Hort Innovation

Final Report

Treatment for Mites on Lychee Fruit Prior to Irradiation for Improved Market Access

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Project code:

LY16002

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Treatment for Mites on Lychee Fruit Prior to Irradiation for Improved Market Access – LY16002

Disclaimer:

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Content

Summary

The project aimed to identify a simple postharvest treatment to ensure fruit destined for quarantine export markets (the USA and New Zealand) are free of non-target quarantine pests that are unknown to be neutralized by the irradiation protocol (400 Gy) determined for target quarantine pests.

The target for the project work is lychee growers and associated industry members who wish to export or are exporting to quarantine markets.

The key activities undertaken included evaluation of a postharvest dip and or flood spray of paraffinic oil (Prospect®) for its efficacy to reduce fruit surface dwelling micro insect pests and mites. The oil flood sprays were also trialled in combination with pre and post high pressure/volume water sprays to physically clean fruit of surface pests prior to oil treatment and/or post oil treatment. The effect of the oil in combination with irradiation on fruit quality was also evaluated.

The quarantine gas Vapormate® was also assessed in an exploratory trial.

Key outputs included:

- two interim project reports in the lychee grower magazine 'Living Lychee', published by Fruit Tree Media
- presentations of project findings at HIA Lychee SIAP meetings
- a presentation to growers at a Lychee industry AGM in October 2017
- a prototype in-line roller spray unit, which included a flood-spray unit for application of oil and high pressure/volume water sprays for pre cleaning or post oil application cleaning. This concept, developed in the experimental prototype, was further developed for a commercial trial.

Project trials have identified that Prospect® oil at 3% concentration applied as a 30 second dip or flood spray does reduce the incidence of surface insects and mites on lychee fruit. However, 100% elimination of pests has not been achievable. The use of oil dips potentially exacerbates the development of surface fungi (rots), predominately around the stalk end of fruit, from about 10 days after treatment. Using a high volume flood spray to apply the oil appears to eliminate the fungal problem associated with dipping fruit. Using a systems approach, which included fruit washing (moderate pressure/high volume) before and/or two minutes after the application of oil, can further reduce the incidence of surface pests. A trial using a prototype in-line water spray, oil flood spray and post oil water sprays significantly reduced the count of live mites relative to a standard packing system. However, the two minute time lag required for the oil to be efficacious prior to a water spray to remove dead or dying pests impacted negatively on packing rates.

The industry have expressed strong interest and support for the project, with one grower using the oil dip method to support a successful export shipment during the 2016/17 season. Further work to explore the efficacy of high pressure and high volume water sprays without the use of oil to reduce surface pest loads was requested by industry representatives at the May 2018 SIAP meeting.

Keywords

Lychee; mites; insects; micro surface pests; paraffinic oil; water spray; postharvest; disinfestation; irradiation; export fruit

Introduction

Australian lychee (*Litchi chinensis*) was granted market access to the USA in 2013 under a trial protocol that included irradiation as the disinfestation treatment. The first shipment occurred in the 2016/17 season. The industry, ready to take up the challenge, is faced with the issue of non-target quarantine pests; that is, those pests that are **not known** to be effectively treated ("neutralised") by the approved irradiation dose. The presence of any non-target quarantine pest in the pre-treatment inspection cause rejection of the consignment unless identified as a non-quarantine pest. The presence of non-target quarantine pests can: delay shipments until successful identification as a non-quarantine pest; or require fruit to be methyl bromide treated prior to irradiation; or cause fruit to be diverted back onto the domestic market. In the case of export to the quarantine port of New Zealand, the identification of non-target pests can result in methyl bromide treatment post entry.

Mites of various genera can be found on lychee foliage and fruit in Australian and orchards worldwide. In Australia pest mite species include erinose mite (*Aceria litchii*) and tea red spider mite (*Oligonychus coffeae*). The other mite species are harmless and are either predators, fungivores or detrivores. The miticides Vertimec®and Vantal®containing the active ingredient abamectin are permitted for mite control in lychee orchards up to 31 July 2019 under Permit 80539. Abamectin is currently registered in the USA for use on lychee with a MRL of 0.01 ppm. Wettable sulphur (800 g/kg Sulphur), which has a 1 day with holding period, is permitted (PER 14508) for erinose mite and white louse scale (*Unaspis citri*) control until 30 November 2021. Despite appropriate field control measures live mites can make their way onto fruit at low numbers. Live mites have been found on irradiated fruit being exported to New Zealand. Hence, this is an important problem to solve during the pilot stage of fruit exports to the USA.

The irradiation treatment (400 Gy minimum), used as part of the export protocol for control of insect pests, is considered not to be sufficient to kill small arachnid pests. Although it is likely they are sterile following irradiation at 400 Gy, they are still alive and hence considered a quarantine hazard. Higher irradiation levels required to kill mites could potentially lead to irradiation levels in excess of 1000 Gy, which is currently not sanctioned under Australian fruit irradiation protocols.

Alternative postharvest treatments for mites include smothering through the use of paraffinic oils or fumigation. Oil based insecticide products are extensively used in the field and postharvest. The mode of action is based on the capacity of the oil to block the ventricles ("breathing tubes") of the pest and hence act as a smothering agent. The chemistry of the oil determines how effectively it can penetrate the insect and ventricles. Caltex have developed a low viscosity food grade paraffinic oil for use as a postharvest dip in citrus for the control of surface pests (Traverner, 2014). Research carried out on rambutan (Leach *et al.*, 2004) showed that a postharvest treatment of a light paraffinic oil ($_{n}$C13) controlled Citrus mealybug (*Planococcus citri* Risso) following a 30 second dip in fresh 3% solution of CPH (a precursor of Prospect® postharvest oil) with no adverse effects on fruit quality. The use of a postharvest oil based dip for the control of surface dwelling mites and insects in lychee merits investigation. Furthermore, the dip can easily be incorporated into current grading and packing lines.

Lychee exports to New Zealand irradiated at 400 Gy have been intercepted by NZ Quarantine following the identification of the following surface dwelling mites and insects.

Mites; Superorder Acarifomes	Insects
– Family Tydeidae	– Family Coccidae (Scale)
– Order Oribatida	– Family Staphylinidae (Rove beetle)
– Family Stigmaeidae	– Family Phlaeothripidae (Trip)
– Family Bdellidae (Snout mites)	

The industry would benefit if in-shed pre-packing postharvest treatments could be used to reliably disinfest fruit of mites and micro insect pests prior to irradiation and export.

The project examined the efficacy of Prospect® postharvest oil applied as a dip and flood spray on the decontamination of surface pests. The fruit quality of oil treated and control fruit in combination with irradiation was assessed to determine if there was an adverse effect of irradiation on oil treated fruit. In the second season, a systems approach was utilized which examined the effect of pre-washing fruit, oil applied as a flood spray, and a post oil application wash. These treatments were examined alone or in various combinations. The addition of food acid additives was also examined.

The bulk of the work was carried out on the main export variety Kwai Mai Pink (B3).

Methodology

Trial work was conducted over two seasons, 2016/17 and 2017/18. During the 2016/17 season trials investigated the efficacy of postharvest paraffinic oil (Prospect®) dip treatments (rates, dip time, varietal response) on mite survival and the effect of the oil treatment in combination with irradiation on fruit quality post treatment.

2016/17 Season

Trial 1

- Treatment Date: 21 December 2016 (oil); 28 December 2016 (irradiation)
- Lychee variety: Kwai Mai Pink (B3)

Twenty-two cartons (5 kg) of lychee were randomly selected immediately after packing. Twenty cartons were selected for irradiation treatments at 0, 400, 600, 800 and 1000 Gy. Ten of the twenty cartons were dipped in manually agitated 3% Prospect® oil for 30 seconds, drained for five minutes and repacked into USA protocol bags and cartons. Fruit in the remaining 10 cartons were also transferred to USA protocol bags and cartons. The cartons were randomly assigned to differing combinations of irradiation treatments for treatment at the Steritech Irradiation facility in Narangba, south-east Queensland. Treatment cartons were palletised with a standard commercial shipment in the cold room (5°C) and consigned by refrigerated transport the next day to a Brisbane market agent for redistribution to Steritech for irradiation treatment.

Plate 1. (***From left to right***): ***Top row*** **- 3% Prospect® oil dip; draining lychees following dipping;** ***Bottom row*** **- fruit repacked in the cold room with commercial fruit; and oil treated fruit.**

Of the remaining two cartons, one was Prospect® oil dipped and the other remained untreated. Cartons were transferred to the Department of Agriculture and Fisheries (DAF) disinfestation laboratory in Redden Street, Cairns and counts of live, moribund and dead mites were conducted and recorded within 24 hours of treatment.

Treated fruit arrived at the Brisbane Markets on 23 December 2016 and were kept in cold storage over the Christmas holiday period. Fruit were transferred to Steritech on 27 December and stored and irradiated at the five pre-designated rates on 28 December. Fruit were stored at 5°C and visually rated by a commercial lychee grower on 30 December (nine days after oil treatment) and again on 7 January 2017, 17 days after oil treatment.

The remainder of the irradiated fruit were airfreighted to the Cairns DAF disinfestation laboratory on 4 January (14 days after treatment (DAT)) and evaluated for colour using a NIX Pro colour sensor to measure L*a*b*. Five fruit of each treatment were scanned using the NIX Pro and LAB measurements analysed.

Fruit Rating

Fruit ratings conducted at Steritech were subjective and supported with a photograph of representative fruit for each oil × irradiation treatment combination. The idea was to quickly assess whether the oil and in particular the oil plus irradiation treatments adversely affected fruit quality prior to further work being conducted.

Plate 2. Fruit treated with Prospect® oil (3%) and irradiated at 0, 400, 600, 800 and 1000 Gy on (top) 30 December 2016 (9 days after oil dipping) and (lower) 7 January 2017 (17 days after oil dipping).

The subjective analysis suggested that there were no obvious deleterious effects associated with oil dipping and irradiation treatments up to 1000 Gy. This was reassuring as it suggested that oil treatments with or without additional irradiation did not adversely affect fruit quality within the evaluation period after oil dipping and irradiation.

Nix Pro LAB colour measurements were conducted on 6 January, 16 days after oil application and 16 days after irradiation. The colour (L, a, b) was assessed on 40 fruit that were treated with oil 3% and 40 untreated fruit at each irradiation rate. A summary of the mean and standard error (se) for each treatment combination is presented in Table 1.

Table 1. Fruit colour (L, a, b) mean and associated standard errors for fruit treated without or with Prospect® oil and at five irradiation levels (0, 400, 600, 800 and 1000 Gy).

Rate	Treatment	L		a		b	
		mean	se	mean	se	mean	se
0	no oil	47.48	2.080	32.12	1.357	28.07	1.266
	oil	40.29	1.064	22.80	1.059	21.54	1.272
400	no oil	47.78	0.850	31.29	0.706	27.05	0.507
	oil	40.67	1.919	23.10	1.295	22.40	1.783
600	no oil	48.27	1.012	31.22	1.266	27.77	0.599
	oil	41.06	2.009	24.95	1.534	22.86	1.367
800	no oil	49.70	2.305	26.22	1.525	27.71	1.615
	oil	43.12	2.290	21.40	1.547	24.79	1.953
1000	no oil	50.81	0.960	32.02	1.051	28.82	0.744
	oil	38.19	1.441	23.01	1.732	21.48	1.985

The 95% confidence intervals were also calculated for L, a, b for each treatment group. Figure 1 shows the mean and the 95% confidence limits. There is a clear tendency for the fruit treated with oil to have lower mean L, a, b. There is nothing to suggest that irradiation has had an effect on the control fruit or on the oil treated fruit. Where the 95% confidence intervals do not overlap suggests there is evidence of a difference between the two samples. However, it is not true at the 0.05 level that non-overlapping 95% intervals suggest there is no difference. Using the 95% confidence intervals to determine significant differences at the 0.05 level is too conservative.

A principal components analysis (PCA) was also conducted using the L, a and b values and was based on the correlation matrix. The first two PC explained 95.9% of the variability. Figure 2 shows the first two PC and the five colours represent the irradiation rate, while the closed symbols represent the control group and the open symbols the fruit treated with oil 3%. There is a strong grouping of the individual fruit based on the oil treatment, as can be seen in the graph with the majority of open symbols being on the left and closed symbols on the right. There is no obvious grouping of the individual fruit based on irradiation rate. If there were a grouping by irradiation rate the different coloured symbols would form separate groups. All three colour measurements tend to be higher for the oil treatments, as seen by the lines pointing more towards the right of the plot where the points are predominately control samples. This is in agreement with the individual bar charts.

