# Working Paper Series

This working paper forms part of the ACIAR Project AGB/2012/061 *Improving smallholder farmer incomes through strategic market development in mango supply chains in southern Vietnam*

Study: Activity 2.3 Mango productivity and quality improvements in fresh supply chains

Pre-condition fruit for Hot water treatments in Australia

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# Summary

Hot water immersion treatments used to meet market access protocols can lead to heat related injures to the peel of Mango *Mangifera indica L* primarily related to the rapid transfer of heat. This has led the export industry to favour the costlier options of vapour heat and irradiation treatments to meet protocol requirements.

The research determined weather ambient temperature conditions, preheating and varietal differences could be utilised to acclimate the fruit to increase its heat tolerance for hot water treatments protocols.

Conditioning NMBP 4069 fruits for 24 hours under ambient conditions 31.6 $^{o}$C and a SD $\pm$ 7.76 $^{o}$C, prior to hot water treatment of 47$^{0}$C for 15 minutes core temperate produced minimal heat related damage upon ripening. Conditioning R2E2 fruit for 6 hours under ambient conditions 42.0 $^{o}$C SD of $\pm$ 1.62 $^{o}$C, prior to hot water treatment of 46$^{0}$C for 20 minutes also reduced any negative impact of the treatment on external peel quality upon ripening. Hot water conditioning of NMBP-4069 at 40$^{0}$C for 60 minutes prior to hot water treatment of 47$^{0}$C for 15 minutes similar reduced any heat related peel damage.

Pre-conditioning in ambient conditions offers a commercially practical solution for establishing HWT treatment facilities for export. The 60-minute 40$^{0}$C water pre-conditioning of NMBP-4069 in addition to this eliminates the variability of ambient temperature fluctuations.

# Context

## Introduction

Mango Mangifera indica L. is an important horticultural crop for Northern Australia and its production offers some distinct seasonality differences to that of the Northern Hemisphere which accounts for the majority of global production. (FAO 2017). This in turn has create some significant export opportunities for the industry, supplying the off-season market. However, with endemic fruit fly species such as *Bactrocera tryoni* (Queensland fruit fly) and the established exotic species *Ceratitis capitate* (Mediterranean fruit fly), phytosanitary treatments are necessary for access to many of the export markets (Johnson and Duthie 2018).

Heat treatment of mango fruit to meet phytosanitary requirements has become the industry standard since the restrictions on fumigants and only limited markets currently accepting irradiation. Hot water treatment (HWT) and Vapour heat treatments (VHT) are the protocols that have been established to allow Australian mangoes to access the Chinese market in (MICoR 2016). These protocol requirements are either a core temperature of 46°C for 20 minutes or 47$^0$C for 15 minutes. (Johnson and Duthie 2018).

Whilst the industry has progressed with utilising the VHT options, virtually no uptake of HWT has occurred in spite of it being a much simpler, more efficient and cheaper option than VHT. Much of the reasoning can be attributed to previous studies conducted on Kensington Pride (Smith and Chin 1989, Jacobi and Wong 1992, Joyce et al. 1993) indicating that HWT can lead to unacceptably high levels of scalding and other heat associated injuries. The response between cultivars appears to differ greatly as studies on Florida cultivars Keitt and Tommy Atkins (Spalding et al 1986, Sharp 1986) showed minimal heat related damage. HWT is a common commercial treatment for these two cultivars.

Previous studies with Kensington Pride (Joyce and Shorter 1994) found that prior conditioning by raising the core temperature of the fruit to 37$^0$C for a period of $\leq$ 12 hours significantly reduced pulp injury in ripened fruit. However, the study also found the reduction in skin related damage was inconsistent. Jacobi *et al* (1995) found that rapid heating of the fruit to 40$^0$C eliminated the inconsistent skin damage. Holding the fruit at ambient conditions for 24 to 48 hours prior to HWT of 42-48$^0$C for 30 to 90 min has also been found to reduce surface scalding on several cultivars (Smith and Chin 1989).

Further studies (Henriod and Sole 2014) with the National Australian Breeding Program (NMBP) varieties indicated some varietal differenced in their response to HWT. NMBP-1201 was found to be the most susceptible variety to heat damage. This effect was significantly reduced when a preconditioning treatment of 30$^0$C for 8 hours was applied to the fruit prior to HWT treatment.

