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Final Report

Evaluate the potential for low-dose methyl bromide as a postharvest disinfestation treatment for citrus

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Summary

Murcott mandarins were fumigated with methyl bromide (MB) at 16 and 18 g/m^3 for 7-10 hours at 18 and 20°C to determine the effect of low-dose MB both on the mortality of Queensland fruit fly (*Bactrocera tryoni*) and on the quality out-turn of the fruit. After fumigation the fruit were stored at 5°C for 21 days to simulate shipping followed by 7 days at 22°C to simulate retail sales. The fruit were then assessed for external and internal defects, skin gloss, skin colour, weight loss, titratable acidity, total soluble solids and taste. Queensland fruit fly was treated as mature larvae and mortality assessed by pupal survival.

The treatments at 16 and 18 g/m^3 MB for 9 and 10 hours duration resulted in significant injury to internal and external quality, fruit gloss and flavour. Fruit assessments after fumigation at 16g/m^3 at 18°C for 7 and 8 hours showed that the internal and external defects and reduced gloss were absent however there were still adverse effects on flavour.

The assumption is that the off-flavours are due to the MB. However, they could in part be the result of lengthy time in the treatment chamber. In this context, it is possible that the off-flavours were caused or accentuated by CO_2 accumulation during MB fumigation.

Fumigation at 16 and 18 g/m^3 MB for 7 and 8 hours at 18°C did not completely disinfest the fruit of third instar larvae of *B. tryoni*. However there were no survivors from an estimated 31,659 larvae fumigated at of 18g/m^3 MB at 20°C for 8 hours thereby resulting in an efficacy of 99.9905% mortality at the 95%CL. This meets quarantine requirements of Thailand and China. Thus, this treatment would be successful other than for the fruit quality issues.

More investigation may be warranted to determine the nature and cause of the off flavours. If due to high CO_2, then they may have been elevated by the infested fruit, the prolonged storage period and / or the chamber loading being volumetrically high. Possible approaches to rule out a CO_2 effect include testing fruit in the chamber with no MB and / or testing with un-stored fruit.

If MB is judged to be important as a disinfestation protocol, then it may be worth continuing with further research. In this context, the important issue is the treatment effect on fruit quality. Further research could involve looking at the fruit at different times in the season, from different locations, at various maturities and subjected to alternative postharvest handling. This may determine the basis for obviating skin damage and, more importantly, off flavours.

Methyl bromide has been identified as an ozone depleting gas. However, its use for quarantine treatments has been exempted in the current phase-out following the Montreal protocol. Recent advances and commercialisation of capture and destroy technology for MB likely ensures its continued use.

Keywords

Murcott mandarins; citrus; methyl bromide; Queensland fruit fly; *Bactrocera tryoni*; disinfestation; fruit physiology; fruit quality; postharvest; quarantine.

Introduction

The aim of the project was to determine the technical feasibility of low-dose methyl bromide (MB) as a quarantine disinfestation treatment for Murcott mandarins. The treatment is only technically feasible if it can kill the insect of quarantine concern, Queensland fruit fly (QFF), *Bactrocera tryoni* (Froggatt), without injuring the fruit.

Murcott mandarins (*Citrus reticulata*), are currently exported to China and Thailand which are significant markets for Queensland mandarins. The current market access protocols utilizing cold storage disinfestation protocols which require that the fruit be maintained at 1-3°C for 16-21 days. However the industry is experiencing barriers to this trade from logistics, cost and out-turn quality. Methyl bromide was investigated as a potentially more suitable treatment to maintain and increase these markets.

Methyl bromide is currently the predominant fumigation for phytosanitary purposes and is used for disinfestation of many fruits and vegetables (Heather and Hallman 2008). Market access protocols exist for fumigation of Australian citrus for export to Indonesia with 64g/m^3 MB for 2 hours at 21°C (Anon 2015) and for interstate trade within Australia with a schedule 24-48g/m^3 for 2 hours at 10-31°C (MB rate increasing with decreasing temperature) (Anon 2008). Neither protocol is currently in use as they cause fruit injury.

Lingren and Sinclair (1951) showed that 32g/m^3 MB for 2 hours at 26.7°C was not a safe concentration for fumigating Navel oranges, Valencia oranges, grapefruit and coastal lemons, although injury was insignificant in lemons from interior areas. Armitage and Steinweden (1946) reported that fumigation at 40g/m^3 MB at 10-16°C for 2 hours was too phytotoxic for grapefruit and orange. Benschoter (1979) determined that 40g/m^3 (at 20% load) or 56/m^3 (at 80% load) for 2 hour duration was required to kill Caribbean fruit fly (*Anastrepha suspensa*) (Loew) in grapefruit at 21-24°C.

Fourney and Houck (1994) concluded from a review of literature that in general, longer exposure times with lower concentrations of methyl bromide usually cause less injury to a susceptible commodity than short exposures with high concentration. Wyatt *et al*. (2013) tried to reduce injury while still killing QFF in Murcott mandarins by reducing the methyl bromide concentration but increasing the treatment duration. Trials were conducted at 20g/m^3 methyl bromide for 4 hour duration at 18g/m^3, but achieved only 91% mortality of QFF. An increase to 6 hour treatment duration at 20g/m^3 at 19°C resulted in 99.99% mortality (Wyatt *et al*. 2013).

Fruit quality trials were conducted with 20g/m^3 MB at 18°C at 4 and 6 hours (Wyatt *et al.* 2013). Fruit were assessed after 21 days held at 5°C and again after a further 7 days at 22°C. There was no fruit injury from the 4 hour treatment. There was evidence of premature skin senescence and a decrease in taste quality in fruit treated for 6 hours, which was not evident after storage at 5°C for 21 days, but which presented after 7 days at 22°C.

Combining the insect and fruit data shows that 20g/m^3 for 6 hours at 18°C was not sufficient to kill all the insects and was also at the upper threshold for fruit quality (Wyatt *et al.* 2013). The strategy for this research was to further decrease the concentration of methyl bromide to 16g/m^3 while increasing the treatment duration and determine the efficacy against QFF and assess fruit injury.

Methodology

Four fruit quality trials (Trials FQ1-4) were conducted to assess the response on Murcott mandarins to four methyl bromide treatment schedules chosen based on previous research (Wyatt et al. 2013). The fruit was harvested in two batches in two consecutive weeks and treated at 4-6 days from harvest. As final assessments were not made until 28 days after treatment, the four trials were essentially in progress simultaneously. This was because of the need to treat the fruit as soon as possible after harvest.

Assessment of the effect of the four fumigation schedules on fruit quality (external and internal defects, skin gloss, skin colour, weight loss, titratable acidity, total soluble solids and taste) were undertaken 21 and 28 days after treatment.

