Interception and hot water treatment of mites and nematodes on root crops from the Pacific Islands

N.E.M. Page-Weir¹, L.E. Jamieson¹, N.L. Bell², T.C. Rohan², A. Chhagan¹, G.K. Clare¹, A.M. Kean¹, V.A. Davis¹, M.J. Griffin¹ and P.G. Connolly¹

¹*The New Zealand Institute of Plant & Food Research Limited (Plant & Food Research), Private Bag 92169, Auckland*

²*AgResearch Ltd, Ruakura Research Centre, Private Bag 3123, Hamilton*

Corresponding author: Natalie.Page@plantandfood.co.nz

Abstract Root crops are major food crops and export commodities in the South Pacific. However, the presence of mites and nematodes results in rejection or treatment of these crops exported to New Zealand. Current disinfestation methods relying on fumigation result in shorter produce shelf life. This paper summarises the organisms intercepted on root crops from the Pacific Islands and sent for identification in New Zealand, with particular reference to mites and nematodes. Results of a laboratory experiment examining the response of representative mite and nematode species to hot water treatment indicated times of less than 4 min at 48°C or 2 min at 49°C resulted in 99% mortality. The implications of these heat treatments for root crops are discussed. Additionally rearing methods are presented for two mite species: a mould mite and a bulb mite. These species will be relevant for use in future New Zealand and Pacific Island disinfestation studies.

Keywords hot water, disinfestation, mites, nematodes, Pacific Islands, root crops.

INTRODUCTION

Root crops, such as taro, yam, cassava and ginger, are major food crops and export commodities in the Pacific Islands. For example, up to 12,000 tonnes of taro are exported annually from the region. However, the presence of organisms such as mites and nematodes results in a high treatment rate of root crops exported to New Zealand. Current disinfestation methods rely on fumigation of crops with methyl bromide. Often taro corms are fumigated several times, either prior to shipment (R. Masamdu, Secretariat of the Pacific Community, unpublished data) and/or once or twice after arrival (De Lima 2009; McGregor et al. 2011), with each methyl bromide fumigation diminishing quality and shelf life, or completely destroying the produce (De Lima 2009; McGregor et al. 2011).

The main interceptions on root crops exported to Australia and New Zealand from the Pacific Islands are nematodes, taro mites, snails and soil (De Lima 2009). Other species intercepted leading to methyl bromide fumigation include scale insects, mealybugs, mites, beetles, thrips and ants (De Lima 2009). Between 2001 and 2005 up to 80% of taro consignments exported from the Pacific Islands were fumigated, mainly because of the interception of taro mite (*Rhizoglyphus minutus*) (McGregor et al. 2011). In 2003, a report by Landcare Research concluded that it was highly unlikely the tropical taro mite (*R. minutus*) could enter and establish in New Zealand and in the unlikely event that it did survive, the probability of it causing any

damage to New Zealand's horticulture crops was extremely low to near zero (Zhang 2003). In response, the Ministry for Primary Industry (MPI, previously MAF) re-categorised this pest from a regulated pest requiring fumigation to a non-regulated pest requiring no action. However, identification of mite eggs and immature stages to differentiate non-regulated species from regulated species is often not possible; therefore the presence of immature stages of *R. minutus* can still be a market access issue.

Nematodes became a quarantine problem for root crops exported from the Pacific Islands to New Zealand after more rigorous inspection regimes (using high powered microscopes) commenced around 2003 (McGregor et al. 2011). It is difficult to distinguish between parasitic/regulated species (i.e. species that feed on live plant tissue and are not found in New Zealand) and non-parasitic/saprophytic species (i.e. probably not of quarantine importance and may not require action) (McGregor et al. 2011). Therefore all root crops with live nematodes detected are usually fumigated on arrival. Consequently, even with the re-classification of taro mite to a non-regulated species, fumigation rates have not changed, mainly due to the presence of live nematodes and other regulated mite species, i.e. *R. setosus* and *R. singularis*. Fumigation, together with the costs of pest identifications, has increased exporter costs and the price to consumers.

Hot water treatment (HWT) has been identified as the disinfestation technology with the greatest potential to satisfy quarantine and quality criteria for root crops from the Pacific Islands (De Lima 2009). Hot water dips have an advantage over insecticides by not only killing invertebrates on the surface of the plant tissue, but also penetrating into the tissue and therefore controlling nematodes (Hara 2013).

