Evaluation of postharvest removal of dithiocarbamate residues in lemons

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Background

Dithiocarbamates are an important group of protective non-systemic fungicides with surface protection against black spot (*Guignardia citricarpa*). These fungicides only work if the fruit / foliage are thoroughly covered before a fungal spore lands on the fruit or leaf and tries to infect it. There are several products of dithiocarbamates including Dithane®, Antracol®, Zineb etc. Products which have dithiocarbamates as the active ingredients include mancozeb, propineb, zineb. These are usually applied at 6 and 12 weeks after the copper application/s at petal fall. Subsequent applications for the control of mites (Brown Citrus Mite and Citrus Rust Mite) also occur depending on the activity of the pest.

Mancozeb is classified as a dithiocarbamate non-systemic agricultural fungicide with multi-site, protective action. Mancozeb (manganese ethylenebis (dithiocarbamate) polymeric complex with zinc salts) is further classified as an ethylenebisdithiocarbamate (EBDC) fungicide. EBDCs have been regarded as relatively harmless because of their low acute toxicity to mammals, but they are generally unstable in the presence of moisture or oxygen and in biological systems. Under these conditions, several degradation products are formed, including ethylenethiourea (imidazolidine-2-thione, ETU). ETU is the main degradation product by hydrolysis and photolysis of EBDCs. ETU is a relatively stable and very polar (water soluble) metabolite. Because of the report of its carcinogenic, mutagenic, and goitrogenic effects in laboratory animals, ETU has become a major human health concern (López-Fernández et al., 2017). But it is the actual dithiocarbamate residue itself that is measured and considered the MRL issue.

The temporary Australian Maximum Residue Limit (MRL) is 7 mg/kg, but this is only temporary and the previous MRL was 0.2 mg/kg. Different overseas export markets have different tolerances to dithiocarbamates, and reducing the MRL in citrus fruit is a priority for growers and exporters. López-Fernández et al. (2017) showed that the optimum conditions to degrade mancozeb and limit their toxicological impact are pH 2, at 25 °C in the presence of light. A previous Horticulture Innovation project (CT13020) showed some postharvest treatments reduced dithiocarbamate residues but their data were variable and limited postharvest washing treatments were used. Miles (2016) further recommended "*several replicate samples to ensure meaningful results*". Therefore this trial increased the number of replicates to six (with 8 fruit sample size (>1kg fresh weight) – as recommended by Symbio Laboratories, Brisbane).

The trial examined the effect of different common postharvest treatments and sanitisers on dithiocarbamate residues in lemon fruit. Chlorine and peroxyacetic acid (PAA) are widely used as sanitisers in the Australian citrus industry. Chlorine has been a very popular sanitiser for years. Chlorine-based sanitisers include sodium hypochlorite, calcium hypochlorite, bromo-chloro-dimethylhydantoins and chlorine dioxide. In this experiment, sodium hypochlorite was used as a postharvest treatment to reduce the levels of dithiocarbamate in lemons. PAA is a strong oxidizing sanitiser which is widely used as a sanitiser in the citrus industry. PAA is commercially available as Tsunami™ and is a mixture of acetic acid (CH_3CO_2H), hydrogen peroxide (H_2O_2), PAA (CH_3CO_3H) and water (H_2O).

The use of hot fungicides (40-50C) are used by some citrus packers to improve the efficacy of the fungicides (Golding and Singh, 2017), and increased temperature has been shown to improve the degradation of mancozeb (Hwang et al., 2001). This trial examined the effect of higher washing temperatures on dithiocarbamate residues in lemon fruit. In addition different combinations of hot washing with different sanitisers were assessed.

Aim: Reduce dithiocarbamate residues in lemon fruit with standard postharvest treatments.

