3 Results & Discussion

Because there was a discrepancy between the taro treatment parameters we used for our preliminary research (48.5°C for 25 mins) and the taro treatment that has been accepted for New Zealand (48.0°C for 25 mins), the water temperature of the water bath during treatment of taro was monitored. From the temperature profiles (Fig 2.1), it was noted that the temperature of the water bath was consistently maintained above 49.0°C. Although an initial dip in temperature was observed a few minutes after the taro was loaded into the water bath (resulting in the water cooling from the taro), the temperature does not drop below 48.5°C (green dashed line) and throughout the whole run, the water temperature is maintained well above the target 48.0°C water temperature (red dashed line). This pattern holds true for batches 2, 3, 4 and 5. No water temperature profile is available for Batch 1.

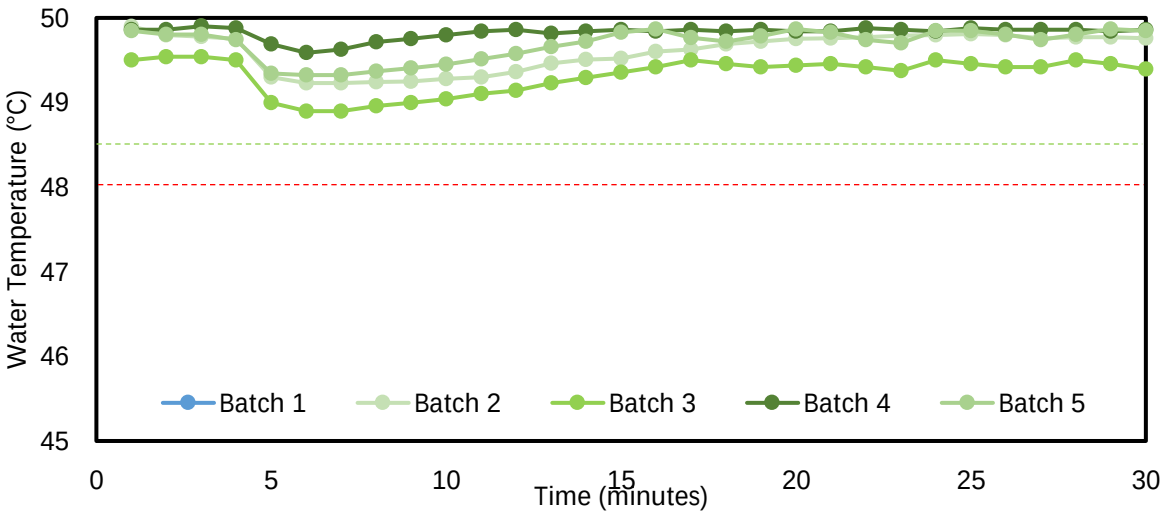

Figure 2.1: Temperature profile of water during hot water treatment

Water temperature of the water bath was monitored during treatment. Temperature probe was inserted into the water bath to measure return water temperature, just prior to the crates of taro being lowered into the water bath. Temperature was monitored for the whole duration of the treatment. Red dashed line indicates 48.0°C target. Green dashed line indicates temperature Phytophthora colocasiae was originally tested at in laboratory-scale research (48.5°C).

When taro corms were removed from the cool store after two weeks, they showed some external rot but generally looked acceptable (Fig 2.2A). However, more rots were observed from corms that were removed from the cool store after four weeks. Rots were primarily observed from the cut ends of the corms and they were often soft rots. The sites of primary fungal infections were observed to be the major wound sites introduced during harvest. It is therefore important to consider proper postharvest handling of corms to reduce the potential of infection from environmental source of Pc. Furthermore, it may be worth considering the option of allowing corms the time to cure, whereby the taro corms develop a cork layer over the wound surface, thereby providing protection against fungal infections (Matthews, 2002). Curing of taro has been found to occur at 34.0°C to 35.0°C (Thompson, 1996) and indeed, it was common practice for taro corms to be dried in the sun immediate after harvest (Matthews, 2002). Fungal growth observed on the surfaces of the corms showed different colouration. Some were observed to be white, others were grey, green, pink or black. This will be discussed further in the next chapter.