HWT has been found to be effective in controlling a variety of mite species on grapevine plants (Szendrey et al. 1995), persimmons (Lester et al. 1997; Lee et al. 2010) and apricots (Jones et al. 1996), as well as nematode species on banana and plantain suckers (Coyne et al. 2010), *Crocus*, *Allium* and *Polianthes* bulbs (Nguyen et al. 2010; van Leeuwen & Trompert 2011) and anthurium

stem cuttings (Tsang et al. 2010). HWT has also been used for disinfestation of both mites and nematodes on strawberry runners and lily bulbs (Hellqvist 2002; Kok et al. 2008; European and Mediterranean Plant Protection Organization 2012). HWT schedules are listed in the United States Department of Agriculture (USDA) treatment manual (USDA 2013) to disinfest a range of fruits, rooted and unrooted plants/cuttings and roots targeting mites, nematodes, mealybugs, fruit flies, snails, leafminers, aphids and other pests.

This paper presents lists of regulated mites and nematodes intercepted in New Zealand from root crop exports originating in the South Pacific. To assess the potential for HWT to replace fumigation disinfestation treatment, the time/mortality responses of a range of hot water treatments are presented using representative mite and nematode species already present in New Zealand.

MATERIALS AND METHODS

Interceptions

Interception records were obtained from the "Interception Database" collected by MPI's Data Analysis Team (previously known as Analysis and Profiling Group, Border Standards Directorate, MAFBNZ). Data were obtained for all border interceptions from root crops from the Pacific from January 2000 – April 2010. Only organisms identified in laboratories have been included in the interception database extracts. They do not include organisms routinely identified at the border by quarantine inspectors and consequently, the most commonly intercepted organisms may not be included.

Data sources that made up the "Interception Database" included: Investigation and Diagnostic Centre (IDC) Reports, Quantum, STARS, AgriQuality, Plant Pest Information Network (PPIN), Laboratory Information Management System (LIMS) extract and The National Plant Pest Reference Laboratory (NPPRL). Data were received in November 2010. Any incomplete interception records from these data sources were excluded.

Data cannot be used accurately for quantitative purposes, for the following reasons:

1. Not all border-intercepted organisms are required to be identified – for many, importers have an option of whether to identify organisms.
2. The interception database was only established in 2003. Although the database contains historical data these may be incomplete before 2003.
3. In 2004 a change of policy was made whereby interceptions made at transitional facilities were classified as border interceptions. Before this time they were classified as post-border interceptions.
4. Occasionally border projects are in place whereby all organisms found on particular commodities must be identified. These commodities vary.

Mould mite rearing

Mould mites (*Tyrophagus putrescentiae* (Shrank)) were acquired from Plant & Food Research, Palmerston North. Initially a method utilising artificial bulb mite diet (Bot & Meyer 1967) was tested, but mites were difficult to remove from the diet for treatment. Subsequently a colony was established at 25°C, 18:6 h light:dark and 65% RH and provided with a layer of cat biscuits (Whiskas® Meaty selection™, 2–3 biscuits deep) in a sealed plastic (ClickClack®) container (152 mm long × 101 mm wide × 68 mm high).

Aggregations of mites on the sides and lids of mature rearing containers were transferred to treatment containers with minimal damage using a fine-tipped paintbrush. A 0.004–0.005 g sample of these mites was found to contain an average of 100–400 adults. Therefore, all mould mite treatments discussed below were provided with adults in this way.

Mite eggs for treatment were provided by transferring 50 adult mites onto a small section of bulb mite diet within a Petri dish. Mites were left for 48 h, after which adults were removed. As eggs were difficult to remove from the diet, and there were concerns about mechanical damage, eggs were treated on the diet.

Bulb mite rearing

Bulb mites (*Sancassania* sp.) were collected from garlic bulbs in the field. Mites were reared on artificial diet (Bot & Meyer 1967) in non-vented Petri dishes (90 × 15 mm) at 20°C in complete darkness. In contrast to mould mites, bulb mites were easily collected using a fine tipped paintbrush because they feed on the outer edges of diet. This was found to be a successful rearing method for use of bulb mites in future trials.