For the commercial adoption of a conditioning treatment, it must produce consistent outcomes whilst being able to practically fit in with current packhouse operating systems. The aim of this study is to determine if a commercially practical condition treatment can be developed that takes advantage of the average ambient day time air temperatures 38-41°C (Northern Australia), for NMBP-4069 and export cultivar R2E2. NMBP-4069 had been identified having export potential (Johnson and Slaven 2014).

## Activities

Methodology

*Fruit*: Experiments were conducted on two varieties R2E2 the main export variety and one of the hybrids from the NMBP, cv4069. R2E2 fruit was collected from a commercial packhouse in Kununurra the same day as it was harvested, NMBP-4069 fruit was harvested from the Frank Wise Institute of Tropical Agriculture, Kununurra (Department of Primary Industries and Resource

Development). Fruit was harvested at a physiologically mature hard-green stage (dry matter content of 15%) in the morning. Treatments commenced shortly after. Fruit peduncles were removed and placed in Mango Wash® solution to neutralise the pH of the sap, aligned with current industry practice. The fruit was then held in the laboratory at 22$^{o}$C, prior to treatments.

*Conditioning:* Conditioning was designed to create a commercially realistic situation this was performed by placing the fruit into a warm non-air-conditioned shed at ambient temperature for the treatment times. Air temperature was recorded and averaged over the duration of the treatment time. Conditioning treatment times of the following 6- and 24-hour periods were used. With the exception of one treatment for NMBP-4069 where fruit was submerged in preheated water of 40$^{0}$C for a period of 1 hour prior to the HWT.

*Treatments:* Two varieties R2E2 and NMBP-4069 were used, two experimental designs were developed to incorporate the different fruit characteristics.

Experiment 1 was with NMBP-4069, each treatment was assigned 32 randomly selected fruit which were split into 4 replications, each consisting of 8 pieces of fruit. Pre-conditioning treatments of 0, 6 and 24 hours at ambient air temperature, an additional water condition treatment of 40$^{0}$C for one hour was also applied. The HWT was applied for 15 min after the pulp temperature next to the seed had reached 47$^{0}$C all treatments apart from one used destemmed fruit, was included to help verify the background sap damage Further treatments of no preconditioning with application of hot water treatment of 20 minutes after the pulp temperature next to the seed had reached 46$^{0}$C and a 60 minutes total time submersion at 48$^{0}$C.

Experiment 2 was with R2E2, each treatment was assigned 24 randomly selected fruit which was split into 4 replications each consisting of 6 pieces of fruit. Pre-conditioning treatments of 0, 6 and 24 hours at ambient air temperature. The hot water treatment was applied for 15 min after the pulp temperature next to the seed had reached 47$^{0}$C. Further treatments of no pre-conditioning with application of hot water treatment of 20 minutes after the pulp temperature next to the seed had reached 46$^{0}$C and a 60 minutes total time submersion at 48$^{0}$C.

The control in each experiment was not exposed to hot water treatment, being placed directly into the ripening fridges at 22$^{0}$C.

*Hot water dipping treatment*: The hot water dipping system consisted of an experimental 12 litre non circulating tank with electronic temperature control, capable of maintaining the temperature at ±0.1°C when fully loaded. Fruit and water temperature were monitored using 3 mm stainless steel sleeved resistance temperature detector (RTD) probes connected to a Hobo data logger. RTD probes, thermometers and the hot water bath were calibrated against a certified thermometer at 5, 20 and 40$^{o}$C to create a calibration curve. Two probes were used for each replication. These were inserted into the stem end of the fruit next to the seed husk to reach the thickest part of the fruit. To ensure complete submersion fruit were held below the water level of the tank with a plastic insert. Treatment times commenced when both probes had reached the core set temperature. After treatment fruit was then hydro-cooled by immersing in 30°C water for 30 minutes. The fruit was then dried and placed in the controlled ripening fridges set at 22$^{0}$C. Ethelene ripening gas was not used to accelerate the ripening process.

*Measurements*. Fruit was assessed prior to treatments to identify firmness, skin colour, any existing sap damage or blemished they may be present.

Post treatment fruit was assessed day 3 and 6 after treatment with the final assessment made after all of the fruit in each treatment was determined to be at an edible ripe stage.

Hand firmness, skin colour, lenticel damage, skin disorders (sap, browning and scalding) and fruit rots assessments were conducted using the same or similar rating scales defined in an Australian mango quality assessment manual (Holmes et al 2009). On the final evaluation a destructive total soluble solids test was performed.