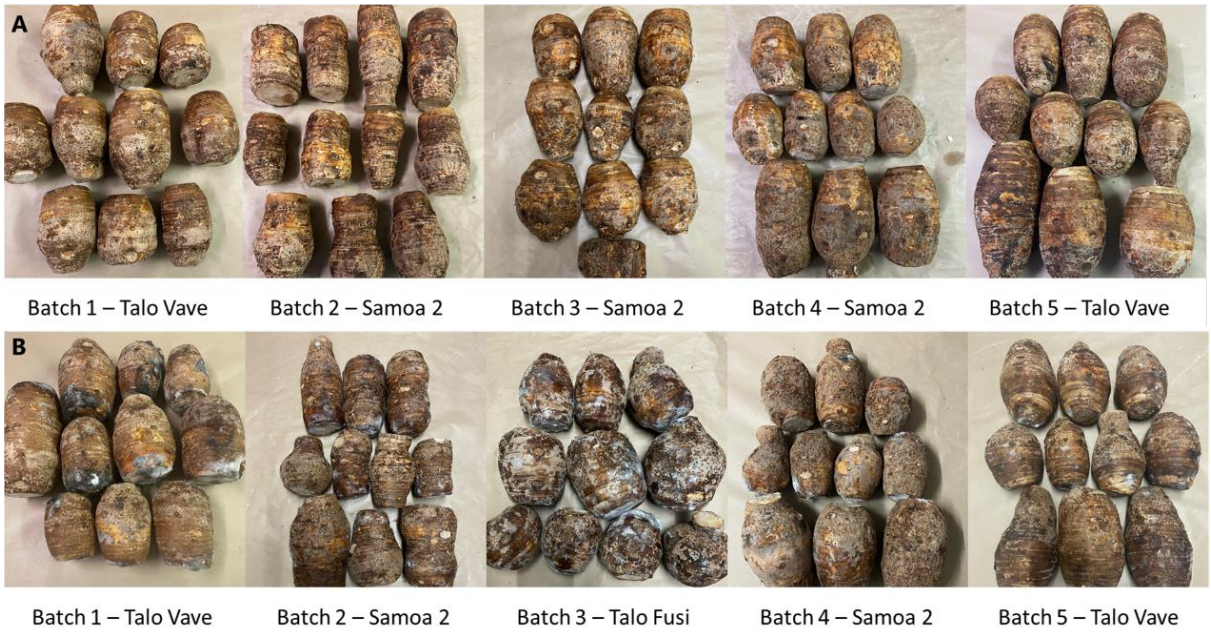

Figure 2.2: External appearance of corms upon removal from cool store after 2 and 4 weeks
After high pressure washing at 50 psi for 15 seconds and hot water treatment at 48.0°C for 25 mins, taro were air dried, then packed into sacks in lots of 10 and moved to 10.0°C for cool store. A: Appearance of corms from batches 1-5 upon removal after being in cool store at 10.0°C for 2 weeks. B: Appearance of corms from batches 1-5 upon removal after being in cool store at 10.0°C for 4 weeks.

After lightly peeling corms upon removal from the cool store, 10 skin slices per corm were excised and plated on to CASPP agar plates. When assessing corms removed after two weeks in the cool store, no Pc was detected from the 500 corms assessed (Table 2.2). This was despite a total of 354 corms out of 500 corms producing mycelial growth on CASPP plates. Of the 354 corms producing mycelial growth, only 348 corms were able to produce growth on V8 media. Of all these, none was detected to be Pc infected. It is however important to note that the number of isolates that were assessed would likely have been less, if all antibiotic consumables had been received on time from the supplier. However, the incomplete panel of antibiotics allowed for the growth of contaminants that would normally have not grown. This is very clear in light of the high amount of mycelial growth observed from plates with missing antibiotics, compared to the number of isolates observed from plates with the full panel of antibiotics required.

Table 2.2: Assessment of week 2 corms by batch for the presence of Phytophthora colocasiae

Each of the 100 corms per batch were lightly peeled, and 10 skin slices per corm were excised and plated on to CASPP agar plates. Where mycelial growth was observed, agar plugs were collected and plated on to fresh V8 plates. Growth was noted and microscopic assessments were conducted at 100X magnification to determine the presence/absence of Pc. *CASPP plates with missing antibiotics due to poor stock availability and shipment issues resulting from COVID-19 lockdowns.

Batch Number	Number of Corms	Number of Corms with mycelial growth (CASPP)	Number of corms with growth on V8 media	Number of Corms from which Pc was detected
1	100	52	51	0
2	100	50	49	0
3	100	52	50	0
4	100	100*	98	0
5	100	100*	100	0

No Pc was detected from taro corms that were removed from the cool store after four weeks (Table 2.3). This was observed despite all 500 corms producing mycelial growth on CASPP, and on V8 media. This is a very high number of isolates and is very likely due to the incomplete panel of antibiotics that was required to inhibit the growth of some common contaminants. Indeed, some of these mycelial growths were very clearly not Pc, based on their colony morphology (e.g., green colonies on V8). All growths on V8 media were assessed microscopically and none showed the distinctive Pc sporangia morphology.