Nematode inoculum

Root knot nematodes (*Meloidogyne* nr *incognita*) were maintained in a glasshouse on tomato plants (cv. 'Rutger's') in a peat potting mix: sand (50:50 by weight) growth medium. Tomato roots heavily galled by *M.* nr *incognita* were washed with tap water, cut into 2–3 cm pieces, placed into plastic trays and covered with 100 ml distilled water. Roots were crushed with a small wallpaper roller then left in the water for 2 h. The resulting suspension was sieved through nested 500 and 20 µm sieves, the first to remove the root debris and the latter to collect the nematode juveniles and eggs. The juveniles and eggs trapped on the fine sieve were thoroughly washed off with distilled water and poured over two layers of Kimwipes® tissues standing on 1 mm wire mesh in a glass Petri dish. Sufficient water was added to the Petri dish to cover the tissues. Dishes were kept overnight to separate juveniles, which moved through the tissue paper, from eggs and root debris, which did not. The juvenile extract was collected and sieved through a 20 µm sieve into a beaker with distilled water, and served as the stock solution. Ten millilitres of stock suspension was transferred into a Doncaster counting dish and groups of 20 active juveniles picked into separate 4 ml glass vials using a pipette ("Brand GMBH & Co" micro-classic pulled pipette), under a microscope at 20–40× magnification, and subsequently filled with 2 ml of distilled water. Vials were sent chilled in insulated polystyrene boxes by courier from the AgResearch Nematology Lab in Hamilton to Plant & Food Research in Auckland to arrive within 24 h. After HWT, vials containing nematodes were returned to AgResearch in Hamilton for mortality assessment.

Hot water treatment

Mites were treated in glass tubes (74 mm length × 24 mm diameter) fitted with fine mesh gauze (200 squares per inch, 0.085 mm aperture) lids at each end. Prior to fitting each lid, Parafilm® was wrapped around the thread to ensure a tight fitting seal. Mites were then weighed into each empty vial and the second lid placed on the end of the tube. Each vial represented a single replicate, with three vials being treated per temperature/time combination during the replicated trial (Table 1). Overall, between 5000 and 7000 adult mites were treated at each temperature (Table 5). The section of diet containing mite eggs was placed within a 4 ml glass vial and filled with water at ambient temperature for treatment. After HWT, each vial was carefully washed out into a funnel lined with filter paper. The filter paper containing both eggs and sections of diet, was placed within a clean Petri dish, sealed with Parafilm® and left for 7 days at 25°C before assessment. Nematodes were treated in 2 ml of distilled water within a 4 ml glass vial (15 × 45 mm).

All treatments occurred within custom made water baths (95 litres), fitted with a grid system of pipes to allow a constant flow of water around the water bath and ensure no area remained unheated. A Grundfos® circulator water pump was used to distribute the water at a rate of 3.5 m³/h, while water bath heaters (Grant Instruments, Cambridge UK) heated the water before entering the pump and being dispersed around the water bath. A data logger (Squirrel, Grant Instruments, Cambridge UK) was used to

record temperature, with probes inside both an empty mite vial and a water-filled vial similar to that in which the nematodes were treated, being placed within each water bath and remaining there throughout the treatment.

All treatment vials were weighted by attachment to a metal grid, with twist ties. This allowed for water flow, while maintaining the vials in a submerged position. After treatment, both mite and nematode vials were placed in a cooling bath (water at ambient temperature) for approximately 30 s. After draining the water, vials were placed horizontally on towels and left to dry for approximately 1 h.

After treatments were completed, nematodes were placed at 4°C to await transport back to AgResearch Hamilton for assessment. Mite vials were placed at 20°C until assessment the next day.

Using temperature and duration data gathered from preliminary research, an experiment with three replicates of each temperature × duration combination was conducted (Table 1).

Mortality assessment

Mites were assessed, using a stereo-microscope at 40× magnification, 24 h after treatment, and classified as live if there was movement when they were gently prodded or dead if there was no movement. None of the assessors made a record of finding any moribund (small twitching movement) mites after treatment. Mite eggs were assessed as either hatched or unhatched 7 days after treatment.

Table 1 Treatment durations and temperatures applied to mites and nematodes using hot water. Each treatment combination was replicated three times.