Skin colour was assessed visually by scoring the percent area of non-blushed skin surface that had attained a yellow colour. Fruit was scored 1 = 0–10% yellow, 2= 10–30% yellow, 3=30–50% yellow, 4=50–70% yellow, 5= 70–90% yellow, 6= 90–100% yellow.
Fruit firmness was measured by applying a mild hand-palm compression on both sides of the fruit. Fruit was scored 0 = hard (no 'give' in the fruit), 1 = rubbery (slight compression with strong pressure), 2 = sprung (flesh deforms by 2-3 mm with moderate pressure), 3 = firm soft (whole fruit deforms with moderate pressure), and 4 = soft (whole fruit deforms with slight pressure).
External injury to the skin such as browning, scald, or sap damage was rated visually using a 0 to 5 scale, 0 = nil damage, 1 = less than 1cm$^2$, 2 = 1-3cm$^2$, 3 = 3 to 12 cm$^2$, 4 = 12cm$^2$ (~10%) to 25%, 5 = more than 25%.
Lenticel spotting was rated on its severity using a 0 to 5 scale based on the accumulated surface area affected, 0=Nil, 1 =Dense, pronounced spots on not more than 5% of the surface, 2 =Dense, pronounced spots on not more than 10% of the surface or scattered pronounced spots on not more than 25% of the surface, 3=Dense, pronounced spots on not more than 25% or scattered, pronounced spots on not more than 50% of the surface, 4=Dense, pronounced spots on not more than 50% or scattered, pronounced spots on more than 50% of the surface, 5=Dense, pronounced spots on more than 50% of the surface.
External rots were scored based on a 0 to 5 rating scale, where 0 = nil, 1 = less than 1 cm$^2$, 2 = 1-3cm$^2$, 3 = 3-12cm$^2$, 4 = 12 cm$^2$ to 25%, and 5 = more than 25%.
Total soluble solids (TSS) assessments were conducted on each piece of fruit per treatment block. TSS was determined using a refractometer with extracted juice obtained by compressing flesh slices. Results were expressed in degree (°) Brix.

*Statistical analysis*: Biometrical analyses of fruit quality were conducted for each variety using the statistical package GenStat. For each fruit quality factor, a one-way analysis of variance (ANOVA) was performed to test the main effect of each heat treatment. Mean differences were separated by the least square difference (LSD) test at the 5% level with non-significant results reported as "ns".

ANOVA was performed for continuous variables for R2E2 and NMBP-4069 varieties. Each rating scale was converted into a continuous variable if it contained numbers or percentages provided in (Holmes et. al 2009). If the rating scale was a range (e.g. skin colour), then the middle value of the range was used as the value, e.g. 10-30% yellow then 20 was used.

Here, we use ANOVA (Wobbrock et al 2011) to analyse all continuous variables separately and determine if the treatments are different. For each replication, eight mangoes NMBP 4069 and six mangoes R2E2 were dipped into a hot water bath, thus the blocking structure is rep/fruit.

Results and discussion

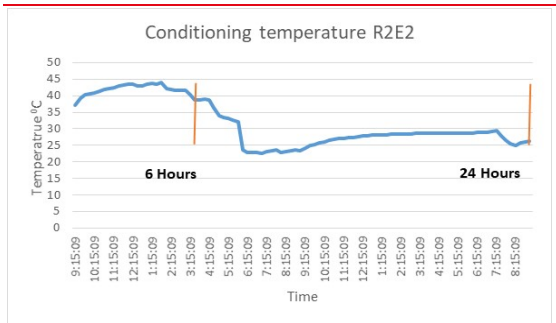


Fig 1 Logged pre-conditioning temperatures for the R2E2 treatments with the holding periods of 6 and 24 hours

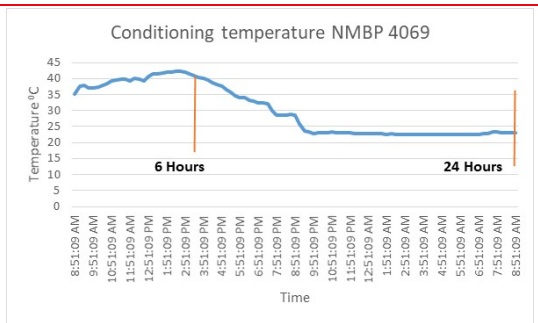


Fig 2 Logged pre conditioning temperatures for the NMBP-4069 treatments with the holding periods of 6 and 24 hours