Table 2.3: Summary of corms assessed by batch that produced mycelial growth and Pc detection.

Each of the 100 corms per batch were lightly peeled, and 10 skin slices per corm were excised and plated on to CASPP agar plates. Where mycelial growth was observed, agar plugs were collected and plated on to fresh V8 plates. Growth was noted and microscopic assessments were conducted at 100X magnification to determine the presence/absence of Pc. *CASPP plates with missing antibiotics due to poor stock availability and shipment issues resulting from COVID-19 lockdowns.

Batch Number	Number of Corms	Number of Corms with mycelial growth (CASPP)	Number of corms with growth on V8 media	Number of Corms from which Pc was detected
1	100	100*	100	0
2	100	100*	100	0
3	100	100*	100	0
4	100	100*	100	0
5	100	100*	100	0

As an extra step for this exercise, an attempt was made to identify some of the fungi and oomycotes observed from the corm rots of taro in this study. It was found that a large proportion of V8 isolates being assessed microscopically were Pythium species (Fig 2.3). Kerz-Moehlendick et al. (1983) identified four Pythium species commonly associated with taro corm rots in Samoa, these being P. middletoni, P. myriotylum, P. splendens and P. vexans. Others fungal species commonly associated with taro corm rots include Lasiodiplodia threobroma, Fusarium oxysporum and F. solani, and Penicillium species (Harris & Fullerton, 1998, Nishimura & Kudo, 1994;

Widodo & Supramana, 2011). Indeed, several of the above species were detected in the assessments, and some of the Pythium species were identified (Fig 2.3) including P. aphanidermatum, P. myriotylum, P. splendens and P. vexans. Also detected were likely Lasiodiplodia and Fusarium but these will be subject to further confirmation, as they are not imperative to this report.