Treatment	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
Air control	2 min	3 min	7 min	9 min	12 min	
Water control (20°C)	2 min	3 min	7 min	9 min	12 min	
45°C	2 min	4 min	6 min	8 min	10 min	12 min
46°C	1 min	2 min	3 min	5 min	7 min	9 min
47°C	1 min	2 min	3 min	4 min	5 min	7 min
48°C	20 s	40 s	60 s	90 s	2 min	3 min
49°C	15 s	30 s	45 s	60 s	90 s	2 min

The nematode suspension was transferred to the centre of a Doncaster counting dish and nematodes counted under a transmitted light stereo-microscope at 20–50× magnification. In many cases not all of the 20 nematodes originally added to vials could be recovered. Nematodes were classified as live if active movement was seen, dead if the body was straight and moribund if the body was still curved, but no movement was seen. Moribund nematodes were categorised as having moderate to low risk of reinvading roots.

Statistical analyses

Separate non-parametric loess smooth curves were fitted to show the relationship between mortality and treatment time for each treatment temperature and the air control. The variability between replicates of any one treatment has been assumed to be uniform and an overall standard error calculated as the root-mean-square of the errors at each treatment time. Mortality has been plotted on the arc sine scale so that an error bar is applicable over the entire range of mortalities. The lethal treatment time to produce 99% mortality (LT_{99}) was calculated for each replicate separately from generalized linear models (GLMs), using the statistical package R (R Core Team). The GLMs used a binomial family with a complementary log log link, and assumed there was no handling mortality. The geometric mean LT_{99} for each treatment temperature and upper and lower 95% confidence limits were calculated assuming a similar variance for all treatment temperatures. Non-overlap of 95% confidence intervals is approximately equal to a test for difference at $P=0.01$.

RESULTS

Interceptions

The results of the collated interception data for organisms found on root crops imported from the Pacific Islands are presented below. Mites represented 25.93% and nematodes 22.75% of the total number of identified organisms (Table 2).

The majority of the intercepted mites belonged to the order Astigmata (Table 2), of which bulb and mould feeding mites were the most represented genera (Table 3). Of the species of mites that were identified, bulb feeding mites

from the *Rhizoglyphus* genus and mould/storage mites from the *Schwiebia* and *Tyrophagus* genera represented the majority of interceptions.

The majority of nematodes intercepted were Secernentians (Table 2), amongst which were many plant-feeding genera (Table 4). Of the intercepted nematodes identified to genus level, the majority belonged to *Aphelenchoides*, along with *Pratylenchus* and *Helicotylenchus*. Fewer specimens of *Meloidogyne*, *Paratylenchus*/*Gracilacus* or *Radopholus* were found. Of the other regulated genera and families, the *Aphelenchus* contain species that feed on fungi, including cultivated forms. The Rhabditidae are a family of bacterial feeding nematodes that includes entomopathogenic forms (*Steinernema* and *Heterorhabditis*).

Mite and nematode mortality trials

Preliminary experiments using temperatures of 40, 45, 46, 47, 48, 49, 50 and 55°C for durations between 15 s and 20 min, suggested that the optimum temperature for 99% pest mortality within 10 min lay between 45 and 50°C (data not shown).

Results from the replicated hot water treatment experiment (Figures 1 & 2; Table 5) indicated that both mites and nematodes had a similar response to HWT with mean predicted time to achieve 99% mortality (LT_{99}) between 3min4s and 3min52s at 48°C and between 1min51s and 1min52s at 49°C. The wide confidence limits showed that there was high variability in the mortality data between replicates. This may have been due to the extrapolation of 99% mortality from mortality points below 90% in some replicates, even though the subsequent exposure time point was 100% mortality.

DISCUSSION

The border interception data indicate that *Rhizoglyphus* were the most abundant intercepted mite genus on root crops imported into New Zealand from the Pacific Islands from January 2000 until April 2010. In nature, bulb mites such as *Rhizoglyphus* are generally not a primary pest, but rather a pest that takes the opportunity to enter wounds caused either by other pests or mechanically by handling (NCSU 2013). Studies

Table 2 Organisms intercepted in New Zealand from root crops imported from the Pacific Islands and sent for identification during the period January 2000 – April 2010.