When the results of these trials were collated it was apparent that fruit quality information from additional fumigation schedules was desirable. Two further fruit quality trials (Trials FQ5-6) were conducted under the same fumigation conditions as the first two disinfestation trials against QFF (Trials QFF1-2). A reduced number of fruit quality assessments were made on these fruits, based on the parameters that were identified to be most important from Trials FQ1-4. These trials were conducted on the second batch of fruit, which had by then been stored at 5°C for 4 weeks. While it is not optimal to conduct quality trials on stored fruit, as the harvest period harvest period had passed, this was the only option available to test the fumigation parameters used in FQ5-6.

After the results of trials FQ5-6 and QFF1-2 were collected, a further four disinfestation trials (QFF3-6) were conducted.

Fruit

Murcott mandarins obtained from 2PH Marketing Pty Ltd, Emerald, Queensland were used for all trials. The fruit were harvested into field bins and cooled to 0°C prior to packing. The fruit were washed in a controlled pH 7-8 water tank and sprayed with the fungicide Chief Aquaflo 0.1% in a grading line and waxed with Carnauba wax. The fruit were then transported to Brisbane in refrigerated road transport at 6°C.

Research Facilities

Refrigerated storage and fruit assessments were undertaken at the facilities of Department of Agriculture and Fisheries (DAF), Ecosciences Precinct, Dutton Park, 4102, Queensland. All fumigations were conducted by Hannay Douglas Pty Ltd, 89 Medway Street, Rocklea, 4106, Queensland, a commercial fumigator in a 1.1 m^3 research-scale fumigation chamber.

Fruit quality trials

When the fruit arrived at the laboratory, they were sorted based on uniform initial good quality (viz. intact skin, no blemishes, uniform and normal colour, no stem damage and similar size). Two hundred (200) fruit were selected for each of the trials, with additional fruit being used as fillers in the fumigation treatment chamber. On the day and evening before each treatment, the fruit were held in a temperature controlled room set at 17°C to equilibrate to this fumigation temperature. This action was to minimize the risk of condensation forming on the fruit, which might otherwise increase the risk of damage caused by exposure to MB.

Blemish-free fruit were randomly assigned for fumigation treatments (control and MB fumigated), and fruit holding times of days at 5°C followed by 7 days at 22°C, respectively. Five replicates were used in trials FQ1-4, and four replicates in trials FQ5-6. Each replicate was comprised of 9 individual fruit sub-samples. Thus, the trial structure was 2 fumigation conditions x 2 holding times x 5 (or 4) replicates x 9 sample fruit per replicate. The averages (of the samples) for each replicate were adopted as the experimental units for statistical analyses.

Insect trials.

Insects

Bactrocera tryoni used in our experiments came from a laboratory colony maintained at DAF Ecosciences Precinct laboratories, Dutton Park, Queensland and reared on a carrot-based medium using the method of Heather and Corcoran (1985). Adults were fed water, sugar and autolysed brewer's yeast, separately.

Infestation

To reduce variability of infestation, each fruit was punctured 10 times with a pin (0.5 mm diameter) at the flower end and placed into cages of 12,000-15,000 mature *B. tryoni* (sex ratio of about 1:1 male to female) for 20-80 minutes. Fruit were placed in rows, with one fruit allocated as an untreated control in each row. Additional fruit were also infested for destructive sampling at treatment time to estimate the proportion of each lifestage present in each trial and used as probe fruit to measure core temperature during treatment. After infesting the fruit were held in controlled environment rooms at 26 ±1°C and 70 ±5% RH for 9 days, except for Trial 17 which was held for 8 days, to allow development of the insects to the third instar larvae as this stage has been shown to be the most tolerant to methyl bromide in previous research.

As only one fumigation chamber was available, six large scale trials (QFF1-6) each testing more than 5,000 third instar larvae of QFF were conducted separately.

Fumigation treatments

The fruit for the fruit quality trials were allowed to equilibrate overnight at 15°C prior to treatment. Infested fruit and non-infested filler fruit (to ensure correct chamber loading) for fruit fly trials were cooled at 14°C for 1-3 hours immediately prior to start of treatment. Once the target fruit core temperature had been reached the fruit was packed into lidded cardboard fruit boxes and transported to the fumigation facilities (Hannay Douglas Pty Ltd, Rocklea, Queensland).

All fumigations were conducted in a single 1.1m^3 steel chamber, held inside a refrigerated shipping container to maintain the required temperature. Chamber loads, estimated as a proportion (%) of the volume occupied by the boxes of fruit relative to the chamber volume was 25-32% for both insect and fruit quality trials.

The chamber headspace was sampled at two places in the chamber at the start, mid-point and end of each fumigation treatment and was analysed by gas chromatography. A detailed description of the methods for the fumigation procedure, the analysis of methyl bromide in the chamber and the measurement of fruit pulp and chamber temperature is included in Appendix 1. The nominal dose and the actual concentration of methyl bromide in the chamber headspace at the start, mid-point and end of the treatment from all trials are included in Appendix 2, Tables A2.12 and A2.13. The mean and maximum chamber air and fruit core temperatures for all trials were recorded and are

presented in Appendix 2, Tables A2.14 and A2.15.

For fruit fly trials, after treatment start, 3-6 additional fruit, which had been infested along with the trial fruit and held under the same conditions until treatment start, were dissected. All eggs and larvae were removed and examined under a dissecting microscope. Larvae were identified to instar according to Anderson (1962) counted and the proportion of each lifestage determined for each trial. The proportion of each lifestage is shown in in Appendix 2, Table A2.11.

Post treatment

Fruit quality Assessments.

Treated fruit were returned to the laboratory and along with Control fruit that had remained at 17°C during the methyl bromide treatment period were then divided into 5 replicates each of 9 individual sub-sample fruit for trials FQ1-4 and four replicates each of 9 individual fruit for trials FQ5-6. Fruit were placed on small styrofoam trays and placed inside thick brown paper bags. Fruit were stored at 5°C and 85-90% RH and were assessed on day 21. Thereafter remaining fruit samples were moved to a vented temperature controlled room at 22°C and assessed after another 7 days of storage at day 28.

Ten (10) different parameters were used to assess any differences in fruit quality between the Control and Treated fruit. These parameters were weight loss, titratable acidity (TA), total soluble solids (TSS), flavour, external defects (2 aspects of visual quality and skin injury), internal defects, skin gloss, and skin colour. Some parameters were not assessed for trials FQ5-6, those being weight loss, TSS & TA.

Assessments on fruit were undertaken on days 21 and 28, respectively, for all trials. External and internal defects, skin gloss, and skin colour were assessed on each individual fruit. Weight loss was assessed for composite samples of 9 fruit. For the destructive analyses (i.e., titratable acidity, total soluble solids and taste), one half of all the nine fruit in each sub sample were juiced and treated as a composite sample. Detailed description of the assessment methodology is included in Appendix 1.