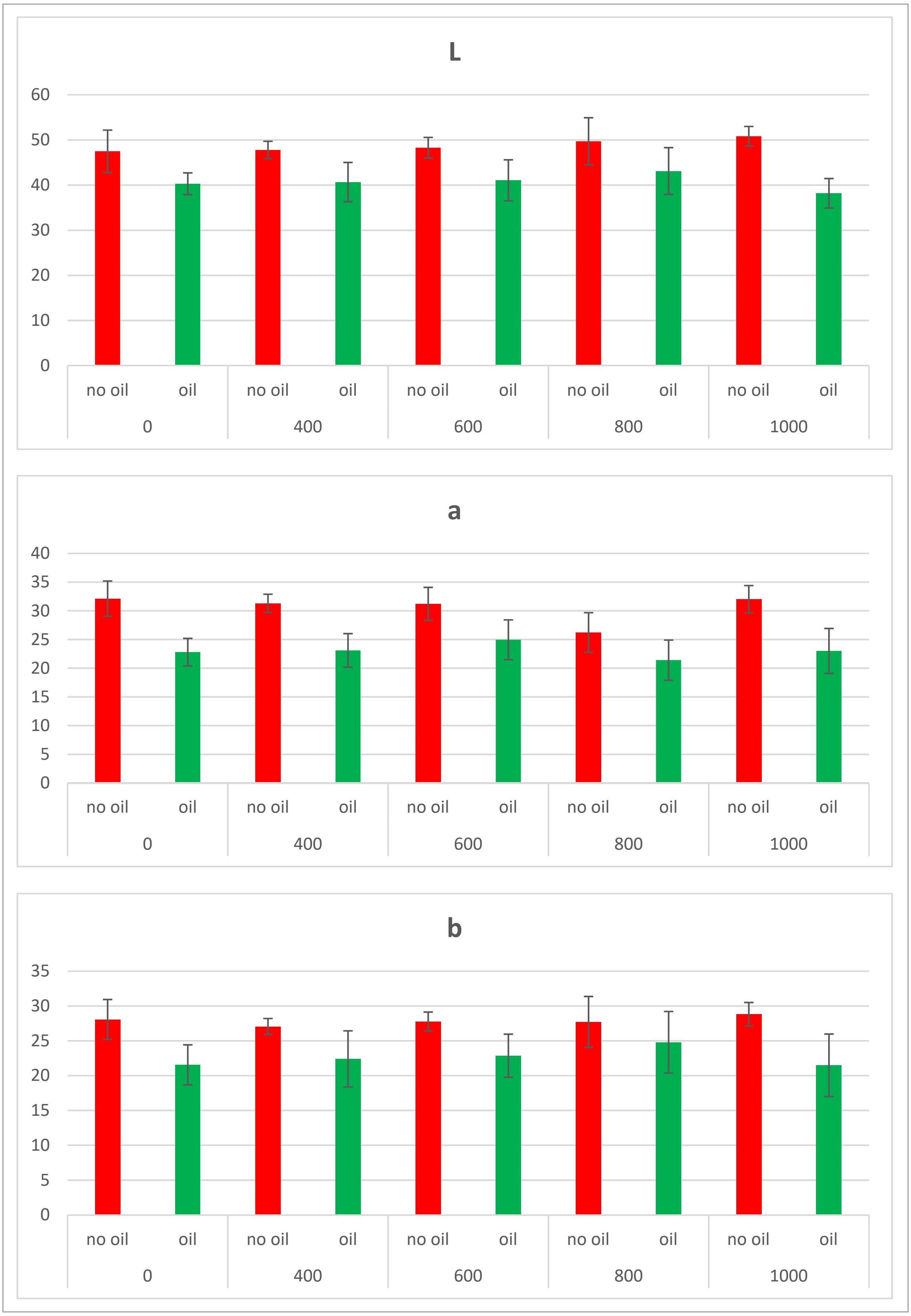

Figure 1. **Mean fruit lightness (L); Mean fruit green-red colour (a); Mean fruit blue-yellow colour (b) and 95% confidence interval for fruit treated without or with Prospect® oil (3%) and at five irradiation levels (0, 400, 600, 800 and 1000 Gy). Note, the higher the "L" value the lighter the fruit; the higher the "a" value the redder the fruit; the higher the "b" value the yellower the fruit.**

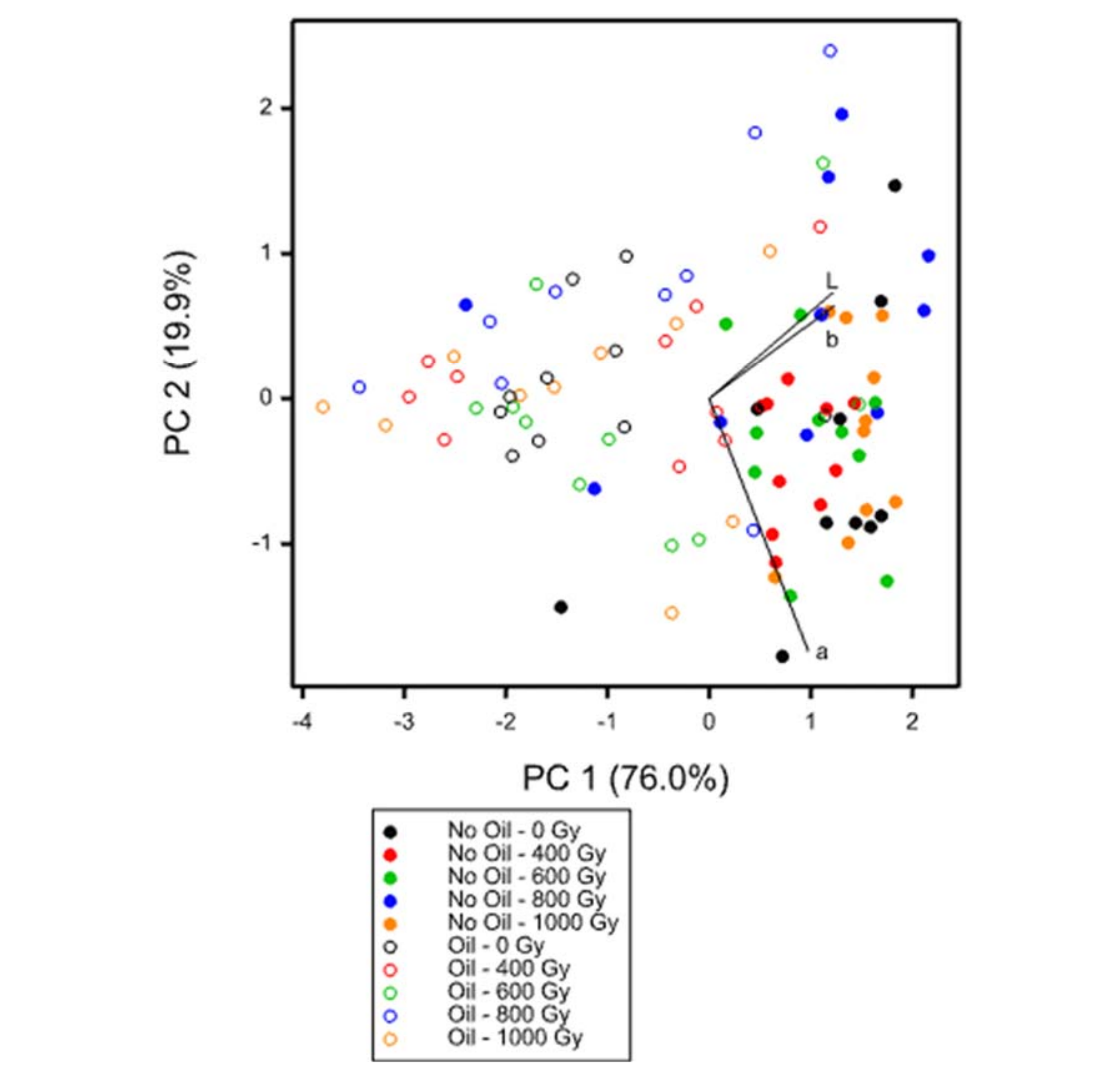

Figure 2. **Principal components analysis (PCA) conducted using the L, a and b values based on the correlation matrix.**

Plate 3. **Fruit at 16 days after oil treatment (*from left to right*): Control (-ve oil, 0 Gy), (-ve oil, 1000 Gy), and (oil, 1000 Gy).**

Total mite counts on 40 inspected fruit on control or oil treated fruit are shown in Table 2.

Table 2. Mite count (dead, moribund or live) 24 hours after oil or control treatment.

Treatment	Mite Status		
	Dead	Moribund	Live
Control	0	0	17
Oil 3%	10	4	1

A Chi-squared (χ^2) test using a random permutation test was conducted and suggested there was a significant correlation between the treatment and the mite status ($p<0.001$). The permutation test was used instead of the standard χ^2 test due to the small counts in many of the cells in the table. The test was conducted based on 4999 random permutations. Given the significance of the test and the counts in the table, it is evident that there are likely to be significantly more live mites in the control compared to the oil treatment, and more dead mites in the oil treatment.

All mites counted on the control fruit were considered alive, while 10 of the total of 15 mites on the oil treated fruit where dead with a further 4 mites in a moribund (near death) state. Oil treatment did reduce the total mite count but not at the 100% level.

Trial 2

- Treatment Date: 12 January 2017

Fruit were picked on 11 January 2017 from three sections of an orchard on the Tablelands of two lychee varieties (Kwai Mai Pink and Sarkan) after the commercial harvest was completed. Fruit were transported on the stalk in clean boxes back to the laboratory at the Centre for wet Tropics Agriculture, DAF, South Johnstone.

On 12 January fruit were destalked, sorted and prepared for treatment. Treatments included:

- Variety: Kwai Mai Pink and Sarkan
- Prospect® oil concentration: 0% (water control), 1%, 3% and 5%
- Replicates: Three
- Sub-samples: Ten fruit per replicate
- Colour measurements: 12 January 2017 (0 DAT) and 27 January 2017 (15 DAT)

The trial was conducted to evaluate the effect of Prospect® oil concentration on fruit quality as assessed by colour. Ten fruit for each replicate were dipped in the appropriate oil concentration for 30 seconds and placed in a rectangular, disposable plastic container and allowed to air dry under an air extraction hood for approximately five minutes prior to sealing with the lid. Colour measurements using the Nix Pro colour sensor were carried out on each sub-sample fruit on the day of treatment and 15 days later on 27 January 2017.

Each fruit was assessed for colour (L, a, b). Fruit were assessed on two occasions. The mean colour measurement for each sample of ten fruit at each assessment was calculated and an analysis of variance (ANOVA) conducted. The position within the orchard was fitted as a random term while the main effects and interaction of oil treatment and assessment date were fitted as fixed effects.

Results for B3 (Table 3) suggest there was a significant interaction between the oil treatment and the two assessment dates for all colour measurements ($p<0.001$). Pairwise comparisons of the means in the interaction table found no significant difference between the treatments for L, a, b at the first sampling date. On the second sampling date the control fruit had significantly higher mean L, a, b compared to the oil treatments. The mean L, a, b decreased significantly over time for the oil treatments, but only the mean L decreased significantly for the control fruit.

For Sarkan (Table 4) a significant interaction of oil treatment and assessment date was only found for colour measurement L ($p<0.001$). At the first assessment 3% oil had a significantly higher mean L, while at the second assessment the control was significantly higher. For all treatments there was a significant reduction in mean L over time.

There was no significant effect of oil or assessment time on colour measurement a, but both main effects of oil treatment and assessment date were significant for measurement b. The mean b decreased significantly over time, and 3% oil had a significantly higher mean b than both the control and 1% treatments (Table 5).

Table 3. Mean L, a, b for Kwai Mai Pink (b3) Fruit.

L - lightness	1%	3%	5%	Control
Oil	44.31 a	44.16 a	43.82 a	49.99 b
Date	12 Jan 17	27 Jan 17		
	53.57 b	37.56 a		
Oil/Date	12 Jan 17	27 Jan 17		
1%	53.76 c	34.85 a		
3%	54.06 c	34.25 a		
5%	53.49 c	34.16 a		
Control	52.99 c	47.00 b		
a – green red	**1%**	**3%**	**5%**	**Control**
Oil	25.27 a	24.32 a	25.43a	30.61 b
Date	12 Jan 17	27 Jan 17		
	30.99 b	21.82 a		
Oil/Date	12 Jan 17	27 Jan 17		
1%	30.86 bc	19.68 a		
3%	30.03 bc	18.60 a		
5%	30.99 bc	19.87 a		
Control	32.07 c	29.14 b		
b – blue-yellow	**1%**	**3%**	**5%**	**Control**
Oil	20.39 a	20.03a	20.37 a	25.19 b
Date	12 Jan 17	27 Jan 17		
	24.63 b	18.36 a		
Oil/Date	12 Jan 17	27 Jan 17		
1%	24.35 b	16.42 a		
3%	25.05 b	15.02 a		
5%	24.61 b	16.14 a		
Control	24.50 b	25.87 b		

Table 4. Mean L, a and b for Sarkan.