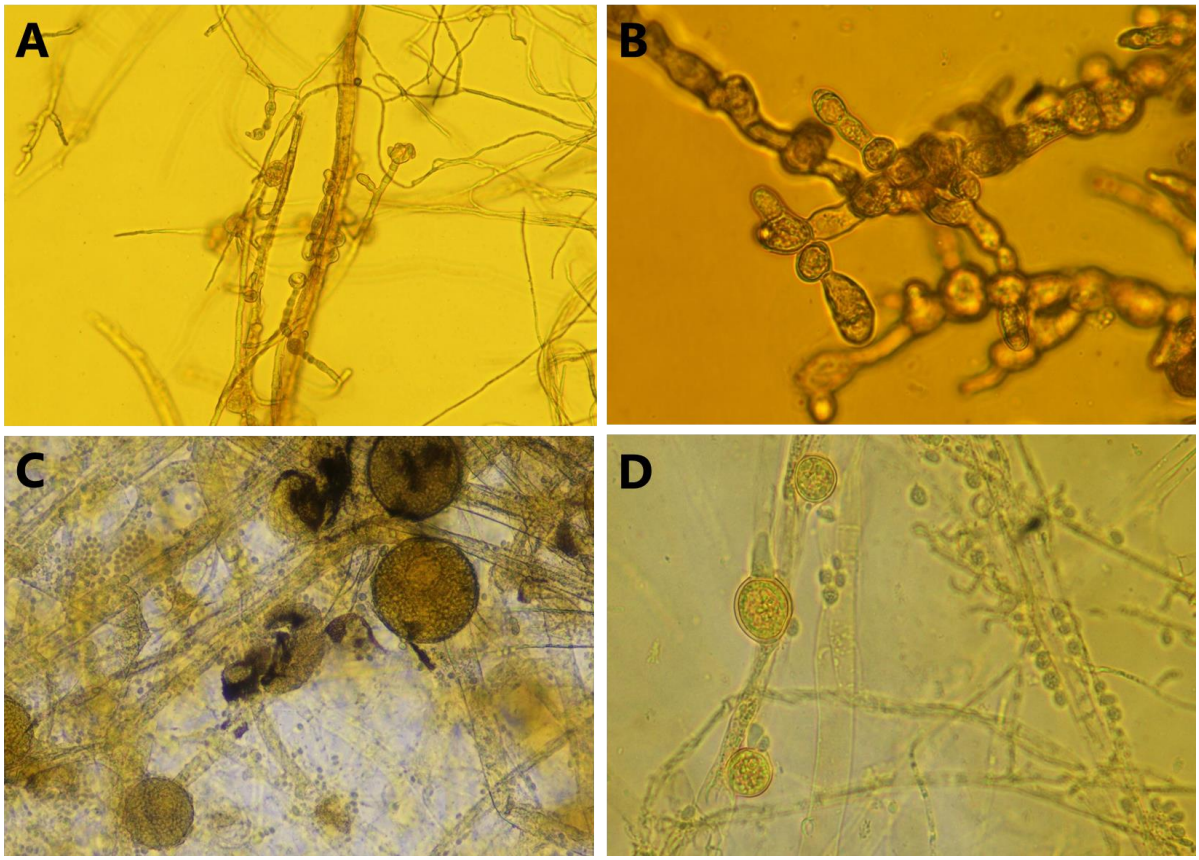

Figure 2.3: Other corm rot species detected from corm rots assessed

A variety of Pythium species were detected from corm rots from which skin slices were excised from. These Pythium species grew successfully on V8 agar. A: P. aphanidermatum toruloid zoosporangia isolated from Batch 2 removed after two weeks, corm 44 slice 4, at 100X magnification. B: P. myriotylum sporangia isolated from Batch 4 removed after two weeks, corm 46 slice 6, at 400X magnification. C: P. splendens sporangia isolated from Batch 1 removed after two weeks, corm 37 slice 1, at 200X magnification. D: P. vexans isolated from Batch 2 removed after four weeks, corm 3 slice 9, at 400X magnification.

3. Assessing the Shelf Life of HPW+HWT Treated Taro

3.1 Background

Many postharvest and biosecurity treatments of fresh produce often affect their quality. Some treatments improve the quality of the produce, while other treatments are detrimental to the shelf-life and quality. A great example of this phenomena was observed when the postharvest quality of breadfruit (Molimau-Samasoni et al., 2019) that had undergone high temperature forced air (HTFA) treatment was assessed, compared to breadfruit that had undergone hot water treatment (HWT). The postharvest quality of breadfruit from both treatments was compared to untreated breadfruit. It was found that while breadfruit treated with HTFA improved the postharvest shelf-life of breadfruit compared to untreated breadfruit, breadfruit which were treated with hot water had reduced quality and had a shorter shelf life compared to untreated breadfruit. On this premise, the shelf-life of taro that had been HPW+HWT treated was also assessed.

The storage temperature for taro corms remains a point of contention amongst researchers. While some researchers recommend taro storage at 4.0°C - 10.0°C (Malaki et al.,2003; Opara, 2003), some researchers recommend a warmer temperature of >10.0°C – 14.0°C (More et al., 2019). Others still recommend non-refrigerated storage of corms at high humidity and warm conditions (Fullerton & Purea, 1982). Although taro appears high quality upon first removal from cool storage, taro quality has been shown to rapidly decline once it is moved to ambient temperatures, attributed to chilling injury (Watson, 1979). Taro is a tropical root crop, and it is unsurprisingly prone to chilling injury which can manifest as rapid weight/water loss, brown discoloration of corm flesh, spongy tissue texture and rapid development of rot (Harris & Fullerton, 1998). At a molecular level, chilling injury was observed as internal browning followed by shrinkage of nuclei, plasmolyses and collapse, and destruction of leucoplasts and amyoplasts (Rhee & Iwata, 1982). Some researchers have postulated that keeping the corm physiologically active affords the corm some level of protection against diseases, reducing the incidence of corm rot during storage (Fullerton & Purea, 1982; Jackson et al, 1979). However, this will lead to corms growing roots during the storage period, which can be undesirable for the consumer, and definitely against Australian requirements of devitalisation (Biosecurity Australia, 2011).

3.2 Methodology

To determine the shelf-life of taro that had undergone HPW treatment at 50 psi for 15 s, and HWT at 48.0°C for 25 mins, taro had this treatment applied and was then moved to a cool store at 10.0°C as per normal practice for Samoan taro export to New Zealand. Due to the variable time required for sea freight, which ranges from two to four weeks, the corms were removed from cool store at these two time points. Each batch had 20 corms each, packed as 10 corms per sack. After two weeks, one sack was moved to 25.0°C; five corms were assessed after three days, and the remaining five corms were assessed after seven days. The sack remaining in the 10.0°C cool store was moved to 25.0°C after four weeks. Five corms were assessed after three days, and the remaining five corms were assessed after seven days.