Methods

Lemon fruit from Queensland with a record of high in-field Mancozeb (dithiocarbamate) usage were harvested and transported to the NSW DPI Centre of Excellence for Horticultural Market Access at Ourimbah, via Sydney markets on 1 May 2018. The fruit were harvested after spraying and not dipped or processed. The lemons were washed with different washing treatments with the custom built high pressure washing system with brushes (Figure 1):

1. No postharvest treatment (fruit just from bin)
2. Cold water wash at 20C x 30 seconds
3. Hot water at 40C x 30 seconds
4. PAA (Tsunami – label rate) at 20C for 30 seconds
5. PAA at 40C for 30 seconds
6. Chlorine wash (50ppm, 20C for 30 seconds)

The fruit were randomly allocated treatments and each treatment was randomly allocated within each replicate. All treatments were independently replicated six times according to a randomized treatments allocation and the washing machine was thoroughly washed at least twice (and cooled/ heated) between treatments. The pH and temperature of wash water was recorded before and during treatment. In addition, the levels of PAA and chlorine were also measured with test strips from samples taken during the washing process.

A total of 48 residue samples were assessed (8 treatments x 6 replicates). After treatment, fruit were drained / air dried for approximately 60 mins at 20C and then frozen at -20C. The samples were coded and frozen samples couriered and analysed for dithiocarbamates (mg/kg) in the same random order at Symbio Laboratories Pty. Ltd. in Brisbane. Symbio are NATA accredited ISO/IEC 17025 laboratory and the lemon samples were analysed with the accredited method for dithiocarbamates (ferbam; mancozeb; maneb; metham-sodium; metiram; propineb; thiram; zineb; ziram) by GC, GC/MS using in-house CR007 method.

Figure 1. Lemons were randomly selected for treatment (top left), washed in a custom built experimental washing unit (top right), with brushes and high pressure nozzles (bottom left), before drying for 1 hour at 20C (bottom right) then frozen (-20C) and sent for dithiocarbamate residue analysis at Symbio Laboratories in Brisbane.

An additional storage trial was conducted to examine the effect of an additional de-greening and storage period on the dithiocarbamate residues following postharvest treatment. There were two additional treatments which were conducted and analysed:

7. Untreated control fruit plus additional postharvest storage
8. PAA at 40C for 30 seconds fruit plus addition postharvest storage

After the washing treatment, fruit were de-greened in ethylene (5ppm) at 25C for 4 days, then stored at 10C for 1 week before the additional residue assessment. This was to simulate the actual postharvest storage and handling of lemons destined to the market.

All fruit samples were frozen (-20C) and sent by courier with freezer packs to Symbio Laboratories (Brisbane) for determination of dithiocarbamate residues.

Statistical design and analysis was conducted by Lorraine Spohr (Biometrician NSW Department of Primary Industries). The effect of washing treatment on dithiocarbamate residue was tested using analysis of variance. Treatment means were compared using the least significant difference procedure with a significance level of p=0.05.

Results

The results of the different postharvest washing treatments on the levels of dithiocarbamates in lemon fruit is presented as a dot-plot of the dithiocarbamate residue raw data along with the means for each washing treatment (Figure 2). These results illustrate the variability of the residues of each sample replicate for each treatment and show that the mean dithiocarbamate concentration in untreated (control) lemon fruit was 2.9 mg/kg. This average dithiocarbamate residue level was lower than the MRL of 3 mg/kg, but the results presented in Figure 2 show that half of the samples (three samples of the six replicate samples)were higher than 3 mg/kg. This illustrates the high variability of residue data (presumably due to spray and orchard factors), and shows the importance of reducing the MRL in fruit with postharvest washing.

There was a significant effect of postharvest washing treatment on dithiocarbamate residues ($F_{7,35} = 41.02$; $p<0.001$), where all washing treatments resulted in lower dithiocarbamate residues, as compared to the non-washed fruit. There was no statistically significant difference between any of the postharvest wash treatments, including hot washing.

Indeed further testing within the different washing treatments, ie using orthogonal contrasts to test potential differences in hot (40C) washing V cold (20C) washing and also the water treatments V PAA treatments, showed there were no significant effects detected for each contrast. This indicates there was no effect of treatment temperature or addition of PAA to the wash on dithiocarbamate residues in the fruit.