Taxonomic group/organism type	Sub-totals	No.	%
Insects	1808		32.83
Undetermined Insecta		9	
Blattodea (cockroaches)		11	
Coleoptera (beetles)		421	
Dermaptera (earwigs)		24	
Diptera (flies, midges)		425	
Hemiptera (bugs)		444	
Hymenoptera (sawflies, wasps, bees, ants)		366	
Isoptera (termites)		4	
Lepidoptera (moths, butterflies)		63	
Neuroptera		3	
Orthoptera (grasshoppers, crickets, locusts)		8	
Psocoptera (booklice, barklice, barkflies)		23	
Siphonaptera (fleas)		2	
Thysanoptera (thrips)		2	
Thysanura (silverfish)		1	
Trichoptera (caddisflies)		2	
Arachnida undetermined	135		2.45
Arachnida (mites)	1428		25.93
Actinedida		18	
Mesostigmata		187	
Oribatida		48	
Sarcoptiformes		13	
Trombidiformes		1	
Astigmata		1161	
Arachnida (spiders)	19		0.35
Collembola	54		0.98
Pseudoscorpiones	1		0.02
Nematodes	1253		22.75
Secernentea		924	
Adenophorea		329	
Millipede/centipede	110		2.00
Worms	45		0.82
Snails & slugs	175		3.18
Pathogens			
Fungi	115		2.09
Bacterium	2		0.04
Other			
Fish	2		0.04
Toad	1		0.02
Bivalves	99		1.80
Crabs, krill, shrimp	22		0.40
Reptiles	6		0.11
Plant	4		0.07
Undetermined	228		4.14
TOTAL	5507		

Table 3 Mites species in the order Astigmata that were intercepted in New Zealand on imported root crops from the Pacific Islands and sent for identification from January 2000 until April 2010 and the status of each species according to the Ministry of Primary Industries Biosecurity Organisms Register for Imported Commodities (BORIC). Total number identified was 1161.

Family	Species	No.	Regulated
Acaridae	Undetermined	70	Y
	<i>Caloglyphus</i> sp.	1	Y ¹
	<i>Cosmoglyphus</i> sp.	2	— ²
	<i>Lasioseius</i> sp.	18	Y ³
	<i>Rhizoglyphus minutus</i>	717	N
	<i>Rhizoglyphus robini</i>	5	N
	<i>Rhizoglyphus setosus</i>	5	Y
	<i>Rhizoglyphus singularis</i>	7	Y
	<i>Rhizoglyphus</i> sp.	199	Y
	<i>Schwiebea</i> sp.	106	Y
	<i>Schwiebea similis</i>	6	Y
	<i>Schwiebea zingiberi</i>	5	Y
	<i>Tyrophagus javensis</i>	1	Y
	<i>Tyrophagus putrescentiae</i>	3	N
	<i>Tyrophagus</i> sp.	6	Y
Chortoglyphidae	<i>Chortoglyphus</i> sp.	1	N
Histiotomatidae	Undetermined	3	Y
	<i>Histiotoma</i> sp.	4	Y ⁴
Saproglyphidae	<i>Suidasia</i> sp.	2	Y ⁵

¹Regulated species in this genus are *Caloglyphus haripuriensis* and *C. krameri*.

²Information as to whether species in this genus are regulated or not was not able to be found.

³Regulated species in this genus are *Lasioseius anthiasae*, *L. bipinosus*, *L. furcisetus*, *L. penicilliger*, *L. queenslandicus*, *L. subterraneus* and *L. yini*.

⁴Regulated species in this genus is *Histiotoma sapromysarum*.

⁵Regulated species in this genus is *Suidasia pontifica*.

suggest that damaged bulbs are more attractive to bulb mites (Diaz et al. 2000). Once within a bulb, the mite population rapidly expands, the bulb offering both a food source and a shelter from fluctuations in environmental conditions (NCSU 2013). Minimising root crop damage is therefore potentially important in managing postharvest mite infestations. An attempt was made to establish a *Rhizoglyphus* mite colony in New Zealand using resident species, but difficulties in sourcing the correct mites and establishing a colony prevented this. Therefore, the decision was made to utilise *Tyrophagus putrescentiae* mites to test the efficacy of HWT.

This mite requires little maintenance time for rearing, it has fast population growth, and this genus is one of the top three genera found on root crops from the Pacific Islands.