Corm assessment was conducted by visual assessment of the external and internal appearance. External appearance was rated based on how much of the corm surface that was covered with fungal growth (Table 3.1). After external assessment, the corms were peeled and cut in half longitudinally to assess the flesh condition, particularly for the presence and prevalence of corm rot (Table 3.1). External and internal qualities were rated based on the following scale:

Table 3.1: Scale for corm assessment (external and internal)
External assessment of corms was made on the basis of how much of the corm surface that was covered with fungal growth. Internal assessment was made on the basis of how much of the corm that was affected with corm rot.

	External Assessment	Internal Assessment
0	No fungal growth on surface	No corm rot
1	1-20% surface covered with fungal growth	1-20% corm rot
2	21-40%	21-40%
3	41-60%	41-60%
4	61-80%	61-80%
5	81-100%	81-100%

3.3 Results & Discussion

The shelf-life of taro that had been HPW treated at 50 psi for 15 s and HWT at 48.0°C for 25 mins was assessed by first comparing the external appearance of the corms. The external appearance of corms removed from cool store after two weeks (Fig 3.1A) did not vary greatly from counterparts removed at the same time then kept at 25.0°C for three (Fig 3.1B) and seven days (Fig 3.1C). Although some fungal growth was observed from corms that were removed after four weeks in cool store (Fig 3.1D), their counterparts that had been kept at 25.0°C for three days (Fig 3.1E) and seven days (Fig 3.1F) showed marked fungal growth on the surface of the corms. A mixture of white, grey, black and green fungi growing on the surfaces of the corms was observed. The huge variability in corm sizes could mean that some smaller corms were more prone to corm rots as a result of compression damage from larger and heavier corms during storage. This makes it important to ensure that uniform standard corm size is adhered to during grading and packing.

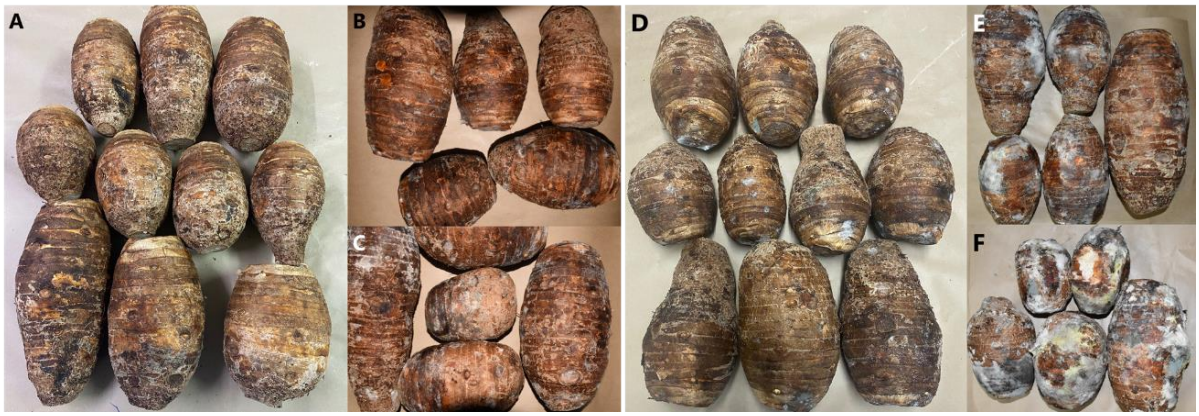

Figure 3.1: Comparison of the external appearance of taro

A: Batch 5 taro directly out of 10.0°C cool store after two weeks. B: Batch 5 taro removed from cool store after two weeks then kept at 25.0°C for three days. C: Batch 5 taro removed from cool store after two weeks then kept at 25.0°C for seven days. D: Batch 5 taro directly out of 10.0°C cool store after four weeks. E: Batch 5 taro removed from cool store after four weeks then kept at 25.0°C for three days. F: Batch 5 taro removed from cool store after four weeks then kept at 25.0°C for seven days.