L - lightness	1%	3%	5%	Control
Oil	40.02 a	41.05 a	40.22 a	42.29 b
Date	12 Jan 17	27 Jan 17		
	44.50 b	37.29 a		
Oil/Date	12 Jan 17	27 Jan 17		
1%	44.18 c	35.86 a		
3%	46.07 d	36.04 a		
5%	44.39 c	36.05 a		
Control	43.37 c	41.21 b		
a – green red	**1%**	**3%**	**5%**	**Control**
Oil	32.65	33.50	33.98	32.36
Date	12 Jan 17	27 Jan 17		
	33.78	32.47		
Oil/Date	12 Jan 17	27 Jan 17		
1%	33.55	31.75		
3%	33.66	33.34		
5%	33.68	34.29		
Control	34.22	30.51		
b – blue-yellow	**1%**	**3%**	**5%**	**Control**
Oil	16.36 a	17.87 b	17.26 ab	16.80 a
Date	12 Jan 17	27 Jan 17		
	17.55 b	16.59 a		
Oil/Date	12 Jan 17	27 Jan 17		
1%	16.79	15.93		
3%	19.02	16.71		
5%	17.87	16.66		
Control	16.55	17.05		

The p-value, standard error (se) of the mean and 95% least significant difference (LSD) are presented in Tables 5. The means are presented below the table. Where a significant effect was found, the 95% LSD is used to make pairwise comparisons. All testing was performed at the 0.05 level.

Table 5. Associated p-value, standard error (se) and 95% least significant difference (LSD) for Tables 3 and 4.

B3		Oil	Date	Oil.Date
Colour L	p-value	<0.001	<0.001	<0.001
	se	0.491	0.347	0.694
	95% LSD	1.488	1.052	2.104
Colour a	p-value	<0.001	<0.001	<0.001
	se	0.544	0.385	0.770
	95% LSD	1.651	1.168	2.335
Colour b	p-value	<0.001	<0.001	<0.001
	se	0.582	0.412	0.823
	95% LSD	1.766	1.249	2.498
Sarkan		**Oil**	**Date**	**Oil.Date**
Colour L	p-value	0.003	<0.001	<0.001
	se	0.376	0.266	0.531
	95% LSD	1.139	0.806	1.611
Colour a	p-value	0.644	0.210	0.464
	se	0.992	0.701	1.403
	95% LSD	3.009	2.127	4.255
Colour b	p-value	0.037	0.012	0.068
	se	0.335	0.237	0.474
	95% LSD	1.016	0.718	1.437

The varieties Kwai Mai Pink (KMP) and Sarkan are different in appearance as fresh fruit. Sarkan has a redder skin colour than KMP. Due to only one block of each variety being sampled, there is no replication of variety and hence no significance testing can or needs to be undertaken to compare varieties.

In summary, the percentage of oil used for the dip had little effect on fruit colour of either variety. Not unexpectedly, fruit darken over time. Plate 4 includes all treatment at 15 days after Prospect® oil dipping. Browning is evident in all treatments as expected, but little discernible colour differentiation between oil concentrations within each variety.

Plate 4. KMP and Sarkan fruit colour & quality following Prospect® oil (3%) treatment at 15 days after treatment.

Trial 3

Trial 3 was designed to examine the effects of Prospect® oil (3%) dip treatment and irradiation (0 and 400 Gy) on mite disinfestation and fruit quality. The trial was initially destined to be part of a commercial export to the USA from a protocol registered farm. However, a breakdown in the farms major cold room instigated the design of a smaller trial. Fruit (cv. KMP) sourced from five parts of the farm were picked, destalked and packed using commercial practices. Twenty cartons, each comprising 5 kg of fresh fruit were selected and 10 cartons dipped using a slotted crate in a manually agitated 3% solution of Prospect® oil for 30 seconds. The treated crates of fruit were removed from the dip and allowed to free drain for 60 seconds back into the tank and then a further five minutes on pallets located next to the dip tank prior to repacking in export protocol bags and cartons (Plate 5). Non-oil treated fruit were packed directly into the appropriate bags and cartons. All cartons were then stored in a cold room at 5°C for 8 hours prior to dispatch overnight in refrigerated (14°C) transport to Steritech irradiation facility in Narangba, south-east Queensland.

- Treatment Date: 17 January 2017
- Treatments:
    - o Oil: Control (no dip), 3% Prospect® oil dip
    - o Irradiation: Control (0 Gy), 400 Gy

The cartons labelled for irradiation (400 Gy) were treated on 18 January. Because the treatments were not part of a standard pallet, the 10 cartons were loaded with irradiation dosimeters and the treatment range recorded was 482 to 607 Gy with an average of 571 Gy for all cartons. This is within the range as expected in an irradiation treatment requiring a minimum of 400 Gy. Because all irradiated fruit (400 Gy) were treated at the same time fruit are considered sub-samples as there is no independent replicates of the irradiation treatment.

All fruit was then stored in a cold room running at approximately 5°C. On 19 January every carton was opened, fruit inspected and four subsample of 12 fruit each were repacked in disposal lidded plastic containers for express shipment of two containers to two locations. Lot 1 was dispatched to DAF Applethorpe facility for mite and insect counts and Lot 2 was dispatched to the DAF Redden Street facility in Cairns for fruit colour observations.

Plate 5 Example of fruit post oil and irradiation treatments, packed in USA protocol bags and cartons.

Mite and insect counts

A total of 120 fruit were inspected for every combination of oil (Control, 3%) and irradiation (0, 400 Gy) treatment. The total mite count for each treatment combination is shown in Table 6. No tests of association between mite status and treatments were conducted due to the large proportion of zeroes in the table. The distribution of mites is random and certainly not due to any treatment effects.

Table 6. Total dead, moribund and live mites recorded on 120 fruit representing each treatment combination.

Rate	Treatment	Mite Status		
		Dead	Moribund	Live
0	Dip	0	0	0
	No dip	1	0	0
400 Gy	Dip	0	0	0
	No dip	0	1	6

Scale Counts

The number of scale insects found on samples of 120 fruit in each of the four treatment combinations, oil (Control, 3%) and irradiation (0, 400 Gy), is shown in Table 7. Fishers' exact test found no significant association between the dip treatment and the irradiation rate on the number of scale insects found (p=0.188).

Table 7. Scale count on 120 fruit representing each treatment combination.

Treatment	0 Gy	400 Gy
Dip	1	0
No dip	2	13

Colour

The colour (L, a, b) was assessed on 120 fruit in each of the four treatment groups on 24 January 2017, 7 days after oil treatment and 6 days after irradiation. A summary of the mean and standard error (se) are presented in Table 8.

Table 8. A summary of the mean L, a, b values and standard error (se).

Rate	Treatment	L		a		b	
		mean	se	mean	se	mean	se
0	Dip	43.96	0.541	29.99	0.609	24.94	0.440
	No dip	42.68	0.457	28.84	0.535	24.00	0.460
400	Dip	47.83	0.373	31.19	0.398	26.59	0.278
	No dip	48.89	0.309	32.22	0.379	27.12	0.252

The 95% confidence intervals were also calculated for L, a, b for each treatment group. Figure 3 shows the mean and the 95% confidence limits. There is a clear tendency for the irradiated fruit to have higher mean L, a, b. The mean difference between the non-irradiated and irradiated fruit appears to be larger for fruit that were not dipped. Where the 95% confidence intervals do not overlap suggests there is evidence of a difference between the two samples. However, it is not true at the 0.05 level, that non-overlapping 95% intervals suggest there is no difference. Using the 95% confidence intervals to determine significant differences at the 0.05 level is too conservative.

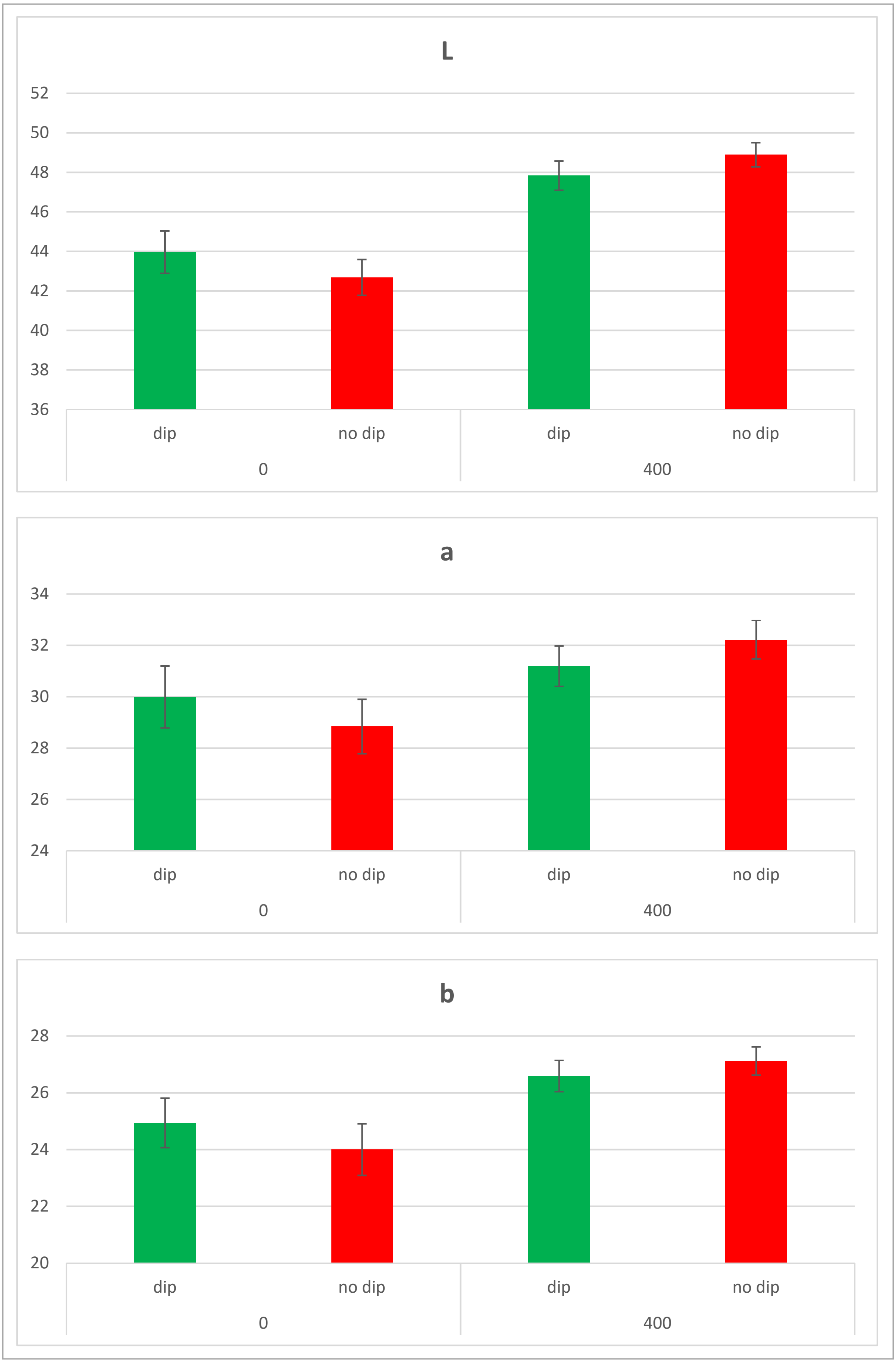

Figure 3. **Mean and the 95% confidence limits for treatment combinations; oil (Control, 3% dip) and irradiation (0, 400 Gy).**

A principal components analysis (PCA) was also conducted using the L, a b values and based on the correlation matrix. The first two PC explained 92.7% of the variability. Figure 4 shows the first two PC. The two colours represent the irradiation rate, while the open symbols represent the fruit that was not dipped and the closed symbols the dipped fruit. There is no obvious grouping of the individual fruit based on either irradiation rate or dipping treatment.

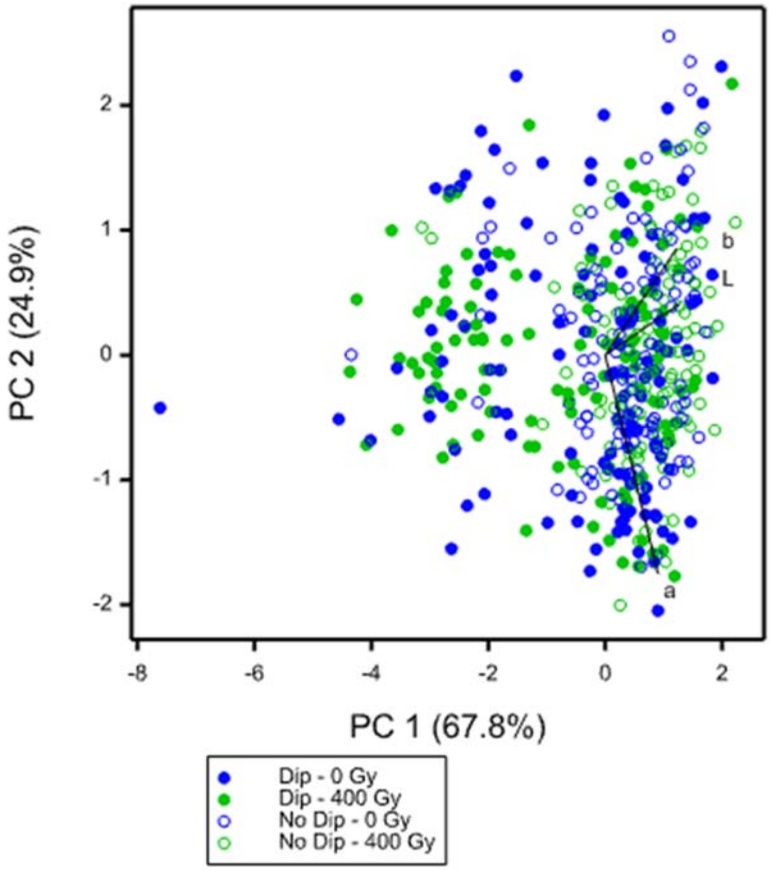

Figure 4. A principal components analysis (PCA) using the L, a b values and based on the correlation matrix.