Determination of insect mortality.

The fumigated fruit and untreated controls were placed on gauzed plastic containers over vermiculite, in ventilated plastic boxes and placed in controlled environment rooms at 26°C ± 1°C and 70% RH for collection of surviving pupae. The number of pupae recovered per control fruit was used to estimate the number of insects treated in each trial.

Statistical Analysis.

The corrected mortality with 95% confidence for fruit fly was calculated using CQT_Stats (Liquido and Griffin 2010) and the methods of Couey and Chew (1986). Corrected mortality was based on the estimated number of insects treated and the number of surviving pupae. For fruit quality assessments, data were subjected to factorial analysis of variance (ANOVA) using GenStat (16^{th} edition, VSN International Ltd, Hemel Hempstead, UK.). Factors used in the ANOVA were treatment and days, with the treatment x day interaction. Where the ANOVA returned a significant F test, least significant differences (LSD) were calculated at the 5% level (i.e. P=0.05) for pairwise comparison of treatment means. Pooled standard errors are also provided to indicate the variability around the means.

Outputs

The output from this research was a data package showing the effect of a range of MB fumigation schedules on the out-turn quality of Murcott mandarins and the mortality of QFF. The trials conducted in this research project are shown in Table 1.

Table 1. Treatment parameters for Fruit Quality (FQ) and Fruit Fly (FF) trials. FQ trials 5 and 6 were treated simultaneously with FF trials 1 and 2, with both lots of fruit in the fumigation chamber together.

Trial	Date	Treatment parameters
FQ1	30/7/14	18g/m^3 MB at 18°C for 9 hours duration
FQ2	31/7/14	18g/m^3 MB at 18°C for 10 hours duration
FQ3	06/8/14	16g/m^3 MB at 18°C for 9 hours duration
FQ4	07/8/14	16g/m^3 MB at 18°C for 10 hours duration
FQ5	28/8/14	16g/m^3 MB at 18°C for 7 hours duration
FQ6	04/9/14	16g/m^3 MB at 18°C for 8 hours duration
FF1	28/8/14	16g/m^3 MB at 18°C for 7 hours duration
FF2	04/9/14	16g/m^3 MB at 18°C for 8 hours duration
FF3	16/10/14	18g/m^3 MB at 20°C for 7 hours duration
FF4	23/10/14	18g/m^3 MB at 20°C for 8 hours duration
FF5	30/10/14	18g/m^3 MB at 18°C for 8 hours duration
FF6	10/12/14	18g/m^3 MB at 20°C for 8 hours duration

Fruit Quality (FQ) Trials.

The results from the analysis of all data are tabulated in Appendix 2, Tables A2.1 – A2.10.

Weight loss

The Treated fruit generally showed significantly more weight loss than the Control fruit at days 21 and 28 for trials FQ1-4. An exception was for trial FQ1 at 21 days, when there was no significant difference. Although the differences were significant due to the low variability, as the differences were small in magnitude at between 1-2%. Weight loss was not recorded for FQ trials 5 and 6 (Appendix 2, Table A2.1).

Titratable Acidity

Titratable acidity was lower in the Treated fruit in all trials (i.e., FQ1-4) and both sample days. Because variability was very low, Treated and Control fruit are significantly different even though the proportional change of citric acid was <0.1% in cases. Titratable acidity was not measure for trials FQ5 and 6 (Appendix 2, Table A2.2).

Total soluble solids

Total soluble solids (TWW%) was less in the Treated than the Control fruit in all trials (i.e., FQ1-4) at days 21 and 28. However, this difference was only significant in Trial FQ1 at day 21, in trial FQ2 at day 28 and in trial FQ4 at day 21. In all cases the difference was < 0.1% between the Control and Treated fruit.

External defects – skin injury area

Minor external skin defects which presented as a darker skin colour were evident on some fruit in all all of the day 21 Treated fruit samples, other than in trial FQ6. However the defect was not prevalent enough to be significant (Appendix 3, Plate 1). At day 28, the external defects were greater for methyl bromide treated fruit in trials FQ1-4. In trial FQ5, a small amount of external damage was evident. But, in trial 6, it was not significantly different between the Treated and Control fruits at day 21. Nonetheless, it was significantly greater in Treated fruit in trials FQ1-4 and trial FQ5 but not trial 6. In trial FQ6, there were no external defects at 21 days and the defect was minimally evident in only 1 piece of fruit from 36, on day 28.

External defects – visual quality

At day 21, there were slight differences only in visual quality with no significant differences found. However, by day 28 the visual quality of all Treated fruit in trials FQ1-4 had declined significantly. Some Treated fruit displayed severe mould development on damaged skin spots and areas (Appendix 3, Plates 2-6,). Control fruit continued to display no external skin defects at 28 days. There were no significant differences between treated and control fruit in trials 5 and 6.

Internal defects

No internal defects were found in trials FQ1-4 at 21 days. However, at day 28 some fruit exhibited a defect in the form of a separation between the skin and the fruit flesh (Appendix 3, Plate 7). Treated fruit from all six trials showed this defect at day 28, to a greater or lesser severity. Roughly half the treated fruit in each trial had this character. This defect was not observed in any control fruit.

Skin gloss

No reduction in skin gloss was observed at day 21 in any of the six trials. In trials FQ1 and 3, roughly half the fruit showed a reduced level of gloss at day 28. Trials FQ2 and 4 had ~ 25% of Treated fruit with a reduced gloss. Trials FQ5 and 6 had 1 and none fruit, respectively, with reduced gloss at day 28.

Skin colour

There was no difference in skin colour between Treated and Control fruit at day 28, so the MB treatment had no effect on colour.

Flavour

The MB treatment caused adverse flavours in treated fruit in all trials at both sample times. Flavour was assessed by tasting juice. For all trials, the juice was tasted and the assessors scored the flavor using a hedonic scale from ‘like extremely’ through to ‘dislike extremely’ (Table A2.9). In all trials and all storage times Treated fruit were scored lower than Control fruit. Off flavours were often discerned by a majority of tasters and sometimes described as being either, bitter, metallic, or having a medicinal type flavour. In general Control fruit were discerned as tasting

sweeter or having more of a fresh flavour.

Additional tasting assessments were undertaken for Trials FQ5 and 6 only. Panellists were each presented with 2 sets of 3 samples of Murcott juice - 1 set contained 2 Control samples and 1 Treated sample and another set contained 2 Treated samples and 1 Control sample. Panellists were asked to identify the different sample in each set of three. Results were expressed as the proportion (%) of tasters correctly identifying the different (Treated or Control) sample from each set (Appendix 2, Table A2.10). Results show that the tasters were correctly able to differentiate between treated and control samples at least 67% of the time. Taste difference test showed that in all cases the majority of persons asked to identify whether Treated and Control samples were different were able to do so. Thus the number of tasters who could differentiate the treated from the control samples was statistically significantly above 33% which would be the correct proportion by chance.