When the assessment of external appearance and internal appearance of corms kept for shelf-life assessments (Fig 3.2) was quantified, some interesting observations were noted. Although the batch observed in Fig 3.1 showed some clear differences in external appearance of corms, when all batches assessed were taken into account, it was found that there was no significant difference in external appearance of corms between those removed from the cool store after two weeks, and those removed after four weeks (Day 3 p-value 0.157; Day 7 p-value 0.312). There was no detection of a significant difference in appearance in internal corm rot between corms kept at 25.0°C for three days and corms kept at 25.0°C for seven days, when removed after two weeks (p-value 0.073) or when removed after four weeks (p-value 0.715). However, a significant difference was observed in internal corm quality between corms removed from cool store after two weeks and corms removed from the cool store after four weeks and kept at 25.0°C for three days (p-value 0.001) or seven days (p-value 0.015). To determine if the quality of corms removed from the cool store after two weeks and kept at 25.0°C for 7 days were comparable to corms removed from the cool store after four weeks and kept at 25.0°C for three days, it was found that the quality of corms removed after two weeks and kept at 25.0°C for seven days was significantly better (p-value 0.046).

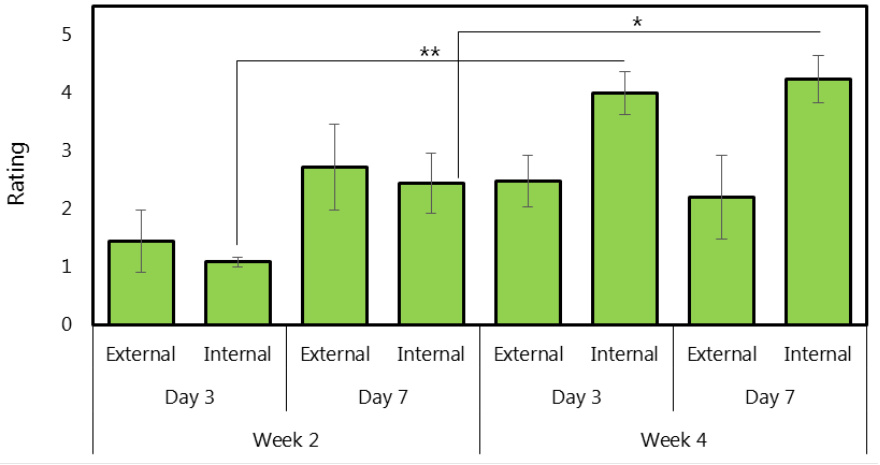

Figure 3.2: Determining shelf-life of corms by comparing of external and internal appearance
Taro corms were removed from 10.0°C cool store and kept at 25.0°C. Half of the corms per batch were assessed after three days, and the remaining half of corms for each batch were assessed after seven days. External appearance was assessed on the basis of how much of the corm surface that was covered with fungal growth. Internal assessment was made on the basis of how much that was affected with corm rot. A rating of 0 – not affected, 1: 1 to 20% affected, 2: 21 to 40% affected, 3: 41-60% affected, 4: 61 to 80% affected, 5: 81 to 100% affected. Error bars indicate standard error, student's t-test was used to assess significance of difference. *: p-value < 0.05; **: p-value less than 0.01

The findings show that corms kept in the cool store for four weeks have inferior quality compared to corms removed from the cool store after two weeks, regardless of days the corms are subsequently kept at 25.0°C (or ambient temperature). These findings suggest that the extra two weeks the corms are kept at 10.0°C cool store affects quality. After being kept at 10.0°C in the cool store for four weeks, corm quality rapidly decreased at ambient temperature. This is likely due to chilling injury which has been observed and reported for corms (More et al., 2019). Chilling injury in taro corms has been reported as rapid water loss of corms after cool store, and the subsequent collapse of corm cells leading to spongy tissue texture of the corm. This was observed in some corms. Furthermore, the collapse of cell structure following chilling injury-related water loss is followed by the rapid invasion and infection of corms with various fungal species. These species include various Pythium species, Lasiodiplodia species, Fusarium species and some Penicillium species, of which some were being recovered from the skin slices during examination.

The findings also suggest that more detailed work is required to identify the optimal temperature for taro storage, particularly if taro were to survive a four-week freight, which is the most likely time frame for shipping taro to Australia. The study should assess temperatures higher than 10.0°C and weight loss can be a simple method to ascertain whether corms have undergone chilling injury or not. In this future study, it may also be worthwhile to consider removing the fungi and fungi-like contaminants that usually cause postharvest corm rots in taro, by exposing the corms to a sodium hypochlorite solution. This will allow any physical manifestations observed from keeping taro at low temperatures to be attributable only to chilling injury. Furthermore, the physical appearance of corm rots associated with poor postharvest handling is not well understood.