Oil and irradiation treatments had little effect on fruit colour as recorded by Commission Internationale de l'Eclairage colour space measurement using a portable NIX Colour Pro. Fruit colour data and supporting images (Plate 6) show that up to 10 days after oil treatment (DAT) and 9 days after irradiation there was no treatment effect on fruit appearance. By 15 DAT oil dipped fruit, regardless of irradiation treatment, had an increased incidence of fungal bloom at the stem end. Irradiation treatment did not negatively impact fruit colour.

B3_S1R1_10 DAT – 27 Jan 17

B3_S1R1_15 DAT – 1 Feb 17

Plate 6. KMP fruit; oil dipped (Control, 3% dip) and irradiated (0, 400 Gy) at 10 and 15 DAT.

Trial 4

A series of small non-replicated trials were conducted to examine the effect of oil treatment on mite and insect control as well as the influence of lychee variety and oil dip temperature.

Trial 4a

- Variety: Waichee
- Treatment: Control (water dip 30 sec); Oil dip (3% for 30 sec)
- Replicates: Two
- Sub-sample: 14 fruit
- Treatment Date: 18 January 2017
- Evaluation date: pest count – 24 January 2017; fruit colour – 1 February 2017

The incidence of mites and scales was least in oil treated fruit where only four dead scale were found on the oil treated fruit in replicate one. In non-treated fruit three mites (live) and 21 scale were found in Rep 1, while a further five scale were identified in Rep 2 fruit (Table 9).

Table 9. Mite and insect counts on lychee variety Wai Chee, six days after treatment.

Treatment	Rep	Mite Count	Eggs	Scale
Oil	R1	0	0	4 (dead)
Oil	R2	0	0	0
Control	R1	3 (live)	0	21 (live)
Control	R2	0	0	5 (live)

Waichee fruit colour was not objectively measured following the application of treatments. However, the Plate 7 shows that in oil treated fruit redness appeared to be enhanced, although fruit were slightly darker than their untreated counterparts. By 14 DAT the incidence of a fungal bloom was starting to be apparent in oil dipped fruit (Plate 8).

Control and treated fruit 9 DAT – 27 Jan 17

Control and treated fruit 14 DAT – 1 Feb 17

Plate 7. Control and 3% Prospect® oil dipped fruit, Wai Chee at 9 and 17 DAT.

Control (left) and treated (right) fruit at 14 DAT

Plate 8. Early signs of fungal bloom development on oil dipped fruit.

Trial 4b

- Variety: Kwai Mai Pink (B3)
- Orchard location: Sunshine coast hinterland, south-east Queensland
- Treatment: Control (ambient water dip 30 seconds); Oil dip (3% for 30 seconds at 2°C, 4°C and 10°C for 300 seconds dip temperature)
- Replicates: One
- Sub-sample: 12-14 fruit
- Treatment Date: 19 January 2017
- Evaluation date: Mite count – 24 January 2017; fruit colour (photograph) – 1 February 2017 (13 DAT)

A number of industry members use hydro-cooling (chilled water flood sprays or baths) to rapidly chill field picked fruit prior to grading and packaging. An ambient temperature oil dip post hydro-cooling would be counterproductive to management of the cold chain. Hence the question of oil temperature and its effect on oil miscibility and subsequent effect on fruit colour/quality was raised.

Twelve fresh field picked and destalked fruit were randomly assigned to four trays. Each tray of fruit was exposed to a different treatment: control (ambient water dip 30 seconds); oil dip (3% Prospect® oil for 30 seconds at 2°C, 4°C and 10°C for 300 seconds). After removal from the dip fruit were allowed to drain for several minutes before being packed into lidded plastic containers for cool transport back to the laboratory. Fruit were stored at 5°C and photographed 13 days after treatment.

Plate 9. **KMP fruit treated at various temperatures (*from left to right*): Control (ambient water dip 30 seconds); Oil dip (3% Prospect® oil for 30 seconds at 2°C, 4°C and 10°C dip temperature) 13 DAT.**

Dip temperatures had no apparent effect on the miscibility of the oil. At 13 days post treatment fruit under all treatments appeared to have maintained their fresh colour with very little evidence of browning. There were signs that oil treated fruit, regardless of dip temperature, were beginning to show early signs of developing a fungal bloom at the stem end.

A mite count conducted five days after fruit were dipped (Table 10) indicated that live mites were only found in the control fruit. Less than half of the control fruit were mite free, whereas 71 to 95% of treated fruit were mite free. In the remaining treated fruit where mites were detected they were either recorded as dead or moribund (mites not free moving). Other surface pests recorded included one mealy bug in the control fruit batch and one egg (unidentified) in the oil treatment (10°C for 300 seconds).

Table 10. **Fruit mite and insect counts for KMP fruit control and oil dipped at a range of temperatures.**

Treatment	Fruit # nil mites	Alive Mites	Dead Mites	Moribund mites	Other pests	Total mite count	Total Fruit count
Control	5 (41.7%)	6	0	0	1 (mealy bug)	6	12
1°C water (3% oil – 30 seconds)	12 (92.3%)	0	1	0	0	1	13
5°C water (3% oil – 30 seconds)	9 (75%)	0	3	1	0	4	12
10°C water (3% oil– 5 minutes)	10 (71.4%)	0	2	1	1 (egg?)	3	14

In summary, treatments had no apparent effect on fruit quality. Oil treatments did not completely rid fruit of mites or insect pests but reduced the incidence of live mites. The presence of dead or moribund mites on oil treated fruit still raises concerns from a quarantine inspection perspective as the presence of any surface pests is likely to trigger rejection or delays depending on pest identification.

Trial 4c

- Variety: Kwai Mai Pink (B3)
- Orchard location: Sunshine coast hinterland, south-east Queensland
- Treatment: Control (30 second water dip at ambient temperature); oil dip (3% for 30 seconds at ambient temperature)
- Replicates: One
- Sub-sample: Half a carton - approximately 120 fruit
- Treatment Date: 19 January 2017
- Evaluation date: Mite counts (20 January 2017 and 27 January 2017)

The primary purpose of the trial was to record the efficacy of the oil dip on surface pest disinfestation. A 5 kg carton of freshly picked and packed grade 2 Kwai Mai Pink was obtained from a grower in south-east Queensland. Fruit were divided into two groups. One lot of fruit was subjected to a 30 second dip in ambient water, drained for 5 minutes and repacked in a labelled polyethylene clip lock bag. The second lot was dipped in a 3% Prospect® oil solution for 30 seconds, drained for 5 minutes and repacked as above. Fruit were stored under cool conditions and transferred the next day to the Cairns DAF postharvest laboratory. On 20 January 17 (1 DAT) a mite count was conducted on 20 randomly selected fruit from each treatment, which were discarded after the count. The remaining fruit were restored at 5°C and a recount conducted on 20 randomly selected fruit from each treatment on 27 January 17 (8 DAT).

Mite counts (Table 11) conducted at 1 and 8 DAT suggested that the oil dipped fruit had a higher percentage of mite free fruit (65%) compared to the control fruit (45%). At 1 DAT one live mite was recorded from a sub-sample of 20 fruit with a further three recorded as dead and three as moribund. At 13 DAT no live mites were detected, whereas seven were recorded as dead and two moribund. The mite counts on control fruit were 11 and 13 alive at 1 and 8 DAT respectively. No dead or moribund mites were recorded.

Table 11. Mite count of KMP fruit (control, dipped in Prospect® oil 3% for 30 seconds) at 1 and 8 DAT.

Treatment	Count Date	# fruit nil mites	Total mite count			Total Fruit count
			Alive	Dead	Moribund	
Control	20 Jan (1 DAT)	9 (45%)	11	0	0	20
	27 Jan (8 DAT)	9	13 (multiple mites per fruit)	0	0	20
Dipped	20 Jan	13 (65%)	1	3	3	20
	27 Jan	13	0	7	2	20

Trial 5

- Variety: Kwai Mai Pink (B3)
- Orchard location: Sunshine coast hinterland, south-east Queensland
- Fruit picked and packed: 8 February 2017
- Treatment Date: 9 February 2017
- Treatment:
  - Oil dip (0% (control), 3%, 5% and 10%);
  - Dip Time (30s, 120s and 300s)
- Replicates: Three
- Sub-sample: Ten fruit

Two 5 kg cartons freshly picked and packed into standard industry packaging on 8 February were transported to the DAF, Centre for Wet Tropics laboratory, South Johnstone on the same evening. The next day (9 February) fruit were randomly divided into 36 lidded plastic containers each containing 10 fruit. Punnets were then randomly assigned to one of the 12 treatments (treatment x dipping time), giving three replicates of each treatment. After treatment fruit were allowed to air dry before placement of container lids and then randomly placed in a 5°C cold room.

Colour measurements (L ,a, b) using a NIX Pro colour system were conducted at 1, 7, 14 and 20 days after treatment (DAT).

Photographs of at all treatment combinations were taken at 1, 6 and 16 DAT.

A fruit rot rating (count of fruit with fungal hyphae clearly present) was conducted on 1 March at 20 DAT. A sample of fruit were forwarded to the DAF pathologist based at Mareeba for identification of fungi.

Colour

Colour was assessed on four occasions (1, 7, 14 and 20 DAT). Hue, Chroma, L, a and b were measured. A repeated measures analysis of variance (ANOVA) was used to compare the effects of treatment and dipping time on colour development over time. The mean colour measurement per punnet was the variable analysed. For all variables there was a significant interaction of date and treatment ($p \leq 0.003$). A significant main effect of dipping time was found for L, a and Chroma ($p \leq 0.051$). No interactions involving dipping time were significant ($p > 0.05$).

The means for the main effect for dipping time and for the interaction of date and treatment are shown in Table 12 along with the associated standard errors (se) (Table 13). Pairwise comparisons were performed using the 95% least significant difference (LSD).

The five minute dipping treatment had a significantly lower mean L, a and Chroma, but there was no significant difference between the 30 second and two minute dipping treatments.

Due to inconsistencies with identifying pairwise differences within the interaction tables of means for treatment and date, comparisons are made within each date (letters underneath the means) and within each treatment (letters beside each mean). The 3%, 5% and 10% treatments showed significant reductions in mean L and b as time progressed, while the control only showed a significant reduction from 16 to 23 February (7 to 14 DAT).

Similarly, the mean a reduced significantly between each assessment for 3%, 5% and 10% treatments, while the control showed no significant reduction in mean a from 16 February to the final assessment on 1 March. The mean Chroma significantly reduced over time for the 3%, 5% and 10% treatments, but there was no significant change for the control. For Hue there was a significant decrease in means from the first assessment to the last assessment for 3%, 5% and 10% but no significant change over time for the control.

Within each time assessment, the control had a significantly higher mean L and b. There was no significant difference between the treatments at the first assessment for a and Chroma, but for all remaining assessments the mean a and Chroma were significantly higher for the control. For Hue the mean was significantly higher for the control at the first three assessments, but by the final assessment there was no significant difference between the treatments.

Table 12. Fruit colour as described by L, a b. LSD comparisons are made within each date* (letters underneath the means) and within each treatment# (letters beside each mean).