Fruit fly (FF) Trials

Six large scale trials were conducted against third instar larvae of *B. tryoni*. The number of Treated and Control fruit used in each trial, the number of surviving pupae from control fruit in each trial, and the estimation of the number of insects treated is also shown in Table 2. Each trial treated more than 5,000 insects. All trials were against third instar larvae which made up between 74-94 % of insects except for trial FF1 which was predominantly second instars. The results of the examination of the instar checks are shown in Table A2.11 in Appendix 2.

Trials FF1, 2, 3 and 5 all resulted in some insects surviving the treatment although the mortality was very high with less than 1% of insects surviving the treatment. Trial FF4 resulted in no survivors from an estimated 20,631 insects treated. This trial was replicated in trial FF6, which also resulted in no survivors from an estimated 11,028 insects treated. According to the methods of Couey and Chew (1986), combining these trials results in no survivors from an estimated 31,659. This equates to a mortality of 99.9905 at the 95% confidence level.

Table 2. Mortality of second and third instar larvae of *B. tryoni* in methyl bromide fumigation trials in Murcott mandarins.

Trial	Stage	Number of fruit		Number of surviving pupae		Estimated no. of insects treated*	Observed mortality
		Control	Treated	Control	Treated		
FF1	L2/L3	59	295	1,227	28	6,135	99.54
FF2	L3	56	293	1,060	15	5,546	99.73
FF3	L3	48	211	3,378	32	14,849	99.78
FF4	L3	59	266	4,576	0	20,631	100.00
FF5	L3	54	229	4,509	9	19,122	99.95
FF6	L3	51	242	2,324	0	11,028	100.00

* Estimated number of insects treated = number of surviving pupae in control/number of control fruit x number of treated fruit.

A summary of treatment parameters and mortality of *B. tryoni* fumigated with MB for each of the trials is shown in Table 3. The MB concentration was not calculated on the weight of MB added to the chamber, instead analysis of samples from the chamber headspace with gas chromatography established the actual treatment parameters. The two samples were taken at the start, mid-point

and end of each treatment. All measurements of gas concentrations measured and calculations to determine CT product are included in Appendix 2, Table A.13. The mean of the two initial samples is included in Table 3. This table shows the number of insects treated, survivors and mortality at the 95% CL for each trial.

The fumigations conducted at 16g/m^3 MB for 7 and 8 hours at 17°C both resulted in survivors, as did the fumigations at 18g/m^3 MB for 7 hours at 20°C and 8 hours at 18°C. The two trials conducted at 18g/m^3 MB at 20°C for 8 hours were successful with no survivors from a combined total of 31,659 insects. This equates to 99.9905% mortality at the 95% CL (Liquido and Griffin 2010).

Table 3. Actual treatment parameters and mortality of *Bactrocera tryoni* fumigated with methyl bromide (MB).

Trial	MB conc* (g/m^3)	Temp (°C)	Duration (h)	CT product g h m^{3**}	Estimated no. of insects treated	Survivors	Corrected mortality (95% confidence)
FF1	16	17**	7	97	6,135	28	99.3743
FF2	16	17**	8	110	5,546	15	99.5835
FF3	18	20	7	110	14,849	32	99.7105
FF4	18	20	8	115	20,631	0	99.9855
FF5	18	18	8	117	19,122	9	99.9179
FF6	18	20	8	125	11,028	0	99.9728

* At treatment start, from analysis of chamber headspace.

** Nominal treatment temperature 18°C

** Calculated using the actual chamber headspace concentrations at the beginning, mid-point and end of treatment. All values in Table A2.13 in Appendix 2.

Outcomes

The expected outcome from this research was a data package which could be used to determine:

- Whether low-dose methyl bromide (MB) can cause quarantine levels of mortality to QFF without causing injury to fruit, and thus whether this technology can be developed as a market access protocol to meet requirements of international quarantine authorities.
- The feasibility of developing a large project sufficient to generate data for a market access submission.

The results from this research showed that:

- There was no combination of MB concentration, treatment duration, and temperature tested in this research which did not adversely affect the fruit, even though the treatment resulted in complete mortality of the insects.
- It is not feasible at this stage to develop a large project sufficient to generate data for a market access submission without conducting further investigations into the adverse flavour results discerned in this research.

The first four fruit quality trials at 16 and 18g/m^3 MB for 9 and 10 hours showed that:

- The fumigation treatments had no effect on fruit quality parameters of weight loss, titratable acidity, total soluble solids and skin colour at any of the assessment times.
- After fumigation plus storage at 5°C for 21 days to simulate shipping, there was no significant effect on internal and external defects or skin gloss.

But:

- Internal and external defects and gloss were significantly reduced in fumigated fruit after the additional 7 days at 22°C, simulating a retail sales period.
- More obvious was that flavour was significantly affected, with the presence of off-flavours after both assessment time intervals.

Positively, the fruit fly trials showed that:

- These schedules were at higher doses than is required to kill fruit flies.
- Quarantine levels of mortality were achieved after fumigation at 18g/m^3 MB at 20°C for 8 hours.

However, the schedules shown to be insufficient to kill fruit flies of 16g/m^3 for 7 and 8 hours applied in FF trials 1 and 2, were also tested in fruit quality trials 5 and 6 and they:

- Showed no adverse effects on internal quality, external quality, gloss, weight loss, titratable acidity, total soluble solids and skin colour.
- But, still caused adverse effects on flavour.

Evaluation and Discussion

Previous research (Wyatt *et al.* 2013) showed that fruit quality trials at 20g/m^3 MB at 18°C for 4 hours produced no injury to Murcott mandarins but an extension of this dose to 6 hours caused premature skin senescence and a minor decrease in taste quality. However the similar dose of 20g/m^3 MB at 19°C for 6 hours did not achieve complete mortality of QFF with 1 survivor from 6,685 treated. Combining the insect and fruit data showed that 20g/m^3 MB for 6 h at 18°C was not sufficient to kill all the insects, but was also at the upper threshold for fruit quality (Wyatt *et al.* 2013). Previous research (Lingren and Sinclair 1951) showed that 16g/m^3 MB was a safe dose for a range of citrus, although Murcott mandarins were not specifically tested.

Fourney and Houck (1994) concluded from a review of literature that the critical fumigant concentration (C) multiplied by the exposure time (T) that is efficacious for an insect pest can be derived from numerous combinations of concentration and exposure times at the same temperature. However, in general, longer exposure times with lower concentrations of MB usually cause less injury to a susceptible commodity than do short exposures with high concentration.