It is likely that some level of drying of the outside of imported root crop material would occur during shipping transit. Therefore, any organisms that are able to tolerate desiccation are likely to be a greater risk than those that do not. The *Aphelenchoides* nematode genus had the greatest number of individuals intercepted in root crops and these are well known as being able to withstand desiccation, including on plant tissue (Hunt 1993). Some species of the

Table 4 Nematode species that were intercepted in New Zealand on imported root crops from Pacific Islands and sent for identification from January 2000 until April 2010 and the status of each species according to the Ministry of Primary Industries Biosecurity Organisms Register for Imported Commodities (BORIC). Total number identified was 1253.

Class	Order	Family	Species	No.	Regulated
Secernentea	Aphelenchida	Undetermined	Undetermined	3	Y
		Aphelenchidae	Undetermined	3	Y
			<i>Aphelenchus</i>	64	Y
		Aphelenchoididae	Undetermined	8	Y ¹
			<i>Aphelenchoides</i>	268	Y
	Diplogasterida	Diplogasteridae	Undetermined	18	N
	Rhabditida	Undetermined	Undetermined	228	Y ²
		Brevibuccidae	<i>Brevibucca</i>	22	N
		Cephalobidae	Undetermined	84	N
		Panagrolaimidae	Undetermined	35	N
		Rhabditidae	Undetermined	80	Y ³
		Teratocephalidae	Undetermined	1	N
	Tylenchida	Undetermined	Undetermined	15	Y
		Anguinidae	Undetermined	1	Y ⁴
			<i>Ditylenchus</i>	1	Y
		Hoplolaimidae	<i>Helicotylenchus</i>	19	Y
			<i>H. californicus</i>	2	Y
			<i>H. egyptiensis</i>	1	Y
			<i>H. microcephalus</i>	1	Y
			<i>H. mucronatus</i>	5	Y
			<i>H. pseudorobustus</i>	1	N
			<i>Rotylenchulus reniformis</i>	2	Y
		Meloidogynidae	<i>Meloidogyne</i>	9	Y
			<i>M. incognita</i>	1	N
		Paratylenchidae	<i>Paratylenchus</i>	1	Y
			<i>Gracilacus</i>	3	Y
		Pratylenchidae	<i>Pratylenchus</i>	10	Y
			<i>P. coffeae</i>	27	Y
			<i>Radopholus</i> sp.	1	Y
			<i>R. similis</i>	2	Y
		Tylenchidae	Undetermined	2	Y ⁵
			<i>Tylenchus</i>	6	Y
Adenophorea	Araeolaimida	Undetermined	Undetermined	3	N
		Plectidae	Undetermined	2	N
	Chromadorida	Chromadoridae	Undetermined	3	N
	Desmodorida	Cyatholaimidae	Undetermined	1	N
	Dorylaimida	Undetermined	Undetermined	293	N ⁶
		Aporcelaimidae	Undetermined	2	N

Table 4 continued

Class	Order	Family	Species	No.	Regulated
		Dorylaimidae	Undetermined	8	N
			<i>Dorylaimus</i> sp.	1	N
			<i>Eudorylaimus</i> sp.	1	N
		Mononchidae	Undetermined	2	N
			<i>Mylonchulus</i> sp.	1	N
		Thornenematidae	Undetermined	1	N
	Enoplida	Undetermined	Undetermined	3	N
		Prismatolaimidae	Undetermined	2	N
	Monhysterida	Undetermined	Undetermined	1	N
		Monhysteridae	Undetermined	5	N

¹Includes species of *Bursaphelenchus*, *Rhadinaphelenchus* and *Schistonchus*.

²Includes species of *Heterorhabditis* and *Steinernema* and many animal parasites.

³Includes species of *Caenorhabditis* and *Phasmarhabditis*.

⁴Includes *Anguina tritici* as a “Potential Vector”.

⁵Includes species of *Aglenchus*, *Boleodorus*, *Cephalenchus* and *Filenchus*.

⁶Assuming species of Longidoridae and Trichodoridae would have been identified separately and that this category includes only those nematodes generally considered to be weakly or non-plant feeding.

Pratylenchus and *Helicotylenchus* genera have also been observed to withstand desiccation in soil, including in soil on shipping containers and imported machinery (Aalders et al. 2012; McNeill et al. 2012). It is expected that some proportion of the population leaving the Pacific Islands on the outside of root crops may be able to survive shipping transit to New Zealand and this appears to be the case from the interception data. Nematodes in the *Meloidogyne* genus are not known for their ability to tolerate desiccation, but some species can survive for long periods in soil without a host (Gaur & Sehgal 1993), while others are found within root crop tissue (e.g. *M. chitwoodi* (Perry et al. 2009)) and might survive shipping transit within the plant. This genus of nematodes contains some of the world’s most plant damaging species so even small numbers of intercepts are a concern, and are the reason they were chosen as test subjects in the HWT trials.