Lightness "L" (the higher the L value the lighter the fruit colour)				
	Dip Time			
	30 sec	2 min	5 min	
	43.4 b	43.6 b	42.1 a	
Date	Treatment			
	3%	5%	10%	Control
10-Feb-17	45.8 d# a*	45.7 d a	45.0 d a	50.5 b b
16-Feb-17	42.3 c b	41.3 c b	39.7 c a	51.1 b c
23-Feb-17	39.9 b b	38.7 b ab	38.2 b a	49.3 a c
01-Mar-17	38.3 aa	37.7 aa	36.9 aa	48.8 ab

Green-Red "a" (the higher the a value the redder the fruit)				
	Dip Time			
	30 sec	2 min	5 min	
	28.5 b	29.0 b	26.7 a	
Date	Treatment			
	3%	5%	10%	Control
10-Feb-17	33.5 d a	33.6 d a	33.2 d a	33.2 b a
16-Feb-17	27.9 c b	26.4 c ab	26.0 c a	32.8 ab c
23-Feb-17	25.4 b b	23.2 b a	23.6 b a	32.0 a c
01-Mar-17	23.2 a b	21.5 a a	21.2 a a	31.9 a c

Blue – Yellow "b" (the higher the b value the yellower the fruit)				
	Dip Time			
	30 sec	2 min	5 min	
	24.2	24.4	23.2	
Date	Treatment			
	3%	5%	10%	Control
10-Feb-17	27.4 d a	27.2 d a	26.6 d a	29.4 b b
16-Feb-17	23.3 c b	22.2 c ab	21.2 c a	29.4 b c
23-Feb-17	21.7 b b	20.5 b ab	19.4 b a	28.6 a c
01-Mar-17	20.3 a b	19.1 a ab	17.9 a a	28.2 a c

Table 13. Chroma and Hue LSD comparisons are made within each date* (letters underneath the means) and within each treatment# (letters beside each mean).

Chroma				
	Dip Time			
	30 sec	2 min	5 min	
	37.5 b	38.1 b	35.5 a	
Date	**Treatment**			
	3%	**5%**	**10%**	**Control**
10-Feb-17	43.5 d a	43.6 d a	42.7 d a	44.6 a a
16-Feb-17	36.5 c b	34.8 c ab	33.7 c a	44.3 a c
23-Feb-17	33.6 b b	31.1 b a	30.7 b a	43.2 a c
01-Mar-17	31.0 a b	28.9 a a	27.8 a a	42.8 a c
Hue				
	Dip Time			
	30 sec	2 min	5 min	
	40.4	40.2	40.8	
Date	**Treatment**			
	3%	**5%**	**10%**	**Control**
10-Feb-17	39.3 a a	39.1 a a	38.8 a a	41.7 a b
16-Feb-17	39.9 a a	39.8 a a	39.2 a a	42.1 a b
23-Feb-17	40.7 b ab	41.0 b a	39.4 ab a	42.0 a b
01-Mar-17	41.2 b a	41.4 b a	40.1 b a	41.7 a a

The p-values for all main effects and interactions for the five colour measurements in Tables 12 and 13 are shown below in Table 14.

Table 14. The p-values for all main effects and interactions for the five colour measurements.

	L	**a**	**b**	**Chroma**	**Hue**
Treatment	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.070**
Dipping Time	**0.051**	**0.003**	**0.219**	**0.008**	**0.709**
Treatment.Dipping Time	0.347	0.436	0.273	0.327	0.249
Date	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Date.Treatment	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.003**
Date.Dipping Time	0.213	0.150	0.298	0.184	0.215
Date.Treatment.Dipping Time	0.306	0.579	0.753	0.690	0.414

Nix colour measurements indicate that oil treated fruit were slightly darker than control fruit at 1 DAT and all fruit progressively darkened over time up to 20 DAT (Table 12). Fruit redness as indicated by a, was the same for oil and control treated fruit at 1 DAT, however redness declined at an increasing rate in oil treated fruit. Similarly for b, which indicates the strength of the blue-yellow spectrum with oil treated fruit becoming less yellow (range -0 to +50) over time than control fruit.

Fruit visual appearance differences were not so clear (Plate 10). In subjective terms fruit appeared visually satisfactory up to 6 DAT, although oil treated fruit regardless of dip time were slightly darker in appearance. At 16 DAT oil treated fruit, regardless of concentration or dip time, were more likely to be displaying a mild fungal bloom around the stem end. A relatively consistent trend over a series of experiments.

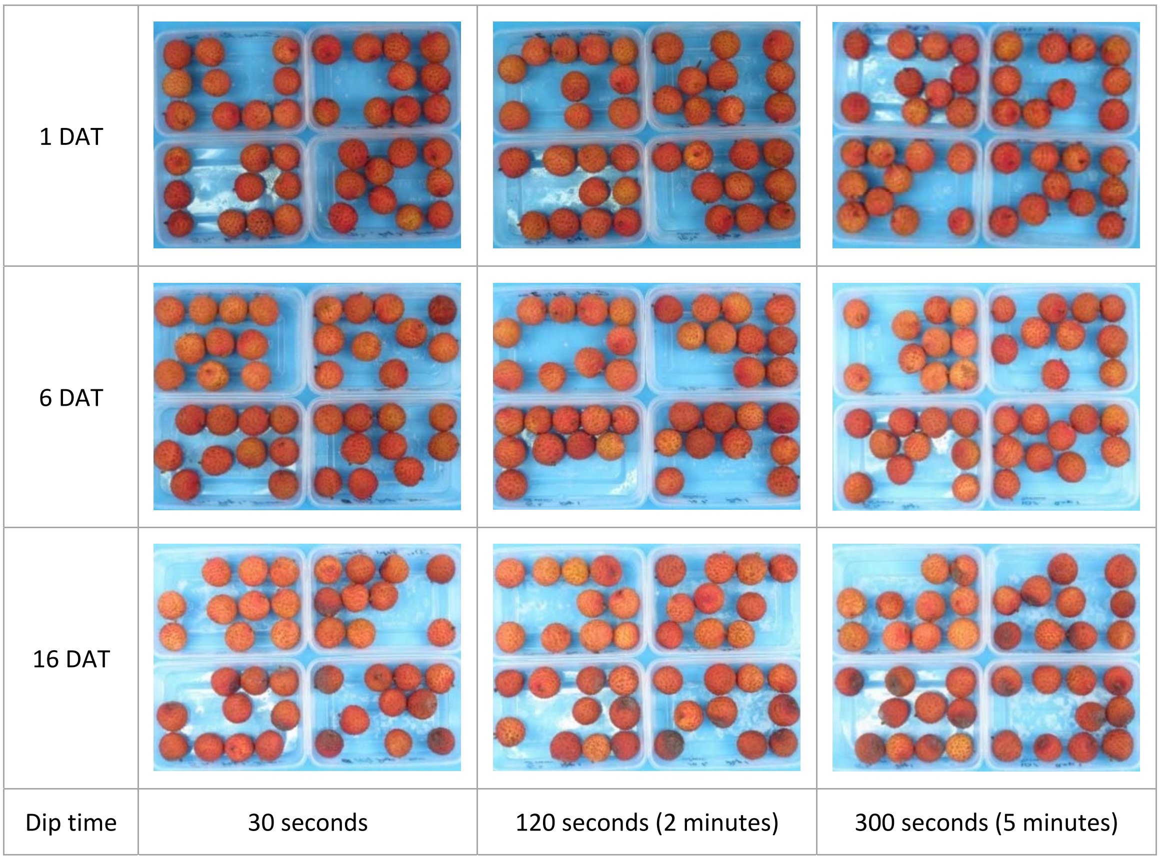

Plate 10. Fruit appearance (Rep 1) following dip treatments (*from left to right*) for 30, 120 and 300 seconds at Prospect® oil concentrations. Within each image oil concentrations vary (clockwise from top left) 0%, 3%, 5% and 10%.

Mould

The number of fruit in each punnet that showed signs of rot was recorded at 20 DAT. A generalised linear model (GLM) assuming a binomial distribution and logit link function was used to compare the proportions affected. Treatment and dipping time were fitted in the fixed effects model. The results suggest there was a significant main effect of both treatment ($p<0.001$) and dipping time ($p=0.004$). The interaction of treatment and dipping time was not significant and subsequently dropped from the fixed effects model ($p>0.05$). The predicted means for the main effects are presented In Table 15. Pairwise comparisons using the 95% LSD found a significantly higher mean proportion of fruit had rot when dipped for 5 minutes compared to 30 seconds, and the control fruit had a significantly lower mean incidence of rot compared to all treated fruit. The 10% oil treatment had a significantly higher mean proportion of fruit with rot compared to the other treatments.

Table 15. Proportion of fruit (%) exhibiting mould at the stem end at 20 DAT.

	Predicted Mean and proportion of fruit with mould	se
Dipping Time		
30 s	0.667 ab (66.7%)	0.0393
2 m	0.667 ab (66.7%)	0.0373
5 m	0.758 a (75.8%)	0.0341
Oil Concentration		
3 %	0.722 b (72.2%)	0.0464
5 %	0.778 b (77.8%)	0.0432
10 %	0.889 a (88.9%)	0.0328
Control	0.289 c (28.9%)	0.0469

Plate 11. Left image: Stem end "fungal bloom"
Right image: Internal stem end view (left) free of fungal bloom; (right) with fungal bloom.

In summary, oil treatment at 3%, 5% and 10% concentration darken fruit relative to control with a negative impact on the red and yellow end of the spectrum as days after treatment progressed. However, subjectively, fruit from all treatments were commercially sound at 6 DAT. In general terms fruit initially appeared commercially sound at 16 DAT except for early signs of the development of a stem end fungal bloom (Plate 11). By 20 DAT fungal blooms were more pronounced in control fruit exposed for longer dip times (120 and 300 seconds) and were significantly more pronounced in oil treated fruit, increasing with oil concentration. Isolation of the fungi present in the stem bloom indicated the presence of *Cladosporium*, *Alternaria*, *Curvularia* and *Epicoccum* species (Vawdrey and Grice pers com, 2017).

Moisture Loss

Moisture loss for each punnet was measured on three occasions (7, 14 and 20 DAT). A repeated measures ANOVA was used to investigate the effect of treatment and dipping time on moisture loss over time. The results found a significant interaction of assessment time and treatment (p=0.008), but the main effect of dipping time and all interactions involving dipping time were not significant (p>0.05).

Pairwise comparisons using the 95% LSD for the interaction of assessment time and treatment found inconsistencies when allocating labels to the means. Due to this, pairwise differences were conducted within each treatment (letters beside the mean) and between treatments within each assessment (letters underneath the means). Results indicate a significant increase in moisture loss over time for all treatments. At 7 and 20 DAT, the control had a significantly higher mean moisture loss than the other treatments. At 14 DAT the control was only significantly higher than the 5% and 10% treatments (Table 16).

Table 16. Mean moisture loss (%) per punnet of ten fruit at 7, 14 and 20 days after the application of oil treatments. LSD comparisons were conducted within each treatment* (letters beside the mean) and between treatments within each assessment# (letters underneath the means).

Days after Treatment	Oil Dip concentration			
	3%	5%	10%	Control
7	0.89 a* a#	0.72 a a	0.86 a a	1.18 a b
14	1.41 b bc	1.16 b a	1.27 b ab	1.63 b c
20	1.56 c b	1.29 c a	1.34 c ab	1.83 c c

Following a series of Prospect® postharvest oil dip trials (T1 to T5) it was apparent that lychee fruit dipped in oil did not guarantee that all mites would be controlled, regardless of concentration or dip time. The treatment dramatically reduced the movement of mites, with live mites being best described as moribund (near death). As a substitute disinfestation treatment the methyl bromide alternative Vapormate® was trialled. A commercial (BOC) gas mixture combining the active ingredient ethyl formate plus carbon dioxide (CO_2) has been successfully utilised to control insects and mites in strawberries, grapes, citrus and banana (Ryan and De Lima, 2014).

Trial 6 – Vapormate® trial

- Variety: Kwai Mai Pink (B3)
- Fruit source: Sunshine coast hinterland, south-east Queensland
- Fruit picked and packed: 14 February 2017
- Treatment Date: 16 February 2017
- Treatments:
  - Two package types at fumigation: a) loose fruit in slotted field crates; b) Carton packed with US approved liner
  - Temperature of fruit prior to gassing: a) non refrigerated, b) ex cold room at 5°C
  - Vapormate® concentration: 0, 160, 240 and 420 g/m³ for two hours
- Replicates: Three cartons per treatment
- Sub-sample for transport to Cairns, mite counts and photography: Ten fruit

A Vapormate® treatment facility was available on the Sunshine Coast (Cedar Hill). Fruit were sourced from a nearby orchard and prepared for treatment as outlined above. Key treatments from a grower convenience perspective included how fruit were presented for the gas treatment:

- a) packed in 5 kg cartons using the approved US liner, or
- b) loose fruit in slotted plastic field crates. The packed fruit would be most convenient; however, there was pre-trial concern that the gas treatment would not be able to fully penetrate the packaging, thereby effecting the efficacy. The loose fruit was a pre grading and packaging alternative, allowing fruit to be fully exposed to the gas concentration. Additional to packaging, fruit were either pre-cooled overnight at 5°C or left unrefrigerated.

Following the Vapormate® treatment at 0, 160, 240 and 420 g/m³ for two hours fruit were randomly sub-sampled from each treatment and replicate combination into lidded containers containing ten fruit each for airfreight to the Cairns DAF postharvest laboratory. On arrival fruit were rapidly assessed for live mites and quality.

Full measurement of mite counts and fruit colour were abandoned because all fruit presented for gassing in open slotted crates were fully brown within 24 hours of treatment regardless of gas concentration. Conversely, mite checks in packed cartons indicated that live mites were present, regardless of gas concentration.