The strategy for this research was to investigate lower concentrations of MB combined with increased treatment duration. This was so that fruit injury could be avoided, but higher mortality levels would result from the increased CT product. The fruit quality trials commenced with the MB concentration reduced from the 20g/m^3 applied in earlier trials (Wyatt *et al.* 2013) to 16g/m^3 MB but with extended treatment durations of 9 and 10 hours. After the treatment, the fruit was held at 5°C for 21 days to simulate shipping and then a further 7 days at 22°C to simulate retail sales.

Assessment of fruit quality conducted at both holding times showed that weight loss, titratable acidity, total soluble solids and skin colour were not affected by the treatment. Internal and external defects were not apparent after storage at 5°C for 21 days. However, a significant amount of injury occurred during the additional 7 days at 22°C, which simulated the retail sales period, suggesting no storage capacity at market arrival. The gloss of the fruit was also slightly reduced in ~ 50% of the fruit. The damaged skin evidently also allowed entry of pathogens (Plates 3-6).

Armitage and Steinweden (1946) have reported that injury was slow in appearing after fumigation with 32g/m^3 MB and resulted in deep pitting due to gradual breaking down of oil cells and intercellular tissues. Lindgren and Sinclair (1951) reported injury to citrus from 32g/m^3 MB showing as a pitting and subsequent browning. Hatton and Cubbedge (1979) using very high rates of 40 and 56g/m^3 MB described the effect of MB fumigation on oranges as resulting in a water-soaked appearance and green mould develop profusely, indicating that MB had injured the peel.

The next series of trials (FQ5 and 6, FF1 and 2) were conducted assessing fruit injury and fly mortality. The dose was maintained at 16g/m^3 MB but treatment duration reduced to 7 and 8 hours. Fruit assessments from these trials showed that the internal and external defects and the issue with gloss were absent with the reduced treatment duration. However, there were still adverse effects on flavour, albeit less so. Unfortunately these doses were not sufficiently efficacious against QFF with survivors in both the 7 and 8 hour treatments.

Further insect trials also resulted in survivors when the concentration of methyl bromide was increased to 18g/m^3 for 7 hours at 20°C and 18g/m^3 for 8 hours at 18°C. It was important to at least find a treatment that was efficacious against the fruit flies. Thus, a treatment of 18g/m^3 MB for 8 hours at an increased temperature of 20°C was tested. This dose was effective against third instar

QFF. Accordingly, a second trial using these parameters was conducted. Combining the results of these trials using the methods of Couey and Chew (1986) showed that this dose meets quarantine requirements with no survivors from a combined total of 31,659 insects, which equates to 99.9905% mortality at the 95% Confidence Level (Liquido and Griffin 2010).

The fruit injury trials (FQ5 and 6) at 16g/m^3 MB for 7 and 8 hours showed that only flavor remained a problem. In addition to using a ranking system for taste, panelists were also asked if they could identify the odd sample when given a choice of two treated samples and a control or two control samples and a treated. Even though the off flavours were much reduced from the earlier trials, the tasters could still differentiate between the treated and control samples. The off flavours in trials FQ1-4 were very 'bitter' and 'metallic', although they were less intense in the trials FQ5 and 6. Only fruit with no indication of mould were tasted, and so it is likely that off flavours were not due to the mould.

Off-flavours are the most significant issue from these trials. It is possible that the off-flavours were caused or accentuated by CO_2 accumulation during MB fumigation. Based on typical Murcott respiration rates of about 40ml/kg/hr at 20^{o}C (Hofman *et al.* 2013), 90 kg of fruit in the treatment chamber and 30% of the chamber being filled with fruit, it is estimated that 3.2-3.7% CO_2 would have accumulated after 7-8 hr. This relatively low concentration and the treatment duration by itself was unlikely to result in a lasting effect on off-flavours after 21-28 d storage. However, there could conceivably have been a MB by elevated CO_2 interaction. In addition, waxing, even with carnauba-based waxes, can result in off-flavours depending on storage condition and duration. However, lower storage temperatures and holding conditions after treatment may reduce the risk of off-flavours. Further evaluation of these factors (e.g. off-flavour testing at several stages from fumigation to after simulated retail shelf conditions) may possibly be warranted with a view to possibly identify treatment combinations for an acceptable MB treatment.

Fruit fly trials at 18g/m^3 MB for 8 hours at 20°C resulted in no survivors from more than 30,000 insects tested, which meets quarantine requirements of Thailand and China. Thus, this treatment would be successful other than for the fruit quality issues. More investigation may be warranted to determine the nature and cause of the off flavours. If due to high CO_2, then they may have been elevated by the infested fruit, the prolonged storage period and / or the chamber loading being volumetrically high. Possible approaches to rule out a CO_2 effect include by testing fruit in the chamber with no MB and / or testing with un-stored fruit.

MB has been identified as an ozone depleting gas. However, its use for quarantine treatments has been exempted in the current phase-out following the Montreal protocol Recent advances and commercialisation of capture and destroy technology for MB likely ensures its continued use.

Recommendations

If methyl bromide is judged to be important as a disinfestation protocol, then it may be worth continuing with further research. In this context, the important issue is the treatment effect on fruit quality.

Further research could involve looking at:

- Fruit at different times in the season, from different locations, at various maturities
- Fruit subjected to alternative postharvest handling
- The levels of CO_2 in the chamber and effect of CO_2 on fruit quality.

This may determine the basis for obviating skin damage and, more importantly, off flavours.

There is strong positive insect control data to provide commercial justification for more in-depth fruit biology and acceptance research. Nonetheless, a few confirmatory insect trials to confirm efficacy would be needed.

The long treatment times of 8 hours are still a logistical improvement on the current market access protocols utilizing cold storage disinfestation which require that the fruit be maintained at 1-3°C for 16-21 days.

Scientific Refereed Publications

None to report.

Intellectual Property/Commercialisation

No commercial IP generated.

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Appendices

Appendix 1.Detailed Methodology

Appendix 2. Detailed results

Appendix 3. Plates

Appendix 1. Detailed Methodology

A1.1 Fumigation procedure.

Liquid MB entered the dispenser from a 25 kg commercial cylinder, and a measured amount was slowly allowed to flow into the vaporiser from where the MB entered the chamber as a gas and was dispersed by a fan at the rear of the chamber. Once the gas had entered the chamber, a 50 ml sample of the chamber headspace was taken with a gas tight syringe with Luer lock (SGE Analytical Science), from each of two sampling valves, at the front and back of the chamber, and also at the mid-point and at the end of the treatment. CT product for each trial was calculated by the method of Bond (1984). After treatment duration , the chamber was vented for 1 hour to remove the MB and the fruit were then transported back to the laboratory.

A1.2 Methyl bromide analysis using Gas Chromatography.