Pre-conditioning temperatures for the R2E2 treatments (Fig 1) resulted in the 6-hour treatment with a mean temperature of 42.0 °C standard deviation (SD) of ± 1.62 °C, the 24-hour treatment was influenced by a late afternoon rain event and the lower night time temperatures, having a mean value of 31.6 °C and a SD ± 7.07 °C. Pre-conditioning temperatures for the NMBP 4069 treatments (Fig 2) resulted in the 6-hour treatment with a mean temperature of 39.6 °C standard deviation (SD) of ± 1.98 °C, the 24-hour treatment was influenced by the drop in the early evening

temperatures, having a mean value of 31.6 ºC and a SD ± 7.76 ºC. The data showed that for 6-hour treatments a relatively constant preconditioning temperature within the range of 39-42 ºC is able to be maintained without additional temperature controls or modifications. This does become increasingly variable as falling evening and night time temperatures influence the mean value for 24-hour treatments.

Table1 Effect of pre conditioning treatments on peel disorders, internal attributes and rots for variety NMBP-4069, original means with mean letter comparisons when the linear mixed effect model showed the y-variate to be significant.

| Treatments | | Peel disorders | | | | | Internal attributes | |
|---|---|---|---|---|---|---|---|---|
| Number | | Colour | Skin browning | Scalding | Sap damage | Lenticel spotting | Brix | Fruit Rots |
| 1 | No conditioning, hwt 47$^0$C@15min | 5.47 ab | 1.03 ns | 2.03 c | 2.33 c | 0.4 ab | 15.46 ab | 0.17 ns |
| 2 | Conditioning 6hrs, hwt 47$^0$C@15min | 5.47 ab | 0.94 ns | 0.41 ab | 2.16 bc | 0.47 b | 15.87 b | 0.0 ns |
| 3 | Conditioning 24hrs, hwt 47$^0$C@15min | 5.63 b | 1.03 ns | 0.06 a | 1.19 b | 0.19 a | 15.67 ab | 0.s ns |
| 7 | No conditioning, no hwt (*Control* ) | 5.56 b | 0.22 ns | 0.0 a | 0.31 ab | 0.a | 17.2 c | 0.38 ns |
| 8 | No conditioning, hwt 46$^0$C@20min | 5.72 b | 0.22 ns | 0.81 ab | 0.81 ab | 0.28 ab | 15.99 b | 0.0 ns |
| 9 | No conditioning, hwt 46$^0$C@60min (total immersion time | 5.66 b | 0.25 ns | 1.44 b | 0.25 a | 0.06 a | 15.77 b | 0.0 ns |
| 13 | Hot water conditioning 40$^0$C 1hr,hwt 47$^0$C@15min | 5.78 b | 0.31 ns | 0.26 a | 0.84 ab | 0.16 a | 16.07 b | 0.47 ns |
| 14 | No conditioning, hwt47$^0$C@15 (*no desapping* ) | 4.97 a | 0.63 ns | 2.16 c | 0.25 a | 0.22 ab | 15.07 a | 0.5 ns |

ns=not significant

Conditioning times and HWT had no significant difference on fruit firmness (data not shown), skin browning or fruit rots for any of the 8 treatments with variety NMBP-4069 Table 1. Apart from treatment 14 (T14) skin colour development was not inhibited by the treatments. T14 mean score of 4.97 whilst recorded as significant ($P \leq 0.05$) is a relatively minor variation. This treatment also scored the lowest total soluble solids which indicates that there may have been a slight maturity variation within the treatments. Conditioning combined with HWT has a significant ($P \leq 0.05$) negative impact on TSS, reducing it by just over 1$^0$brix, which is aligned with a similar response reported by Henriod and Sole (2014) for NMBP-4069. Previous work has suggested that the rapid heat transfer as occurs in HWT can interrupt the metabolism of starch to sugars during the fruit ripening process (Jacobi et al. 1995b). Varietal response has also been reported to vary significantly (Henriod and Sole 2014).

Non-de-sapping of the fruit in T14 significantly ($P \leq 0.05$) reduce the sap damage when compared to treatment 1 (T1) an identical treatment that included de-sapping. T1 recorded significant sap damage ($P \leq 0.05$), which was reduced in the 6- and 24-hours conditioning treatments. Whilst it appears as the conditioning times had an effect of reducing the impact of the damage, this was not observed in treatments 8 (T8), 9 (T9), or 13 (T13), which were also not subjected to a pre-conditioning treatment. Other factors during the harvest procedures, such diurnal variations in sap turgor pressure and composition, can influence the severity of sap related injury (San et al 2019).