Out of curiosity the three replicate lidded trays for each treatment combination were stored for two weeks and appearance recorded (Plate 12).

<table>
<tr><td colspan="3" rowspan="3"></td><td>Control</td></tr>
<tr><td>Packed fruit</td></tr>
<tr><td>Slotted crate</td></tr>
<tr><td>160 g/m³</td><td>240 g/m³</td><td>420 g/m³</td><td></td></tr>
</table>

Plate 12. Fruit appearance at 14 DAT for control and Vapormate® treated fruit at 160, 240 and 420 mg/m³ presented as packed fruit or loose in slotted trays.

Vapormate® fumigation was not successful. Mites were only controlled at the maximum fumigation rate on unpacked fruit, but fruit quality was dramatically impacted and fruit fully brown with 24 hours of treatment. Fruit packaged in cartons using the US liners were exposed to approximately half the fumigant rates at each dosage. While fruit appearance of packed fruit remained sound live mites were recorded in all treatments and control fruit.

2017/18 Season

During the 2017/18 season Prospect® postharvest oil was applied as a recirculated flood spray incorporated with a test or commercial prototype roller conveyer system (Plate 13). Additional pre-wash and post oil high pressure/volume water treatments were evaluated in addition or absence of the oil treatment.

Usual treatment combinations trialled for fruit surface pest elimination included:

- Control (non-treated fruit)
- Pressure wash, to waste, at the start of the packing line to remove dust, surface pests and plant residues using a centrifugal pump
- Oil flood spray (3% Prospect® oil for 30 seconds) recirculated using a helical rotor pump to eliminate separation “break point” of oil and water that can result from excess agitation as occurs using a centrifugal pump system (Taverner pers com, 2017)
- A post oil pressure spray, to waste, applied approximately two minutes after oil application to remove surface pests that may have been killed or adversely affected by the application of oil
- Combination of pre-wash, oil and post oil wash treatments
- In the last trial, various food grade acids were incorporated at 1% concentration with the oil treatment so the efficacy of the amendments could be evaluated.

Plate 13. Experimental and commercial prototype inline oil flood spray and high pressure water sprays.

Four trials were conducted to compare disinfestation treatments on the presence of mites and other insects on fruit destined for export markets. Replicate samples were collected from different parts of the farms where fruit was acquired and each replicate consisted of 10 sub-sample fruit. Post treatment counts of mites and other insects were recorded, as well as whether the insects were alive, moribund or dead.

For each trial six analyses were conducted. The variables analysed are summarised in Table 17.

Table 17. Variables analysed for each of the four trials conducted during the 2017/18 lychee season.

Variable	Mites/Insect Count	Insect Status
Total Count of Live Mites	All live orbatid, predator and other mites	Alive
Total Count of Mites	All orbatid, predator and other mites	Alive, moribund, dead
Total Count of Other Live Insects	All live scale, mealy bug and others	Alive
Total Count of Other Insects	All scale, mealy bug and others	Alive, moribund, dead
Total Count of All Live Insects	All live pests (mites, scale, mealy bug and others)	Alive
Total Count of All Insects	All pests (mites, scale, mealy bug and others)	Alive, moribund, dead

Statistical Analysis

The total counts were analysed using a generalised linear mixed model (GLMM) with a Poisson distribution and log link function. If under-dispersion was present, the dispersion parameter was fixed at one. If there was evidence of large over-dispersion the Negative Binomial distribution and log link function was used. Under and over-dispersion are not uncommon and indicate whether there is less or more variation present than expected from a Poisson distribution. All GLMM had treatment fitted as a fixed effect and replicate as a random effect. If the random effect was found to be constrained to zero, the model was simplified to a generalised linear model (GLM) with no random term. The 95% least significant difference (LSD) was used to make pairwise comparisons if the main effect of treatment was found to be significant ($p<0.05$).

The results from each GLMM include the mean counts on the log scale (Predicted Mean), associated standard errors (se) and back-transformed mean counts (BT Mean). The BT Mean is the mean count per ten pieces of fruit. The results from a GLM includes the predicted mean count (mean count per ten pieces of fruit) and associated standard error.

Trials 1 and 3 had low counts of mites and insects, with many samples of ten fruit recording no surface pests. Where it was deemed the number of non-zero samples was too small for a sensible GLMM or GLM analysis, a two-part conditional GLM was performed. To overcome analytical problems arising from numerous zero values, a binomial model with the logit link function was used for the presence/absence component, and a conditional gamma model with the log link was used for the non-zero counts that were actually observed. The binomial model simply considers whether the mite/insect of interest is present. The conditional gamma model only analyses the count data from treatments that have samples of ten fruit with a non-zero count. If no non-zero counts are observed for a treatment the standard error associated with the conditional gamma component of the model cannot be estimated. Combining the means and standard errors of these binomial and conditional gamma models enables a more accurate and meaningful estimation of the model effects. The results presented include the predicted proportion of samples with the surface pest species of interest present, as obtained from the binomial GLM. The predicted mean counts and standard error from the full two-part conditional GLM is also presented.

Trial 1

- Variety: Kwai Mai Pink (B3)
- Orchard Location: Far north Queensland
- Date picked: 13 December 2017

Treatments applied 14 December after fruit were stored in cartons and industry standard "crispy bag" overnight in an air conditioned laboratory (approximately 22°C).

- Treatments:
    - Control
    - Pre-wash only
    - Oil flood spray
    - Pre-wash + oil
    - Oil + post wash
    - Pre-wash + oil flood spray + post wash
- Replicates: Three replicates with ten sub-sample fruit sourced from different areas in the orchard
- Measurements: Mean total mite count, live mites, total insects (all types) and live insects

Treated fruit were packed in lidded plastic containers and stored overnight at 5°C. Mite and insect counts were conducted the following day approximately 24 hours after treatments were applied.

A significant effect of treatment was observed in all analyses (Table 18). All samples from the control treatment contained live mites, resulting in the mean proportion of samples with live mites present for this treatment being significantly higher than all other treatments. When the presence of moribund and dead mites are also included all treatments still had a significantly lower mean proportion of samples with mites present compared to the control.

Live insects other than mites were only found in the control and pre-wash treatments. The mean proportion of samples with other live insects was significantly higher for these two treatments compared to all other treatments. In terms of the mean count of other live insects, the mean in the pre-wash treatment was significantly higher than in the control.

The analysis of the proportion of samples with any live insects also found these two treatments to have a significantly higher mean proportion than the other treatments. The mean total count of live other insects in the pre-wash was significantly higher than the control treatment. The control treatment was not significantly different to any other treatment.

When the total count of all insects (including mites) is analysed, the control had a significantly higher mean count than the oil + post wash and pre-wash + oil + post wash treatments (data not shown).

Table 18. Trial 1: Mean total count of all mites (alive, moribund or dead), live mites, all other insects (alive, moribund or dead) and live insects.

Treatment	Mean total count of all mites (alive, moribund or dead) (P=0.003)	Mean total count of live mites (P=0.001)	Mean total count of all other insects (alive, moribund or dead) (P=0.010)	Mean total of all live insects (P=0.001)
Control	1.00 a	1.00 a	1.75 bcd	1.00 a
Pre-wash	0.25 b	0.25 b	6.75 a	1.00 a
Oil	0.00 b	0.00 b	4.00 abc	0.00 b
Pre-wash + Oil	0.25 b	0.00 b	4.75 ab	0.00 b
Oil + Post-wash	0.00 b	0.00 b	1.00 cd	0.00 b
Pre-wash + Oil + Post-wash	0.00 b	0.00 b	0.25 d	0.00 b

Trial 2a

- Variety: Kwai Mai Pink (B3)
- Orchard Location: Bundaberg, Queensland
- Date picked: 9 January 2018

Treatments applied immediately after picking.

- Treatments:
    - Control
    - Pre-wash only
    - Oil flood spray
    - Pre-wash + oil
    - Oil + post wash
    - Pre-wash + oil flood spray + post wash
- Replicates: Four replicates (sourced from different areas in the orchard) with ten sub-sample fruit.
- Measurements: Total mite count, live mites, total insects (all types) and live insects.

Treated fruit were sampled and packed in lidded plastic containers and stored overnight at 5°C. The next day fruit were airfreighted to the Cairns DAF postharvest laboratory and stored at 5°C overnight. Mite and insect counts were carried out on 12 January approximately 48 hours after treatment application.

A significant effect of treatment (Table 19 and 20) was only observed on the total count of all live insects. The control treatment had a significantly higher mean count of live insects than treatments T2, T4 and T6.

Table 19. Trial 2a: Mean total count of all mites (alive, moribund or dead), live mites, all other insects (alive, moribund or dead) and live insects.

Treatment	Mean total of all mites (P=0.384)	Mean total all live mites (P=0.431)	Mean total count of all insects (P=0.661)	Mean total count of live insects (P=0.103)
Control	1.90 ns	1.92 ns	8.93 ns	5.40 ns
Pre-wash	0.24	0.24	5.95	0.74
Pre-wash + oil	1.65	1.33	9.49	4.75
Pre-wash + oil + post-wash	0.48	0.24	9.84	0.49
Oil	1.43	1.20	4.37	0.74
Oil + post-wash	0.24	0.00	5.33	0.98

Table 20. Trial 2a: Mean total count of all pests (alive, moribund or dead) and live pests.

Treatment	Mean total count of all pests (mites + insects) (P=0.665)	Mean total count live pests (P=0.038)
Control	11.22 ns	7.58 a
Pre-wash	6.24	0.99 b
Pre-wash + oil	11.02	5.93 a
Pre-wash + oil + post-wash	10.74	0.74 b
Oil	2.89	1.99 ab
Oil + post-wash	5.81	0.98 b

Trial 2b

- Variety: Kwai Mai Pink (B3)
- Orchard Location: Bundaberg, Queensland
- Date picked: 10 January 2018
- Treatments: Applied 10 January 2018
    - A. Control - Shed standard system
    - B. Pre-wash + Oil (2 minutes) + Post-wash
    - C. Pre-wash + Oil (1 minutes) + Post-wash
- Replicates: Six replicates from different areas in the orchard with ten sub-sample fruit per replicate
- Measurements: total mite count, live mites, total insects (all types) and live insects

Treated fruit were sampled and packed in lidded plastic containers and stored overnight at 5°C. The next day fruit were airfreighted to Cairns DAF postharvest laboratory and stored at 5°C overnight. Mite and insect counts were carried out on 12 January approximately 48 hours after treatment application.

A GLMM was used to analysis the total count of live mites and the total count of all mites. All other analyses were performed using a GLM due to the random effect being constrained at zero. There was also evidence of over-dispersion which was not accounted for by the Poisson distribution so a GLM with a negative binomial distribution was fitted.

Results suggest there is an effect of treatment on the count of live mites. The overall count of live mites is significantly higher using treatment A (conventional line) compared to treatments B (pre-wash + oil (2 min) + post wash) and C (pre-wash + oil (1 min) + post wash).

The GLMM analysis of the total count of mites (alive, moribund and dead) found no significant effect of treatment (p=0.147).

The GLMM analysis of the total count of live mites found a significant effect of treatment (p=0.030). Pairwise comparisons using the 95% LSD found the mean number of live mites per ten pieces of fruit was significantly higher in treatment A (conventional line) compared to treatments B and C (Table 21).

The negative binomial GLM on the total count of other insects (live, moribund and dead) found no significant effect of treatment (p=0.819).

The negative binomial GLM on the total count of other live insects found no significant effect of treatment (p=0.375).

Table 21. Trial 2b: Mean total count of all mites (alive, moribund or dead), live mites, all other insects (alive, moribund or dead) and live insects. Back transformed means shown.

Treatment	Mean total count of all mites (live, moribund and dead) (P=0.147)	Mean total count of live mites (P=0.030)	Mean total count of insects (live, moribund, or dead) GLM (P=0.819)	Mean total of count live insects GLM (P=0.375)
Control	2.17 ns	2.32 a	8.50 ns	4.67 ns
Pre-wash + Oil (2 minutes) + Post-wash	0.51	0.27 b	6.00	1.83
Pre-wash + Oil (1 minutes) + Post-wash	1.02	0.42 b	8.67	6.17

Trial 3

- Variety: Kwai Mai Pink (B3)
- Orchard Location: Sunshine Coast hinterland, Queensland
- Date picked: 9 February 2018
- Treatments: 12 treatments applied 20 February 2018 (Table 22)
- Replicates: Four replicates from different areas in the orchard with ten sub-sample fruit per rep
- Measurements: Total mite count, live mites, total insects (all types) and live insects

Table 22. Treatments applied to KMP Trial 3.