Gas samples were injected into a Perkin Elmer 580 Gas Chromatograph with Pneumatic Pressure Control (PPC) using a gas tight syringe (50 ml) with Luer lock (SGE Analytical Science), and a 1 ml sampling loop via a 6 port GSV sampling valve. Separation was achieved using an Elite – Q Plot column (0.53 µm x 30 m) (Perkin Elmer, USA). Chemical analysis was performed using a Flame Ionisation detector (FID) at 220°C, with flows of carrier gas of 8 ml/min He and flame, gases of 45ml/min H2 and 450 ml/min air. The MB retention time was 2.8 min. System control and data analysis was by a dotLINK integrator and TotalChrom Workstation Software (Perkin Elmer, USA). Calibration curves were obtained by analysis of sample cylinders of known concentration of bromomethane (MB) in ultrapure air as obtained from Scott-Marrin, Riverside, CA, USA.

A1.3 Temperature recording.

Tinytag® Ultra 2 temperature data loggers (Type TGU-4510) (Gemini Data Loggers (UK) Ltd Chichester, West Sussex, England.) with two sensors (chamber air and thermistor probe for fruit core), were used to monitor temperature at 5 minute intervals during treatment.

A1.4 Fruit Assessment

Weight Loss -
Fruit weight was measured with a Mettler PE 3600 electronic balance. All weight losses were subsequently expressed as a proportion (%) of the original weight for each sub sample of 9 fruit. Weight loss was not recorded for Trials 5 and 6.

Titratable Acidity (TA)
TA expressed as % citric acid equivalents was determined by hand titration with 0.1N NaOH solution of 10 ml composite juice samples containing 3 drops of 0.2% Phenolphthalein to a definite, consistent and persistent colour change end point.

Total Soluble Solids (TSS)
% Total Soluble Solids was measured with a handheld refractometer (Otago Pocket refractometer PAG-1).

External Defects
External defects scores were made for all fruit in each post-treatment assessment for all 6 trials. The visual subjective assessment was performed by one technician to ensure consistency. Two rating systems were utilised to try and ensure that any and all external defects were being evaluated. Fruit were assessed for external defects based on **skin injury area** estimated in sq cm. 0=Nil, 1=0.5 sq cm, 2= 1 sq. cm, 3= 1.5 sq. cm, 4= 2 sq. cm. up to 9= 4.5 sq cm.(increments of 0.5 sq. cm.), and, for **visual quality** where 1 =Extremely poor, not useable,3 = Poor, excessive defects, 5=Fair, slight to moderate defects, 7= Good, minor defects, 9= Excellent , essentially free from defects

Internal Defects
All fruit were sliced laterally in half to expose the middle of the segments to reveal any internal defects present. Individual fruit were rated as per the following scores:
0=Nil. 1=Slight, 2= Moderate, and 3=Severe.

Skin Gloss
Fruit gloss was assessed for all treatments as per the following rating scores: 3 = Highly glossy, 2 = Reduced gloss, and, 1 = Dull, no gloss.

Skin Colour
One technician assumed responsibility for rating all the fruit through all the trials in order to ensure consistency. Skin colour was assessed using the following visual rating scale.
Skin colour 0-5 scores were:5 = Deep orange, 4 = Orange, 3 = Yellow/orange, 2 = Yellow, 1 = Light yellow/ Green and 0 = Light green.

Flavour
One half for each of the 9 fruit in each sub-sample was juiced and the five sub samples combined to result in two samples (Control and Treated) for each assessment period (i.e. days 21, and 28) for Trials 1-4. A panel of either 6 (Trial 2&3) or 10 (Trial 1&4) untrained people were used from a small pool of persons available throughout the trials. The participants were invited to make general comments and to score flavour using a hedonic 1-9 Likeability Scale: 1 = Dislike extremely,2=Dislike very much, 3=Dislike Moderately, 4= Dislike slightly, 5 = Neither Like nor dislike, 6=Like slightly, 7=Like moderately, 8=Like moderately and 9 = Like extremely.

Additional tasting assessments were undertaken for Trials 5 and 6 only. Available panellists were each presented with two sets of three samples of Murcott juice - One set contained two Control samples and 1 Treated sample and another set contained 2 Treated samples and 1 Control sample. Panellists were asked to identify the **different sample** in each set of three. Results were expressed as a proportion (%) of tasters identifying the correct different sample (Treated or Control) from each set.

Appendix 2. Detailed results

A2.1 Fruit Quality Experiments.

Table A2.1. Weight loss as a proportion (%) of initial fresh mass

Fruit weight loss (%)		DAY 21[#] Mean	DAY 28[##] Mean	Pooled s.e.
Trial FQ1: 18g/m^{3}MB at 18°C for 9 h	Control	2.31	3.76^a	0.120
	Treated	2.56	5.96^b	
Trial FQ2: 18g/m^{3}MB at 18°C for 10 h	Control	2.31^a	3.79^a	0.058
	Treated	2.58^b	5.04^b	
Trial FQ3: 16g/m^{3}MB at 18°C for 9 h	Control	2.67^a	3.43^a	0.103
	Treated	3.04^b	4.76^b	
Trial FQ4: 16g/m^{3}MB at 18°C for 10 h	Control	2.20^a	2.84^a	0.053
	Treated	2.57^b	3.82^b	

[#] Constantly held at 5°C. [##] First 21 d at 5°C; moved to 22°C for final 7 d.
Within trials and days, means with different superscripts are significantly different (P<0.05).

Table A2.2. Titratable Acidity expressed as the percentage of citric acid

Titratable acidity (%)		DAY 21[#] Mean	DAY 28[##] Mean	Pooled s.e.
Trial FQ1: 18g/m^{3}MB at 18°C for 9 h	Control	0.47	0.52	0.012
	Treated	0.44	0.51	
Trial FQ2: 18g/m^{3}MB at 18°C for 10 h	Control	0.63^a	0.56^a	0.015
	Treated	0.57^b	0.50^b	
Trial FQ3: 16g/m^{3}MB at 18°C for 9 h	Control	0.62	0.62^a	0.019
	Treated	0.56	0.54^b	
Trial FQ4: 16g/m^{3}MB at 18°C for 10 h	Control	0.67^a	0.59	0.013
	Treated	0.62^b	0.56	

[#] Constantly held at 5°C. [##] First 21 d at 5°C; moved to 22°C for final 7 d.
Within trials and days, means with different superscripts are significantly different (P<0.05).

Table A2.3. Total Soluble Solids % (TSS).