Scalding is one of the more common heat related injuries contributed to HWT. Pre-condition of the fruit for 24 hours and 60-minute hot water conditioning of T3 and T13, respectively, effectively eliminated scald damage when following the 47$^0$C for 15 min protocol. The impact of reducing the temperature by 1$^0$C to follow the 46$^0$C for 20 min protocol resulted in a significant ($P \leq 0.05$) reduction of scald damage. Non de sapping did not have any significant ($P \leq 0.05$) impact in reducing scald damage. R2E2 did not record any significant ($P \leq 0.05$) difference in scald damage from any of the treatments, indicating that the variety may be slightly more resistant to scald damage than NMBP 4069.

All treatments on NMBP 4069 appeared to have slight levels of lenticel damage with the 6 hour preconditioning treatment being significantly higher over the control. R2E2 did record a background level of lenticel spotting which appeared to be significantly ($P \leq 0.05$) exacerbated by the 24-hour conditioning 47$^0$C treatments. Varietal differences in there lenticle responses to treatments such as hot water can vary greatly. (Rymbhi et al 2012) the data is indicating that R2E2 is more predisposed to lenticle damage then what was recorded with NMBP 4069.

There was also a significant increase in rots (P≤ 0.05) with R2E2 T4 and T6, whilst the level was still relatively low it is not immediately apparent why this was happening.

Both T9 with NMBP 4068 and T12 with R2E2 failed within the 60 minutes to reach the core pulp temperature of 46$^0$C therefore would not meet the protocol, it is unlikely this would treatment would work even with preconditioning as T2 and T13 recorded an internal pulp temperature of 40$^o$C after conditioning but still requiring 75 min of immersion time to meet the protocol requirements.

Table 2 Effect of pre conditioning treatments on peel disorders, internal attributes and rots for variety R2E2, original means with mean letter comparisons when the linear mixed effect model showed the y-variate to be significant..

| | Treatments | Peel disorders | | | | | |
|---|---|---|---|---|---|---|---|
| Number | | Colour | Skin browning | Scalding | Sap damage | Lenticel spotting | Fruit Rots |
| 4 | No conditioning, hwt 47$^0$C@15min | 4.46ns | 0.08ns | 0ns | 0.38ns | 2.61b | 0.88b |
| 5 | Conditioning 6hrs, hwt 47$^0$C@15min | 4.56ns | 0.48ns | 0.36ns | 0.04ns | 2ab | 0a |
| 6 | Conditioning 12hrs, hwt 47$^0$C@15min | 4.29ns | 0.30ns | 0.5ns | 0.30ns | 2.88c | 0.54b |
| 10 | No conditioning, no hwt (*Control*) | 4.33ns | 0.25ns | 0ns | 0.17ns | 1.67a | 0a |
| 11 | No conditioning, hwt 46$^0$C@20min | 3.96ns | 0.33ns | 0.67ns | 0.21ns | 1.79a | 0.13a |
| 12 | No conditioning, hwt 46$^0$C@60min (total immersion time | 4.71ns | 0.58ns | 0.13ns | 0.13ns | 1.58a | 0.042a |

ns=not significant

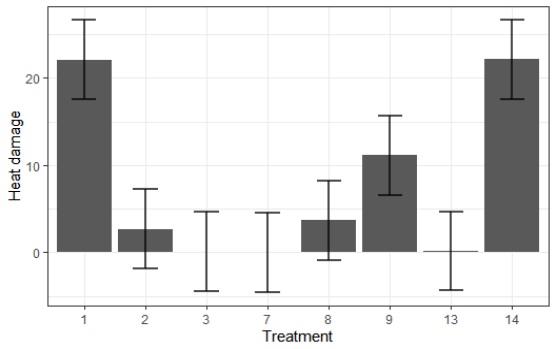


Figure 3 NMBP 4069 mean heat damage (± 8.674 LSD) for each treatment. Comparing between 2 treatments, if the LSD does not overlap then these two treatments are significantly different

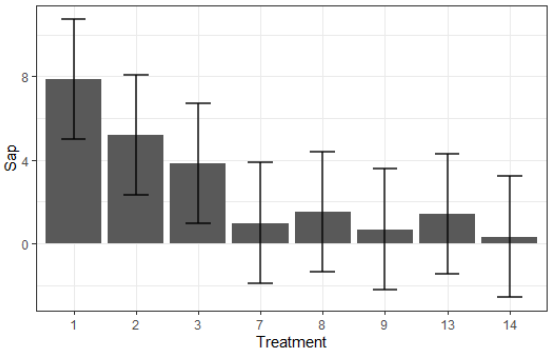


**Figure 4 NMBP 4069 mean sap (± 2.798 LSD) for each treatment. Comparing between 2 treatments, if the LSD does not overlap then these two treatments are significantly different.**