T#		T#	+ Citric acid 1%	T#	+ Lactic acid 1%	T#	+ Malic acid 1%
1	Control	4	Pre-wash + Oil + Citric acid	7	Pre-wash + Oil + Lactic acid	10	Pre-wash + Oil + Malic acid
2	Pre-wash	5	Pre-wash + Oil + Citric acid + post wash	8	Pre-wash + Oil + Lactic acid + post wash	11	Pre-wash + Oil + Malic acid + post wash
3	Pre-wash + oil	6	Pre-wash + Citric acid	9	Pre-wash + Lactic acid	12	Pre-wash + Malic acid

Fruit were sourced from a farm located on the Sunshine Coast hinterland, a late fruit producing region. Following packing and storing at 5°C, fruit were airfreighted to the DAF laboratory at the Centre for Wet Tropics Agriculture, South Johnstone and stored in commercial packaging at 5°C for a week. The delay in application of treatments was due to the late arrival of the food acids, which had been ordered several weeks earlier.

On the day of treatment, fruit from the four replicate populations randomly subdivided in to treatment/replicate lots and treatments applied. Control (untreated), pre-wash, Prospect® oil (3%) and post wash were in various combinations were combined with the addition of three food grade acids (citric, lactic and malic). The acids were either added to the oil flood spray or applied independently as a flood spray where oil was not part of the treatment combination. The addition of food acids was trialled to test if they improved the pest control efficacy of the oil treatment and fruit quality. Research reported by Taverner (2015) indicated that the addition of food acids to Prospect® oil at 1 to 4% concentration decreased the hatching percentage of Fullers Rose Weevil eggs, a pest of citrus. The successful use of food acids for postharvest management of lychee browning has been reported in a range of publications (Jiang and Fu 1998, Silva et al. 2012). Hence the use of food acids as adjuvants to Prospect® oil may have had a dual effect of surface pest control and fruit red-life enhancement.

The addition of all three food acids to the Prospect® oil caused a thickening and frothing of the solution (Plate 14). However, there did not appear to be any adverse effect on fruit the next day.

Plate 14. Thickening and frothing of the Prospect® oil following the addition of food acids.

Following treatment fruit were stored in lidded disposable containers at 5°C. The next morning the treated fruit were transferred to the Cairns DAF postharvest laboratory for mite and insect counts as described earlier.

Surface pest results

Fruit obtained for this trial had a very low incidence of mites and insect pests with many replicate of ten fruit having a zero count.

Live mites were only found in the control treatment and, therefore, this data has not been analysed. Intuitively this would suggest there is a higher mean count of live mites in the control than the other treatments.

A two-part conditional GLM was required to analysis the total count of mites, total count of other live insects, and total count of all live insects. This is due to the large number of samples that recorded a zero count. The analyses of the total count of other insects and the total count of all insects were performed using a GLMM.

A significant effect of treatment was observed on the proportion of samples with mites present, other live insects present and the proportion of samples with any live insects present. All samples from the control treatment contained live mites, resulting in the mean proportion being significantly higher for this treatment compared to all other treatments.

With respect to the analysis of other live insects, no significant difference was identified between the mean proportion of samples for the control, high pressure spray (HPS), HPS + oil and HPS + citric acid. All other treatments recorded no other live insects and were found to be significantly different to the control.

The analysis of the proportion of samples with any live insects found no significant difference between the control, HPS, HPS + oil and HPS + citric acid. All other treatments were found to be significantly different to the control

The two-part conditional GLM found a significant effect of treatment (p=0.013) with respect to the presence of mites (live, moribund and dead). Pairwise comparisons using the 95% LSD found all treatments had a significantly lower mean proportion of samples with mites than the control. The significance of the conditional gamma GLM was just outside the 0.05 level of significance (p=0.052). The highest mean count of mites was found in the control samples (Table 23).

Table 23. Total mean count of mites and insects.

Treatment	Total count of mites (live, moribund or dead) Predicted proportion GLM (P=0.013)	Total count of all live mites (not analysed)	Mean total count of insects (live, moribund or dead) Back Transformed mean (P=0.752)	Mean total count of live insects Predicted proportion GLM (P=0.017)
Control	1.00 a	6	4.59 ns	0.75 a
Pre-wash	0.20 b	0	2.41	0.50 ab
Pre-wash + oil	0.25 b	0	2.17	0.25 ab
Pre-wash + oil + Citric acid	0.00 b	0	2.66	0.00 b
Pre-wash + oil + Citric acid + Post-wash	0.00 b	0	2.41	0.00 b
Pre-wash + citric acid	0.25 b	0	4.35	0.50 ab
Pre-wash + oil + Lactic acid	0.00 b	0	2.90	0.00 b
Pre-wash + oil + Lactic acid + Post-wash	0.25 b	0	4.10	0.00 b
Pre-wash + Lactic acid	0.25 b	0	0.97	0.00 b
Pre-wash + oil + Malic acid	0.00 b	0	2.66	0.00 b
Pre-wash + oil + Malic acid + Post-wash	0.00 b	0	1.21	0.00 b
Pre-wash + Malic acid	0.00 b	0	3.38	0.00 b

Post treatment fruit colour measurements were abandoned due to the age of the fruit prior to treatment. At the time of treatment and the day after, during pest counts the fruit appeared in good marketable condition (Plate 15).

Plate 15. Fruit appearance the day following treatment application and pest counts. Treatments are (*from left to right*): *Top row* - T1, T2, T3 and T4; *Centre row* - T5, T6, T7 and T8; *Bottom row* - T9, T10, T11 and T12. All fruit are from Replicate 2.

A count of fruit rots (Plate 16) was conducted on 2 March 2018, 10 DAT for each treatment and replicate combination. The mean number of fruit exhibiting rots is shown in Table 24.

Table 24. Mean number of fruit per Punnett of 10 fruit with rot at 10 DAT

Treatment #	Food acid addition	Mean number of fruit with rots
1	None	2.00
2	None	2.50
3	None	2.50
4	Citric	3.00
5	Citric	2.25
6	Citric	3.50
7	Lactic	4.25
8	Lactic	3.25
9	Lactic	3.75
10	Malic	3.25
11	Malic	3.50
12	Malic	3.00

Plate 16. Example of rot damage at 10 DAT for 9 of the 12 treatments. Treatments are (*from left to right*): *Top row* - T1, T6 and T8; *Bottom row* - T9, T11 and T12.

By day 10 DAT, or 21 days after picking, fruit from the bulk of treatments was unmarketable. Interestingly the Control fruit were in the best condition.

Quarantine Statistics

In the 2017/18 season four trials were conducted to compare disinfestation treatments on micro mites and other insects on fruit destined for export markets. Samples of fruit were collected from different parts of a block. Counts of micro pests (mites and other insects) were recorded, as well as whether the mites and insects were alive, moribund or dead.

The number of fruit with the presence of live mites, any mites (live, moribund and dead), any live insect, and any insect in any physical state were calculated. Based on the number of fruit sampled for each treatment in the trials and the number of fruit infested, the maximum infestation level (%) was calculated based on 95% confidence. Using this maximum infestation level, the expected maximum number of infested fruit an inspector may find in a sample of 180 fruit was determined. The sample size of 180 is based on six cartons being selected from a pallet and 30 fruit from each carton being inspected.

All calculations were performed using the freeware CQTStats (Liquido and Griffin, 2010), which uses the formulas of Couey and Chew (1986).

Trial 1

Six treatments were trialled in this experiment:

T1. Control

T2. Pre-wash only

T3. Oil flood spray

T4. Pre-wash + oil

T5. Oil + post wash

T6. Pre-wash + oil flood spray + post wash

Live mites were only found in treatments T1 and T2. Based on the maximum infestation level calculated for each treatment the expected maximum number of fruit that might have live mites in a sample of 180 fruit is 62 for the control treatment (T1), 14 for treatment T2 and eight for the remaining treatments. Treatments T3, T4, T5 and T6 have reduced the expected maximum number of fruit in a sample of 180 that may be found to have live mites by approximately 87%.

Taking into account other live insects the expected maximum number of fruit that may be found with any live insect in the control treatment is 87 out of the 180 fruit sampled. Treatments T3, T4, T5 and T6 have the greatest impact by reducing this expected maximum down to eight. Treatment T2 had no impact on the proportion of fruit found to have other live insects.

Trial 2a

Six treatments were trialled in this experiment:

- T1. Control
- T2. Pre-High Pressure Wash (HPW)
- T3. Pre-HPW + oil
- T4. Pre-HPW + oil + post-HPW
- T5. Oil
- T6. Oil + post-HPW

Treatment T6 had the largest reduction in the proportion of fruit infested with live mites, with no fruit infested compared to 15% for the control treatment. From a sample of 180 fruit the expected maximum number of fruit that might be found to have live mites in the control treatment is 42 (maximum infestation rate of 29.61%). This is reduced to eight fruit (maximum infestation rate of 7.49%) for treatment T6.

Trial 2b

Three treatments were trialled in this experiment:

- A. Conventional line (fruit destalked, graded and lightly misted prior to packing)
- B. Pre-wash + oil (2 min) + post wash
- C. Pre-wash + oil (1 min) + post wash

Sixty fruit were sampled for each treatment and 16.67%, 3.33% and 3.33% were found to have live mites in treatments A, B and C respectively. This equates to a maximum infestation level of 28.27%, 10.49% and 10.49% respectively (based on 95% confidence). Using these maximum infestation levels on a sample of 180 fruit the expected maximum number of fruit that might be found to have live mites is 39, 12 and 12. Both treatments B and C have reduced the expected maximum number of fruit infested with live mites by approximately 70%.

Treatment C has had no impact on the presence of other insects, with the proportion of fruit infested with any form of live insect being slightly higher compared to treatment A.

Trial 4

Twelve treatments were trialled in the Houser experiment:

1. Control
2. High pressure spray (HPS)
3. HPS + oil
4. HPS + oil + Citric acid
5. HPS + oil + Citric acid + post HPS
6. HPS + Citric acid
7. HPS + oil + Lactic acid
8. HPS + oil + Lactic acid + post HPS
9. HPS + Lactic acid
10. HPS + oil + Malic acid
11. HPS + oil + Malic acid + post HPS
12. HPS + Malic acid

No fruit were found to be infested with live mites for treatments T2 to T12. Only the control treatment (T1) had fruit with live mites. Based on the maximum infestation level calculated for each treatment the expected maximum number of fruit that might have live mites in a sample of 180 fruit is eight for treatments T2 to T12, and 42 fruit for the control. The treatments have reduced the maximum number of fruit in a sample of 180 that may be found to have live mites by approximately 80%.

Taking into account other live insects, the expected maximum number of fruit that might be found with any live insect in the control treatment is 57 out of the 180 fruit sampled. All treatments suggest they will reduce this number, with treatments 4, 5, 7-12 having the most impact.

The results for the four trials are presented in Appendix 1.

Summary

2016/17 season

1. The mites found on fruit sampled, in order of frequency included:
    i. Oribatid mites, which are detrivores (detritus eaters) commonly found in/on soil litter and plants
    ii. Predator mites - Order Mesostigmata e.g. Phytoseiidae
    iii. Mite eggs- possibly Spider mite, family Tetranychidae
    iv. Other mites not identified were are most likely herbivore or fungivore in nature
    v. No erinose mites were found.
2. A Prospect® oil dip solution did not guarantee that all mites would be controlled, regardless of oil concentration or dip time. However, the bulk of mites were killed. The treatment also dramatically reduced the movement of mites, with live mites being best described as moribund (near death).
    i. All mealy bug or scales present on fruit were killed by the oil treatment.
3. The oil dip and irradiation treatments were not deleterious to the appearance of fruit. The extent of change depended on the variety being treated. Within several hours of treatment fruit were not oily to touch. Post oil irradiation treatments at 200, 400, 600, 800 and 1000 Gy did not negatively impact fruit appearance.
    i. Kwai Mai Pink (KMP) fruit - The bulk of fruit treated were Kwai Mai Pink (B3), the variety most likely to be exported due to reliability and volume of fruit production and relatively low-priced fruit availability from southern growing areas post-Christmas/New Year. Oil treated KMP fruit were slightly darker in appearance. The difference between treated and untreated fruit was small and possibly only noticeable by an observer who knows lychee fruit well.
    ii. For red varieties tested in small observation trials (Wai Chee, Sah Keng and Salathiel) treated fruit were a brighter and more vivid red, in most cases initially enhancing the appearance of the fruit.
4. Oil treated fruit, when stored between 5 to 10°C remained in good condition up to 14 days post treatment. After two weeks many oil treated fruit developed “fungal blooms” around the stalk end. Isolation of the fungi present in the stem bloom. Fungal isolation indicated the presence of *Cladosporium*, *Alternaria*, *Curvularia* and *Epicoccum* species.
5. The oil treatment appeared to be compatible with cold water (1, 5 and 10°C). Hence there is a possibility that the treatment could be incorporated with hydro-cooling.
6. In one oil experiment conducted at the DAF Centre for Wet Tropics Agriculture, South Johnstone we did experience “gelling” (clag-glue in appearance) of the product at 1, 3, 5% oil concentration. The reason is unknown but may be related to water quality. This made agitation and dipping difficult but treated fruit appeared normal within a few hours of treatment. Communication with the oil manufacturers suggested this problem has been recorded previously but the reason for the gelling has not been identified. We suggest grower’s trial a small volume with their water supply before making up a large volume of solution.
7. Two commercial shipments to the USA were treated with 3% Prospect® postharvest oil in a 30 second dip prior to irradiation. The first shipment moved through the supply chain to the US without any problems. Unfortunately, in the second shipment, a mite detection occurred so the shipment was diverted to the domestic market prior to irradiation. For both markets there were no issues raised with the fruit quality of oil dipped fruit.