It has also been reported that poor postharvest handling of taro, although not readily detectable soon after harvest, can cause corm rots (Underhill, 2015). However, how to distinguish these rots from rots associated with disease or rots associated with chilling injury is unclear. It is also likely that the compromised corm structure resulting from poor handling makes the corms more susceptible to infection and pathogen-related corm rots. Being better able to distinguish between the different types of postharvest corm rots of taro and their associated causes will allow the industry to better pinpoint and identify the parts of the taro value chain that require improvement to ensure delivery of a quality product to export markets.

4. Conclusions and recommendations

The research findings provide the following conclusions:

- The preliminary study demonstrated that hot water treatment at 48.5°C for 25 mins is effective in managing Phytophthora colocasiae following inoculation of the surface of taro corms.
- The pilot study demonstrated that the high pressure washing at 50 psi for 15 seconds, in combination with a hot water treatment at 48.0°C for 25 mins is successful in managing the biosecurity risk of Phytophthora colocasiae should it be present on the surface of taro corms.
- The study has also shown that treated corms kept at 10.0°C for two weeks will have sufficient shelf life to be sold a week after removal from cool store.
- However, the results have also demonstrated that treated corms kept at 10.0°C for four weeks will not be of acceptable quality for the market upon removal from cool store.

Should Samoa wish to further improve on this new pathway for the Australian market in the future, further research is recommended to identify the optimal temperature for taro corm cool storage and to explore any other opportunities including, but not limited to:

- Investigate phenotype of poor handling damage to corms;
- Varietal-dependent tolerance to treatment and cool store; and
- Cork development and potential to provide protection against corm rot.

5. References

Biosecurity Australia. (2011). Draft review of import conditions for fresh taro corms.

Environmental Protection Agency (2021). Decision: modified reassessment of methyl bromide.

Fonoti, P.; Tofinga, M. P.; Hunter, D. G. (2008). Screening a cycle 1 breeding population of taro (Colocasiae esculenta L.) for resistance to taro leaf blight in Samoa. Research Journal of Biological Sciences. 3(8): 888-891

Fullerton, RA. & Purea, M. (1982) Non-refrigerated shipment of taro. Report to the Research Advisory Committee, Totokoitu Research Station, Rarotonga.

Fullerton, RA. & Tyson, JL. (2003). The biology of Phytophthora colocasiae an implications for its management and control. In: 3rd Taro Symposium, 21-23 May 2003, Nadi, Fiji, pp 107-110

Fullerton, RA. & Tyson, JL. (2014). New access for Samoan taro to Australia. (PHAMA Report)

Fullerton, RA.; Tyson, JL.; Molimau-Samasoni, S.; Tugaga, A.; Maslen-Miller, A.; & Mellow, K. (2019). Defining the biotic constraints to fresh taro from Samoa gaining market access to Australia. Final Project Report to the Australian Centre for International Agricultural Research

Harris, FM. & Fullerton, RA. (1998) Optimum handling and storage conditions for Fijian dalo. Report to Balthan International (Fiji) Ltd.

Jackson, GVH. & Gollifer, DE. (1975). Storage rots of taro (Colocasiae esculenta) in the British Solomon Islands. Ann. Appl. Biol. 80: 217-230

Jackson, GVH.; Gollifer, DE.; Pinegar, JA. & Newhook, FJ. (1979). The use of fungicides against postharvest decay in stored taro in the Solomon Islands. In Small-Scale Processing and Storage of Tropical Root Crops. CRC Press

Jamieson, L. E.; Page-Weir, N. E. M.; Wilkinson, RT.; Redpath, SP.; Hawthorne, AJ.; Brown, SDJ.; Aalders, LT.; Tunupopo, F.; Tugaga, A.; Toomata, T.; Shah, F.; Armstrong, JW. & Woolf, AB. (2018). Developing risk management treatments for taro from the Pacific Islands. New Zealand Plant Protection. 71: 81-92

Kerz-Moehlendick, FH.; Fukegem FH.; & Schloesser, E. (1983). Morphology and pathogenicity of four Pythium species isolated with root and corm rot of taro, Colocasiae esculenta (L.) Schott in Western Samoa. Alafua Agriculture Bulletin 8(3): 53-62

Malaki, I.; Hunter, D.; Tuivavalagi, N.; Ullah, MW. & Amosa, F. (2003). The effects of sodium hypochlorite dipping, temperature and duration of storage on the quality of fresh taro corms destined for overseas markets. University of the South Pacific Repository.