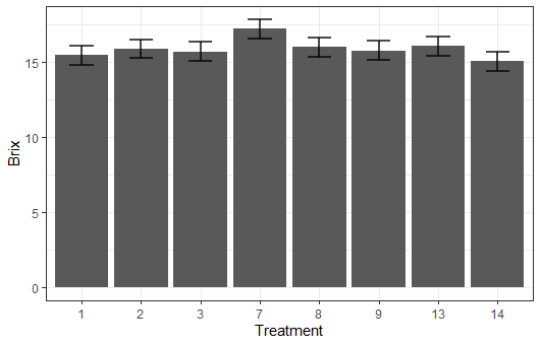


**Figure 5 NMBP 4069 mean brix (± 0.63 LSD) for each treatment. Comparing between 2 treatments, if the LSD does not overlap then these two treatments are significantly different.**

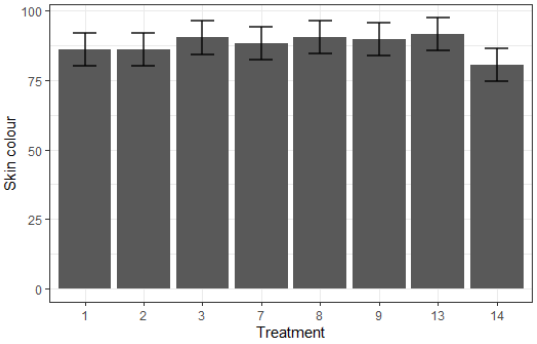


**Figure 6 NMBP 4069 mean skin colour (± 5.886 LSD) for each treatment. Comparing between 2 treatments, if the LSD does not overlap then these two treatments are significantly different.**

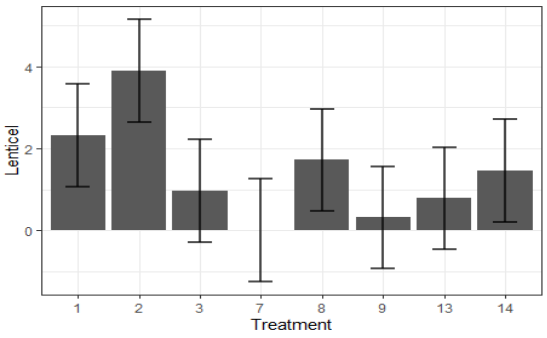


**Figure 7 Mean lenticel (± 2.510 LSD) for each treatment. Comparing between 2 treatments, if the LSD does not overlap then these two treatments are significantly different.**

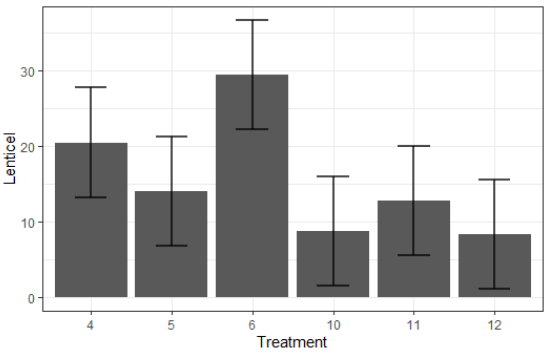


**Figure *8* R2E2 mean lenticel (± 7.25 LSD) for each treatment. Comparing between 2 treatments, if the LSD does not overlap then these two treatments are significantly different.**

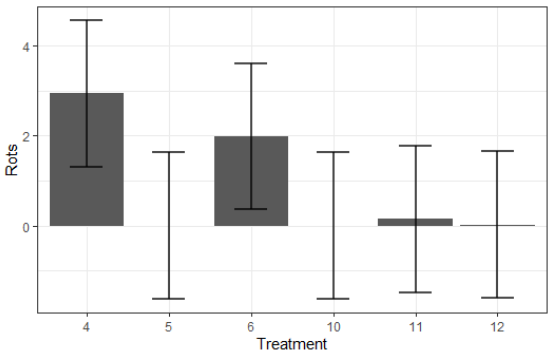


**Figure *9* R2E2 mean rots (± 1.624 LSD) for each treatment. Comparing between 2 treatments, if the LSD does not overlap then these two treatments are significantly different**

Pre-conditioning 24 hours and 60 minutes at $40^0$C on NMBP-4069 for HWT of $47^0$C for 15 d min and 6 hours for HWT of $47^0$C for 15 or $46^0$C for 20 min on R2E2 seemed to reduce any negative impact of the treatment on external peel quality. Internally with NMBP 4069 there was a slight reduction in TSS but no observations of starch deposits or internal browning this was the case for all of the treatments.