8. All oil dip applications were carried out with manual agitation of the oil immediately prior to dipping and agitation during dipping. This may result in excess application of oil on the fruit due to the lack of continuous agitation potentially improving the disinfestation efficacy of the oil but exacerbating the development of a fungal bloom at the stem-end.
9. The alternative Vapormate® fumigation treatment was not deemed successful. Mites were only controlled at the maximum fumigation rate on unpacked fruit but fruit quality was dramatically impacted and the fruit were unsalable. Fruit packaged in cartons and US approved plastic bags were exposed to approximately half the fumigant rates at each dosage. While fruit appearance of the packed fruit remained sound, live mites were recorded in all gas treatments.

2017/18 season

1. During the 2017/18 season Prospect® oil (3%) was applied to fruit as a recirculating low-pressure, high-volume flood spray for 30 seconds, as occurs for export citrus.
2. A helical rotor positive displacement pump was used to recirculate the oil, which is recommended for oil/water solutions to maintain the solution at "break point" and at the correct concentration during application. Note: A centrifugal pump is not recommended.
    i. The application of Prospect® oil as a recirculated flood spray appears to have improved the final appearance of the fruit relative to dipping and eliminated the occurrence of stem end fungal bloom after two weeks of storage.
3. The washing of fruit before or after oil application was incorporated into the oil trials.
4. As in the previous season, fruit surface mite and micro insect pressure varied between orchards.
5. The data collected from four trials suggest that a combination of pre-washing, oil flood spray and post oil application (two minute lag time) was an effective method of disinfesting surface pests from lychee. However, the method was not able to guarantee 100% disinfestation.
6. Incorporation of the oil with a two minute lag time prior to post oil washing to allow the oil to incapacitate mites and insects reduced fruit throughput compared to a conventional system. In the system trial carried out, labelled as 2017/18 Trial 2b, 160 kg/hour were packed using system B (pre-wash + oil + post wash) compared to 260 kg/hour in the conventional system.
7. Fruit washing alone was able to reduce the load of surface pests on fruit, suggesting that improvements in fruit washing sprays through higher pressure and/or higher volumes may achieve a similar result to a combination of oil and water washing.
8. The addition of food acids (citric, lactic and or malic) did not improve the efficacy of the oil.

Outputs

Key outputs included two interim project reports in the lychee grower magazine 'Living Lychee' published by Fruit Tree Media:

- Post-harvest control of surface pests on lychee for export to the USA and NZ. *Living Lychee*, Issue 72, March 2017
- AGM Grower meeting presentations – Mite project update, October 2017. *Living Lychee*, Issue 74, November 2017.

Presentations of project findings at Hort Innovation Lychee SIAP meetings.

- Project update at Lychee SIAP, 16 March 2017
- Project update at Lychee SIAP, 27 September 2017
- Project update at Lychee SIAP, 29 May 2018.

Lychee industry AGM, October 2017 was attended by 18 growers.

Equipment development

A prototype in-line roller spray unit which included a flood-spray unit for application of oil and high pressure/volume water sprays for pre cleaning or post oil application cleaning (Plate 17) was developed. This concept was further developed by a lychee grower for a commercial trial (Plate 18 and 19).

Plate 17. Experimental prototype oil flood spray and high pressure water washing (*from left to right*): *Top row* - Roller conveyor with spray hood, water and oil tanks; Twin fan nozzle water spray for pre or post oil application (5 fan nozzles, 1.11 L/s at 13 psi); *Bottom row* - Flood spray for oil application (0.63 L/s at 0 psi); Mono helical rotor pump used for oil application.

Plate 18. Commercial prototype in line oil/spray application conveyor.

Plate 19. Components of the commercial oil/spray prototype (*from left to right*): *Top row* - pre-wash; oil flood spray; *Bottom row* - Oil drip line (two minute transit) until post oil application spray at the far end of the conveyor; final sorting prior to packaging.

Outcomes

The project has demonstrated that postharvest treatment of lychee using Prospect® postharvest fruit treatment (717 g/L Paraffinic Oil) as a dip or flood spray, 3% concentration applied for 30 seconds, does reduce the incidence of micro surface pests (mites, mealy bugs, scale and Lepidoptera) on fresh lychee fruit. The use of fruit washing before and after oil treatment can further reduce the incidence of surface pests. Calculated maximum infestation rate of live mites, based on a 95% confidence interval, for control fruit varied from 28.3 to 42.4 %, whereas in the Pre-wash + Oil + Post wash system the calculated maximum mite infestation rate varied from 7.5 to 11.9%. Similar reductions in live mite populations were calculated for the pre-wash only treatment using high volume fan nozzles operating at moderate pressure and volume. Note: None of the treatments were able to demonstrate 100% disinfestation.

The oil treatment has minimal effects on fruit quality with treated fruit being a little darker than untreated fruit. However, applying the oil in a minimally agitated dip resulted in fruit developing a fungal bloom at the stem end after two weeks of storage. This problem was not present when the oil was applied to fruit as a recirculated flood spray at the same concentration and time (3% solution for 30 seconds).

Oil treatment did not interact adversely with irradiation treatment, suggesting that the oil treatment is compatible with the irradiation protocol required by New Zealand and the USA.

The disadvantage of the oil treatment is that according to research undertaken by Taverner (date unreported) it requires at least two minutes after application for fruit to “drip dry” and work sufficiently on pests to inactivate them prior to further water sprays to wash of incapacitated pests. This delay has a major impact on the packing rate relative to conventional systems, with a 38.5 % reduction in pack rate.

Industry leaders have suggested that further work is carried out in the coming season to examine the effect of higher pressure/volume water sprays without an oil treatment for surface pest decontamination.

Monitoring and evaluation

Target of mite free fruit for export almost met.

Recommendations

In conclusion, the use of Prospect® postharvest insecticide oil for the control of surface micro pests on lychee has potential to succeed, but not guaranteed to be 100% effective.

Discussions with participating growers suggest that the oil treatment could easily be added to existing lychee hydro-cooling, conveyor sorting and packaging systems. Based on project results the application of Prospect® oil as a recirculated flood spray is preferable to dipping, resulting in fruit with improved appearance and a longer storage life. The pump type used to recirculate the solution is important with the potential for oil water separation in a highly agitated solution, which can occur using a centrifugal pump, Tavener (date unreported).

Allowance should be made to drain the oil dripping from fruit after treatment, with the oil being returned to the mixing tank. A minimum of two minutes lag or "drip time" following oil application, as recommended by the citrus export industry (Taverner, date unreported), is considered a problem by industry as it impacts on fruit throughput and hence packing rate.

Data obtained during the 2017/18 season suggests that there is potential for fruit washing alone to provide a suitable reduction of surface pests. Industry members on the SIAP panel have requested DAF submit a project proposal for the 2018/19 season to investigate the efficacy of in-line high pressure/high volume sprayers to decontaminate lychee fruit of micro surface pests.

Based on our project work, experience and discussion with lychee growers the following recommendations are made:

- Undertake pre-harvest pest management to control surface pests including mites.
- Select clean fruit with a low pest load for treatment and subsequent export.
- Pressure wash fruit at the start of the packing line to remove dust, surface pests and plant residues.
- Apply Prospect® postharvest oil using a flood spray shower (agitated solution - 3% v/v for a minimum of 30 seconds).
- Ensure the oil is maintained at "break point" by using a helical rotor, piston or other positive displacement pump.
- Pressure wash fruit to remove insects loosened by the oil treatment, two minutes after application.

High pressure/high volume pressure sprays prior to and post oil treatment should lower the pest load and wash off any ailing pests. The removal of the bulk of the oil may also reduce the stem end rot observed in the oil trials.

Refereed scientific publications

None to report.

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Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

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    - Dr Carole Wright, Senior Biometrician
    - Peter Leach, DAF Market Access Focus Team Leader
    - Neil Wiltshire, Horticulturist (Resigned)
    - Dr James Drinnan, Horticulturist
    - Daniel Sole, Laboratory Technician
    - Peter Nimmo, Senior Experimentalist
    - Richard Piper, Entomologist
    - Andrew Manners, Senior Entomologist.
    - Melisa Frazer, Administrative Officer.

Appendices

Appendix 1

2017/18 Trial 1 - Quarantine statistics and the maximum infestation % at the 95% confidence interval.

Pest	Treatment	Number of fruit sampled	Number of fruit infested	Percentage of fruit infested	Maximum infestation (%) (95% confidence)	Number of infested fruit (n=180)
Live Mites	T1	40	10	25.00	42.41	62
	T2	40	1	2.50	11.86	14
	T3	40	0	0.00	7.49	8
	T4	40	0	0.00	7.49	8
	T5	40	0	0.00	7.49	8
	T6	40	0	0.00	7.49	8
Total Mites	T1	40	10	25.00	42.41	62
	T2	40	1	2.50	11.86	14
	T3	40	0	0.00	7.49	8
	T4	40	1	2.50	11.86	14
	T5	40	0	0.00	7.49	8
	T6	40	0	0.00	7.49	8
Total Live	T1	40	15	37.50	57.75	87
	T2	40	18	45.00	66.73	102
	T3	40	0	0.00	7.49	8
	T4	40	0	0.00	7.49	8
	T5	40	0	0.00	7.49	8
	T6	40	0	0.00	7.49	8
All Insects	T1	40	15	37.50	57.75	87
	T2	40	18	45.00	66.73	102
	T3	40	7	17.50	32.87	47
	T4	40	10	25.00	42.41	62
	T5	40	4	10.00	22.88	31
	T6	40	1	2.50	11.86	14

2017/18 Trial 2a - Quarantine statistics and the maximum infestation % at the 95% confidence interval.

Pest	Treatment	Number of fruit sampled	Number of fruit infested	Percentage of fruit infested	Maximum infestation (%) (95% confidence)	Number of infested fruit (n=180)
Live Mites	T1	40	6	15.00	29.61	42
	T2	40	1	2.50	11.86	14
	T3	30	2	6.67	20.98	28
	T4	40	1	2.50	11.86	14
	T5	40	5	12.50	26.29	36
	T6	40	0	0.00	7.49	8
Total Mites	T1	40	6	15.00	29.61	42
	T2	40	1	2.50	11.86	14
	T3	30	3	10.00	25.85	36
	T4	40	2	5.00	15.74	20
	T5	40	5	12.50	26.29	36
	T6	40	1	2.50	11.86	14
Total Live	T1	40	14	35.00	54.72	82
	T2	40	4	10.00	22.88	31
	T3	30	10	33.33	56.54	85
	T4	40	3	7.50	19.38	25
	T5	40	7	17.50	32.87	47
	T6	40	3	7.50	19.38	25
All Insects	T1	40	24	60.00	84.38	132
	T2	40	19	47.50	69.70	107
	T3	30	15	50.00	76.99	120
	T4	40	17	42.50	63.75	97
	T5	40	15	37.50	57.75	87
	T6	40	13	32.50	51.67	77

2017/18 Trial 2b - Quarantine statistics and the maximum infestation % at the 95% confidence interval.

Pest	Treatment	Number of fruit sampled	Number of fruit infested	Proportion of fruit infested (%)	Maximum infestation (%) 95% confidence	Number of infested fruit (n=180)
Live Mites	A	60	10	16.67	28.27	39
	B	60	2	3.33	10.49	12
	C	60	2	3.33	10.49	12
Total Mites	A	60	10	16.67	28.27	39
	B	60	4	6.67	15.26	19
	C	60	5	8.33	17.52	23
Total Live	A	60	20	33.33	48.44	72
	B	60	9	15.00	26.18	36
	C	60	21	35.00	50.42	75
All Insects	A	60	27	45.00	62.06	95
	B	60	18	30.00	44.49	66
	C	60	31	51.67	69.73	107

2017/18 Trial 3 - Quarantine statistics and the maximum infestation % at the 95% confidence interval.

Pest	Treatment	Number of fruit sampled	Number of fruit infested	Percentage of fruit infested (%)	Maximum infestation (%) (95% confidence)	Number of infested fruit (n=180)
Live Mites	1	40	6	15.00	29.61	42
	2	40	0	0.00	7.49	8
	3	40	0	0.00	7.49	8
	4	40	0	0.00	7.49	8
	5	40	0	0.00	7.49	8
	6	40	0	0.00	7.49	8
	7	40	0	0.00	7.49	8
	8	40	0	0.00	7.49	8
	9	40	0	0.00	7.49	8
	10	40	0	0.00	7.49	8
	11	40	0	0.00	7.49	8
	12	40	0	0.00	7.49	8