In the side experiment which examined the effect of an additional storage time of de-greening and storage of lemons, the results showed that treating fruit PAA at 40C for 30 seconds fruit before de-greening and storage had similar residue levels, as compared to fruit where the analysis was conducted immediately after treatment (Table 1). This suggests that the initial postharvest washing was an important factor in reducing the dithiocarbamate levels from the fruit, and all washing treatments were statistically similar. Even the addition of a de-greening and storage simulation did not further reduce dithiocarbamate residues in the fruit.

It is interesting to note that the non-washed stored fruit had significantly higher dithiocarbamate residues (5.4 mg/kg) than the non-washed fruit that were immediately analysed (2.9 mg/kg). This observation is difficult to reconcile. The loss of water from the fruit during de-greening and storage would not be enough to contribute to the increase in dithiocarbamate residues (as expressed as mg/kg) observed in stored non-washed fruit. This observation could simply be due to sampling of these fruit or another unknown occurrence.

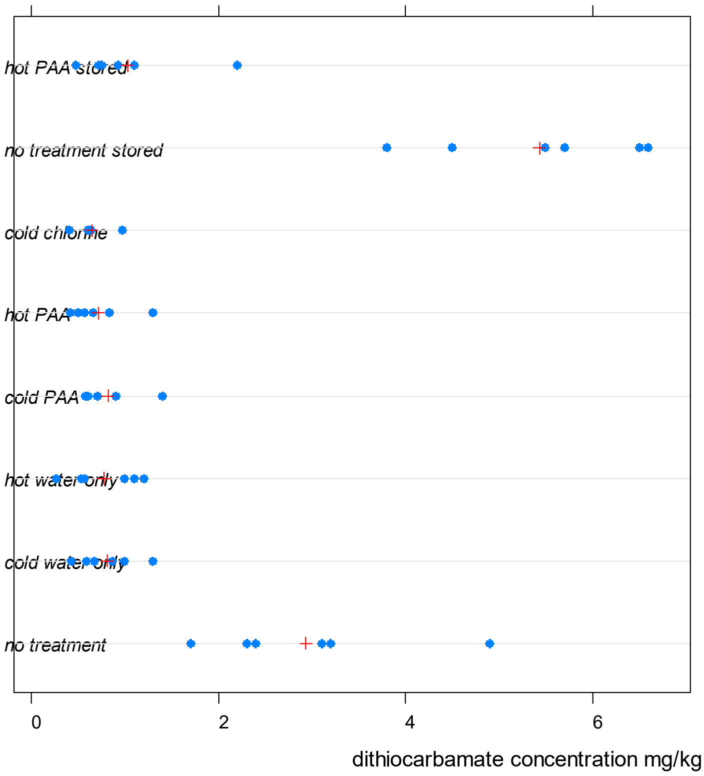

Figure 2. Dithiocarbamate residue raw data and means for each washing treatment (and after de-greening and storage) in lemon fruit.

Table 1. Effect of washing treatment on dithiocarbamate residue concentration in lemon fruit

Treatment	Concentration (mg/kg)	
cold chlorine	0.643	a
hot PAA	0.712	a
hot water only	0.775	a
cold water only	0.810	a
cold PAA	0.817	a
hot PAA – stored	1.027	a
no treatment	2.933	b
no treatment – stored	5.433	c

Treatments with the same letter indicate these treatments are statistically similar (p<0.05)

Conclusion

Any postharvest washing (irrespective of chlorine or PAA, treatment temperature (20C or 40C), or additional postharvest storage time) reduced the dithiocarbamate residues in lemon fruit, as compared to non-washed fruit. This reduction in dithiocarbamate residues was significant and contributes to lowering the dithiocarbamate MRL in lemon fruit. While these levels are below the current temporary MRL, many export markets are sensitive to dithiocarbamate and all efforts should be undertaken to reduce the risk of chemical contamination. Holding the fruit for longer periods (eg de-greening and storage) did not further reduce dithiocarbamate residues.

References

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