Maslen-Miller, A.; Fullerton, RA.; Tugaga, A.; Tunupopo, F.; Molimau-Samasoni, S.; Bowen, JK.; MacDiarmid, RM.; Tyson, JL. (2020). Symptom expression of Phytophthora colocasiae in inoculated taro corms. New Zealand Plant Protection. 73: 1-5

Matthews, P. J. (2002). Taro Storage Systems. JCAS Symposium Series. 16: 134 – 163

Molimau-Samasoni, S.; Vaaiva, V.; Seruvakula, S.; Tugaga, A.; Ortiz, G.; Wallace, S.; Seelye, M.; Waddell, BC.; Brown, SDJ.; Jamieson, LE.; & Woolf, A. (2019). A comparison of postharvest quality of breadfruit (Artocarpus altilis) after disinfestation with hot air or hot water treatments. New Zealand Plant Protection. 72: 67-74

Molimau-Samasoni, S.; Tugaga, A.; Tautua, R.; Vaaiva, V.; Tofete, S.; Tusa, S.; Vaega, E.; Woolf, A.; Fullerton, RA. & Tyson, JL. (2021). Assessing the effectiveness of a hot water treatment to eliminate Phytophthora colocasiae from freshly harvested taro. In Plant Science Central Conference, 5th July, Palmerston North New Zealand

More, S. J.; Ravi, V. & Raju, S. (2019). Tropical Tuber Crops. In Postharvest Physiological Disorders in Fruits and Vegetables. 719-755. CRC Press

Nishimura, N. & Kudo, K. (1994). Fusarium oxysporum f. sp. colocasiae n. f. sp. Causing dry rot of Taro (Colocasiae esculenta). Ann. Phytopath. Soc. Japan. 60: 448-453

Onwueme, I. (1999). Taro cultivation in Asia and the Pacific. Food and Agriculture Organisation of the United Nations, Bangkok Thailand.

Opara, LU. (2003). Edible Aroids: Postharvest Operation. Food and Agriculture Organisation of the United Nations

Pacific Trade Invest. (2012). Pacific Islands: Exporting taro to New Zealand – Market Brief.

Paull, RE. & Chen, CC. (2015). Taro: Postharvest quality-maintenance guidelines. Vegetable and Root Crops. University of Hawaii College of Tropical Agriculture and Human Resources, Manoa Hawaii

Revill, PA.; Jackson, GVH.; Hafner, GJ.; Yang, I.; Maino, MK.; Dowling, ML.; Devitt, LC.; Dale, JP.; & Harding, RM. (2005). Incidence and distribution of viruses of Taro (Colocasiae esculenta) in Pacific Island countries. Australasian Plant Pathology. 34: 327-331

Rhee, J. K. & Iwata, M. (1982). Histological observations on the chilling injury of taro tubers during cool storage. Journal of Japanese. Soc. Hort. Sci. 51(3): 362-368

Shepherd, B. (2012). Developing exports of Samoan taro to New Zealand. PHAMA Report

Singh, D.; Jackson, G.; Hunter, D.; Fullerton, R.; Lebot, V.; Taylor, M.; Iosefa, T.; Okpul, T. & Tyson, J. (2012). Taro Leaf Blight – A threat to food security. Agriculture. 2: 182-203

Thompson, A. K. (1996). Postharvest Technology of Fruit and Vegetables. Oxford: Blackwell Scientific.

Underhill, S. JR. (2015). Postharvest loss and food safety risks in Samoa's fruit and vegetable value chains. End of Project Report.

Underhill, S. JF. (2017). Better postharvest handling for Samoan smallholder farmers – A practical guide. Westminster Printing, Australia

Wallace, S.; Stevenson, B.; Jamieson, L.; Waddell, B.; Molimau-Samasoni, S.; Vaaiva, V.; Tugaga, A.; Tunupopo, F. & Woolf, A. (2021). Market evaluation of taro high pressure washer and hot water treatment system. PHAMA Report.

Watson, J. (1979). Importing root crops from the South Pacific Islands for New Zealand Markets. In D. L. Plucknett (ed.), Small-scale Processing and Storage of Tropical Root Crops, 151-165. Boulder, Colorado: Westview Press.

Widodo & Supamana. (2011). Fusarium species associated with corm rot of Taro in Bogor. Microbiology Indonesia. 5(3): 132-138

Yusop, MSM.; Saad, MFM.; Talip, N.; Baharum, SN.; & Bunawan, S. (2019). A review on viruses infecting taro (Colocasiae esculenta (L.) Schott). Pathogens. 8: 56