Total Soluble Solids (%)		DAY 21[#] Mean	DAY 28[##] Mean	Pooled s.e.
Trial FQ1: 18g/m^{3}MB at 18°C for 9 h	Control	12.16^a	11.38	0.144
	Treated	11.62^b	11.10	
Trial FQ2: 18g/m^{3}MB at 18°C for 10 h	Control	12.06	11.72^a	0.151
	Treated	11.66	11.22^b	
Trial FQ3: 16g/m^{3}MB at 18°C for 9 h	Control	12.4	12.22	0.196
	Treated	11.94	11.9	
Trial FQ4: 16g/m^{3}MB at 18°C for 10 h	Control	12.72^a	11.88	0.172
	Treated	12.04^b	11.72	

[#] Constantly held at 5°C. [##] First 21 d at 5°C; moved to 22°C for final 7 d.
Within trials and days, means with different superscripts are significantly different (P<0.05).

Table A2.4. External Defects - Skin Injury Area.

Skin Injury Area (cm^2)		DAY 21#	DAY 28##	Pooled s.e.
		Mean	Mean	
Trial FQ1: 18g/m^3MB at 18°C for 9 h	Control	0.00	0.00[a]	0.278
	Treated	0.38	5.29[b]	
Trial FQ2: 18g/m^3MB at 18°C for 10 h	Control	0.00	0.00[a]	0.198
	Treated	0.18	4.20[b]	
Trial FQ3: 16g/m^3MB at 18°C for 9 h	Control	0.00	0.00[a]	0.299
	Treated	0.64	4.93[b]	
Trial FQ4: 16g/m^3MB at 18°C for 10 h	Control	0.0	0.00[a]	0.288
	Treated	0.46	3.46[b]	
Trial FQ5: 16g/m^3MB at 18°C for 7 h	Control	0.00	0.00[a]	0.041
	Treated	0.03	0.11[b]	
Trial FQ6: 16g/m^3MB at 18°C for 8 h	Control	0.00	0.00	0.027
	Treated	0.00	0.05	

Constantly held at 5°C. ## First 21 d at 5°C; moved to 22°C for final 7 d.
Within trials and days, means with different superscripts are significantly different (P<0.05).

Table A2.5. External Defects - Visual Quality.

Visual Quality (1-9)		DAY 21#	DAY 0 to 28##	Pooled s.e.
		Mean	Mean	
Trial FQ1: 18g/m^3MB at 18°C for 9 h	Control	9.00	9.00[a]	0.301
	Treated	8.66	3.44[b]	
Trial FQ2: 18g/m^3MB at 18°C for 10 h	Control	9.00	9.00[a]	0.124
	Treated	8.77	5.31[b]	
Trial FQ3: 16g/m^3MB at 18°C for 9 h	Control	9.00	9.00[a]	0.237
	Treated	8.51	3.95[b]	
Trial FQ4: 16g/m^3MB at 18°C for 10 h	Control	9.00	9.00[a]	0.288
	Treated	8.64	5.33[b]	
Trial FQ5: 16g/m^3MB at 18°C for 7 h	Control	7.94	8.05	0.101
	Treated	7.99	7.75	
Trial FQ6: 16g/m^3MB at 18°C for 8 h	Control	8.30	9.00	0.078
	Treated	8.08	8.77	

Constantly held at 5°C. ## First 21 d at 5°C; moved to 22°C for final 7 d.
Within trials and days, means with different superscripts are significantly different (P<0.05).

Table A2.6. Internal Defects.

Internal Defects (0-3)		DAY 21#	DAY 28##	Number of fruit affected on day 28 (received a rating 1)*.
		Mean	Mean	
Trial FQ1:	Control	0.0	0.0	0
18g/m^{3}MB at 18°C for 9 h	Treated	0.0	0.44	20
Trial FQ2:	Control	0.0	0.00	0
18g/m^{3}MB at 18°C for 10 h	Treated	0.0	0.51	23
Trial FQ3:	Control	0.0	0.00	0
16g/m^{3}MB at 18°C for 9 h	Treated	0.0	0.64	29
Trial FQ4:	Control	0.0	0.00	0
16g/m^{3}MB at 18°C for 10 h	Treated	0.0	0.37	17
Trial FQ5:	Control	0.0	0.00	0
16g/m^{3}MB at 18°C for 7 h	Treated	0.28	0.50	18
Trial FQ6:	Control	0.0	0.00	0
16g/m^{3}MB at 18°C for 8 h	Treated	0.47	0.39	14

Constantly held at 5°C. ## First 21 d at 5°C; moved to 22°C for final 7 d.
*From a total of 45 fruit for trials 1-4 and 36 fruit for trials 5 and 6.

Table A2.7. Skin Gloss.

Skin Gloss (1-3)		DAY 21#	DAY 28##	Number of fruit in each skin gloss score*		
		Mean	Mean	1	2	3
Trial FQ1:	Control	3.00	3.00	0	0	45
18g/m^{3}MB at 18°C for 9 h	Treated	3.00	2.57	0	19	26
Trial FQ2:	Control	3.00	3.00	0	0	45
18g/m^{3}MB at 18°C for 10 h	Treated	3.00	2.84	0	8	37
Trial FQ3:	Control	3.00	3.00	0	0	45
16g/m^{3}MB at 18°C for 9 h	Treated	3.00	2.51	0	20	25
Trial FQ4:	Control	3.00	3.00	0	0	45
16g/m^{3}MB at 18°C for 10 h	Treated	3.00	2.82	1	10	34
Trial FQ5:	Control	2.91	3.00	0	0	36
16g/m^{3}MB at 18°C for 7 h	Treated	2.92	2.97	0	1	35
Trial FQ6:	Control	3.00	3.00	0	0	36
16g/m^{3}MB at 18°C for 8 h	Treated	3.00	3.00	0	0	36

Constantly held at 5°C. ## First 21 d at 5°C; moved to 22°C for final 7 d.
*Score: 3 - glossy, 2 - reduced gloss; 1 - dull.

Table A2.8. Skin Colour

Skin Colour (0-5)		DAY 21# Mean	DAY 28## Mean	Pooled s.e.
Trial FQ1: 18g/m^{3}MB at 18°C for 9 h	Control	4.24^a	4.24	0.063
	Treated	4.55^b	4.24	
Trial FQ2: 18g/m^{3}MB at 18°C for 10 h	Control	4.15	4.35	0.083
	Treated	4.20	4.31	
Trial FQ3: 16g/m^{3}MB at 18°C for 9 h	Control	4.33	4.35	0.100
	Treated	4.24	4.46	
Trial FQ4: 16g/m^{3}MB at 18°C for 10 h	Control	4.31	4.31	0.069
	Treated	4.41	4.51	
Trial FQ5: 16g/m^{3}MB at 18°C for 7 h	Control	4.19	4.60	0.064
	Treated	4.30	4.55	
Trial FQ6: 16g/m^{3}MB at 18°C for 8 h	Control	4.55	4.77	0.071
	Treated	4.55	4.66	

Constantly held at 5°C. ## First 21 d at 5°C; moved to 22°C for final 7 d.