The present results show that a 3–6 min HWT at 48–49°C killed 99% of the mites and nematodes tested in this study 95% of the time. However, both mites and nematodes were treated in absence of

plant material and a commercial HWT will most likely need to be more severe when mites and nematodes are treated ‘in situ’ on root crops. Jones & Waddell (1996) found that lethal times to kill 99% of the tydeid mite, *Orthotydeus californicus* (Koch), at 48°C increased from 0.52 min to 1.5 min to 6.2 min when testing mortality responses of this pest ‘off fruit’, adhered to apricots and naturally infested on apricots, respectively.

Previous HWT research targeting nematodes has used lower temperatures (39–43°C) for longer times 1–4 h (Southey 1993; USDA 2013). However, these long exposure times are for the control of nematodes in a completely anhydrous state present deep within bulb tissue. Also, for these bulb treatments the HWT is designed such that the bulbs are still able to germinate and grow post-treatment, presumably indicating minimal damage to the bulb tissue. Preliminary studies (D. Brash, Plant & Food Research, unpublished data) reported that taro is tolerant of HWT up to 60 min at 45°C and 50°C, and up to 30 min at 55°C. A subsequent trial (R. Masamdu, Secretariat of the Pacific Community, unpublished data)

reported a drop in quality after a 24 min exposure to HWT at 50°C. However, taro corms were still in good condition 21 days after a 12 min HWT at 50°C.

Unfortunately the number of pests treated in the present trials did not demonstrate efficacy to the extent required for an acceptable disinfestation treatment. The mite and nematode rearing and HWT methods presented here offer the opportunity to rear large numbers of target species in the Pacific Islands to generate an acceptable efficacy dataset. Additionally, using a programmable heat block system (Neven et al.

2012) may be a faster way to determine the initial relative heat tolerance of regulated species and their life stage in the Pacific Islands.

The work presented indicates that HWT may be a viable and economic disinfestation treatment to control mites and nematodes on root crops from the Pacific Islands. Future work will test the thermal tolerance of a range of regulated species and life stages of mites and nematodes in the Pacific Islands, both on and off plant material and investigate the quality of produce that have undergone HWT. Since there are difficulties in identifying nematodes and mites to the species

Figure 1 Percent mortality of adult mould mites (*Tyrophagus putrescentiae*) after hot water treatment at 45–49°C for 0–12 min. Values are the mean of three replicates. Standard error bars are presented to the right of the figure.

Figure 2 Percent mortality of *Meliodogyne nr incognita* root knot nematodes after hot water treatment at 45–49°C for 0–12 minutes. Values are the mean of three replicates. Standard error bars are presented to the right of the figure.

Table 5 The mean estimated time to reach mortality of 99% (LT₉₉, min and/or s) of mould mites or root knot nematodes, or the percentage of pests killed (where mortality did not exceed 60%). LT₉₉ estimate extrapolated by points below 80% mortality.

Treatment	Mean	SEM	95% confidence limit		n
			Lower	Upper	
Mites					
Air control	0.3–20.5%				6015
Water control	1.0–74%				6025
45°C	13min58s ¹	33 s	7min30s	25min57s	1979
46°C	8min47s	35 s	5min6s	15min6s	5923
47°C	6min14s	38 s	3min37s	10min43s	5976
48°C	3min4s	20 s	1min47s	5min17s	7516
49°C	1min52s	1 s	1min5s	3min12s	7153
Nematodes					
Air control	0–31.5%				277
Water control	0–25.0%				247
45°C	9min24s	2min8s	4min56s	14min25s	295
46°C	6min48s	6 s	4min18s	10min45s	336
47°C	5min12s ¹	2min48s	2min58s	9min8s	212
48°C	3min52s	24 s	2min27s	6min7s	345
49°C	1min51s	5 s	1min10s	2min56s	332

¹Mean LT estimate calculated from two replicates.

level (particularly immature states), it would be prudent to test the tolerance of both regulated and non-regulated species to HWT.

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