The high ambient temperatures experienced in Kununurra during fruit development are likely to be a contributing factor with the overall sensitivity of the fruit to HWT, similarly to what was found with Kensington variety from the Darwin region (Joyce and Shorter 1994), and summer papaya in Hawaii (Paull and Chen 1990). It is plausible that this could be attributed to the high stress growing conditions contributing higher levels of induced gene expression and synthesis of heat-shock proteins in the cells (Al-Whaibi 2011). Further research would be required to confirm this.

Pre-conditioning in ambient conditions offers a commercially practical solution for establishing HWT treatment facilities for export. Pre-conditioning would be subject to fluctuating temperature conditions and may be limited to day time treatments only. Further research would be required to fully test the robustness of this system and produce some specific parameters to work within. The 60-minute $40^0$C water pre-conditioning eliminates this variability and offers a simple commercially practical solution to HWT of NMBP-4069 to meet export protocols.

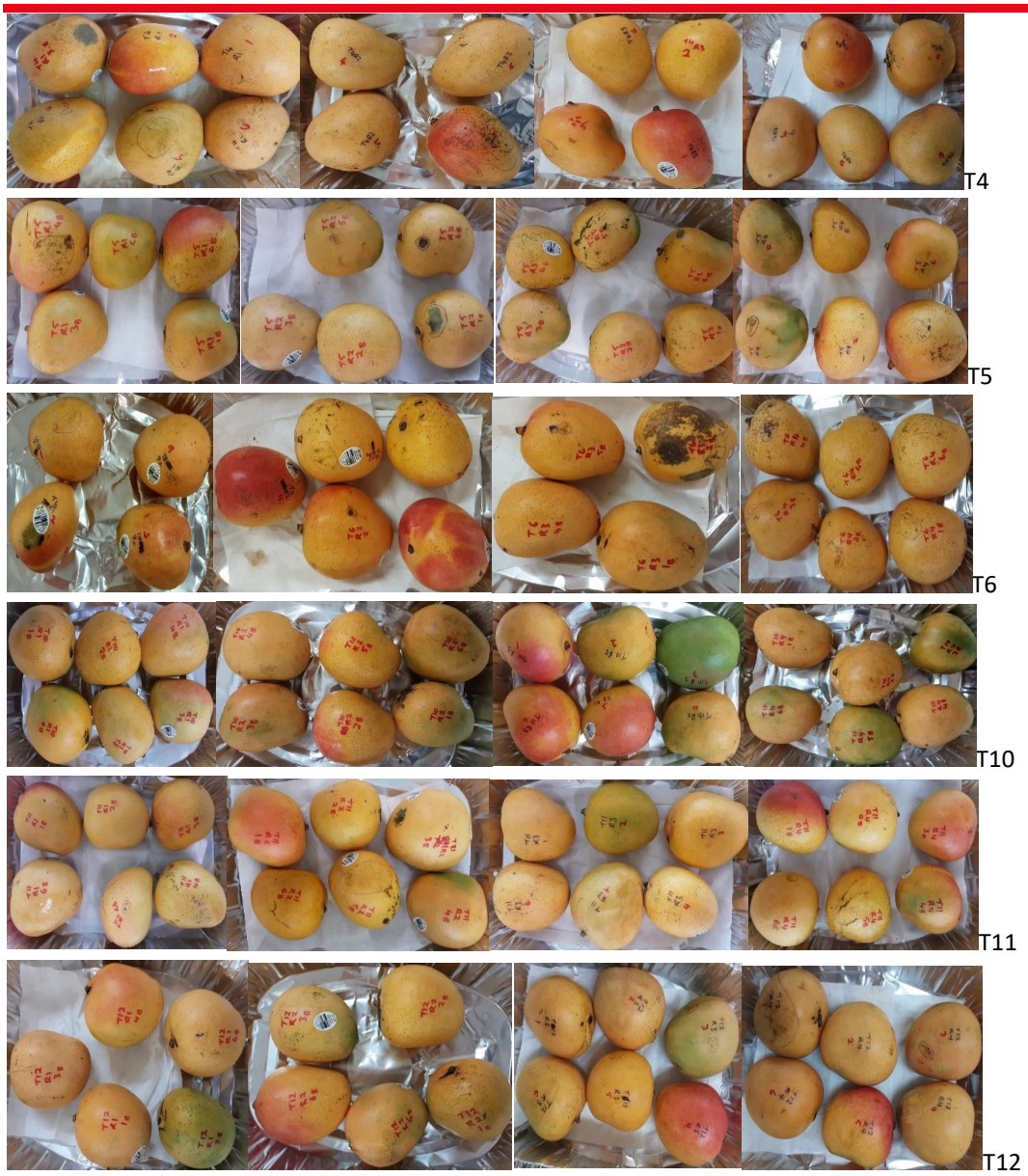


**Figure 10 Images of treatment results R2E2**

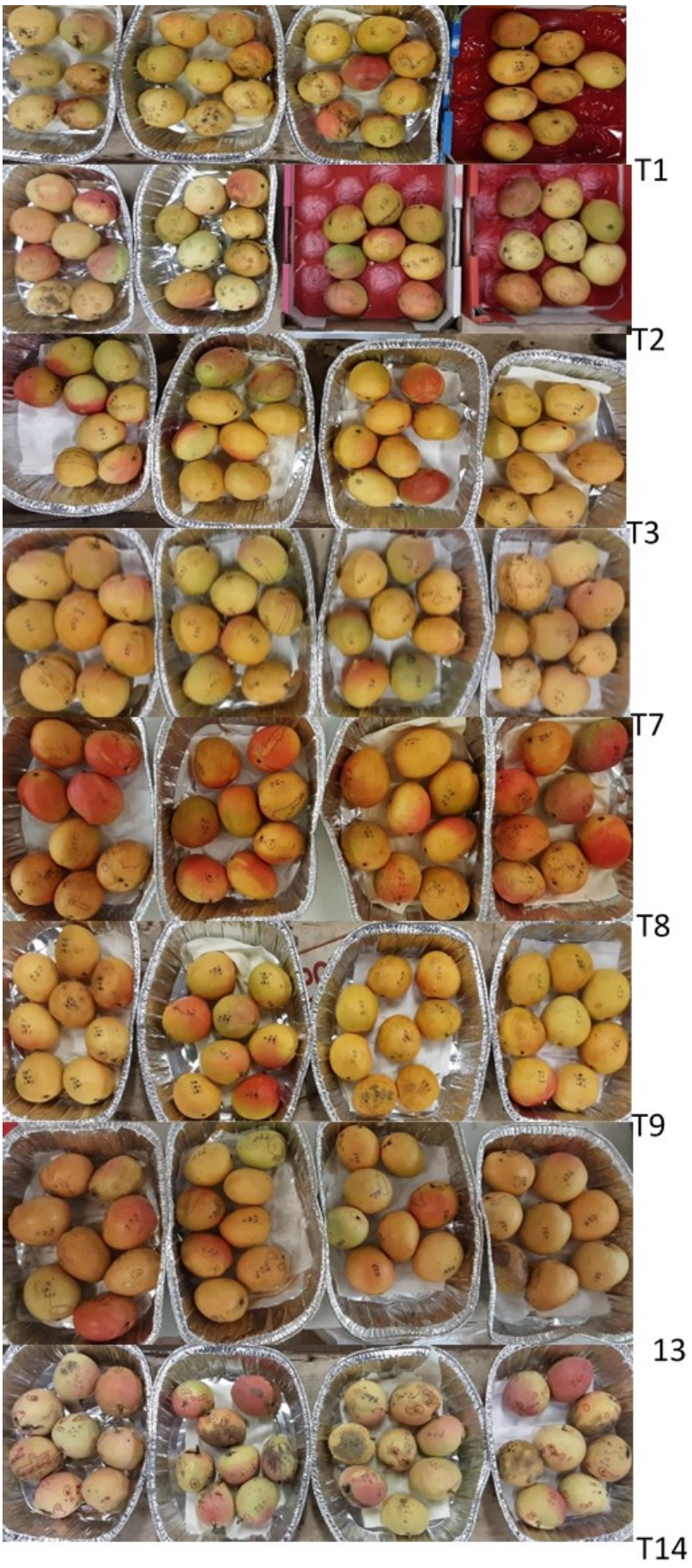


**Figure 11 Images of treatment results R2E2**

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