Table A2.9 Flavour scores

Flavour (1-9)		DAY 21# Mean	DAY 28## Mean	Pooled s.e.
Trial FQ1: 18g/m^{3}MB at 18°C for 9 h	Control	7.0^a	6.6^a	0.26
	Treated	5.4^b	3.0^b	
Trial FQ2: 18g/m^{3}MB at 18°C for 10 h	Control	7.0^{na}	7.0^{na}	NA**
	Treated	5.0^{na}	NA	
Trial FQ3: 16g/m^{3}MB at 18°C for 9 h	Control	7.0^a	7.0^a	0.173
	Treated	3.6^b	3.4^b	
Trial FQ4: 16g/m^{3}MB at 18°C for 10 h	Control	7.0^a	7.0^a	0.156
	Treated	3.6^b	3.8^b	
Trial FQ5: 16g/m^{3}MB at 18°C for 7 h	Control	7.2^{na}	6.9^{na}	NA
	Treated	4.2^{na}	4.6^{na}	
Trial FQ6: 16g/m^{3}MB at 18°C for 8 h	Control	6.9^{na}	7.4^{na}	NA
	Treated	4.7^{na}	3.0^{na}	

Constantly held at 5°C. ## First 21 d at 5°C; moved to 22°C for final 7 d.
na – l.s.d not able to be generated, as statistical assumptions violated.
Trial 5 and 6 l.s.d not able to be generated because juice samples were pooled between replicates..
**No treated fruit from trial 2 were tasted because of either mould growth of an unpleasant smell. Only the 'good' fruit which looked fine were tasted. As only 5 fruit out of 45 were tasted strong statements cannot be made. In trials 5 and 6 all the fruit were tasted.

Table A2.10. Flavour difference test

Trial	Day[#]	No. of successful tasters	Total No. of Tasters	Correct proportion	t-test vs 0.333	Sig. level
FQ5	21	16	20	0.8000	5.22	0.000042
	28	8	12	0.6670	2.45	0.030622
FQ6	21	10	12	0.8333	4.65	0.000563
	28	17	20	0.8500	6.47	0.000003

[#] Constantly held at 5°C. [##] First 21 d at 5°C; moved to 22°C for final 7 d.

Table A2.11. Proportion of third instar larvae present in each trial.

Trial	Age of insects at start of treatment (hours)	Proportion of target lifestage present (%)
FF1	193	*
FF2	216	76
FF3	214	94
FF4	215	74
FF5	214	90
FF6	216	78

*Mixture of second (61%) and third (29%) instar larvae

Table A2.12. Measurement of the methyl bromide concentration in the chamber headspace at the start, mid-point and end of each treatment from front and back sampling valves in Fruit Quality trials .

Trial	Methyl bromide concentration (g/m^3)					
	Start treatment		Mid-point		End treatment	
	Front	Back	Front	Back	Front	Back
FQ1	18.00	17.74	*	*	13.47	13.16
FQ2	18.13	18.12	14.74	14.17	12.61	12.08
FQ3	15.69	15.53	13.43	13.08	11.59	11.21
FQ4	15.66	15.57	13.35	12.94	11.08	10.74
FQ5	16.10	16.24	13.77	13.59	12.11	11.78
FQ6	16.69	16.01	13.72	13.29	12.02	11.72

*Problem with GC therefore no reading available

Table A2.13. Measurement of the methyl bromide concentration in the chamber headspace at the start, mid-point and end of each treatment from front and back sampling valves.

Trial	Duration (h)	Methyl bromide concentration (g/m^3)					
		Start treatment		Mid-point		End treatment	
		Front	Back	Front	Back	Front	Back
FF1	7	16.10	16.24	13.77	13.59	12.11	11.78
FF2	8	16.69	16.01	13.72	13.29	12.02	11.72
FF3	7	17.57	17.46	15.84	15.78	13.42	13.63
FF4	8	18.31	17.03	13.62	13.81	12.65	12.55
FF5	8	17.64	17.62	14.44	14.12	12.54	12.22
FF6	8	18.53	17.68	15.54	15.18	13.96	13.63

Table A2.14 The mean and maximum chamber air and fruit core temperatures in Fruit Quality trials. Mean of two loggers at each data point.

Trial	Temperature (°C)			
	Maximum chamber air	Mean chamber air	Maximum fruit core	Mean fruit core
FQ1	18.2	17.8	17.9	17.5
FQ2	18.2	17.8	17.9	17.6
FQ3	18.1	17.7	17.9	17.5
FQ4	18.2	17.8	17.9	17.6
FQ5	17.5	17.1	17.6	16.9
FQ6	17.4	16.9	17.2	16.6

Table A2.15. The mean and maximum chamber air and fruit core temperatures. Mean of two loggers at each data point.

Trial	Temperature (°C)			
	Maximum chamber air	Mean chamber air	Maximum fruit core	Mean fruit core
FF1	17.5	17.1	17.6	16.9
FF2	17.4	16.9	17.2	16.6
FF3	20.3	19.3	20.2	19.8
FF4	20.4	19.7	20.2	19.4
FF5	18.7	17.9	18.4	17.1
FF6	20.8	20.1	20.7	20.0

Appendix 3. Plates

Plate 1. Darker skin colour was evident on some fruit after storage for 21 days at 5°C in trials FQ1-5.

Plate 2. External defects evident in Treated fruit (T2) from Trial FQ1: 18g/m^3MB at 18°C for 9 h after storage for 21 days at 5°C followed by 7 days at 22°C.

Plate 3. External defects evident in Treated (T2) fruit compared with Control (T1) fruit from Trial FQ2: 18g/m^{3}MB at 18°C for 10 h after storage for 21 days at 5°C followed by 7 days at 22°C.

Plate 4. External defects evident in Treated (T2) fruit compared with Control (T1) fruit from Trial FQ3: 16g/m^{3}MB at 18°C for 9 h after storage for 21 days at 5°C followed by 7 days at 22°C.

Plate 5. External defects evident in Treated (T2) fruit compared with Control (T1) fruit from Trial FQ4: 16g/m^{3}MB at 18°C for 10 h after storage for 21 days at 5°C followed by 7 days at 22°C.

Plate 6. External defects evident in Treated fruit from Trial FQ5: 16g/m^{3}MB at 18°C for 7 h after storage for 21 days at 5°C followed by 7 days at 22°C.

Plate 7. Internal defect in the form of a separation between the skin and the flesh after storage for 21 days at 5°C followed by 7 days at 22°C. This plate shows severe separation from the skin. Some fruit had much less separation both in circumference affected and separation distance. All examples of separation were given a rating of 1, regardless of the severity because it was a measure of internal defect, not separation severity. If there had been additional internal problems they would have scored a 2 or a 3. Skin separation was the only internal defect found.