Horticulture Innovation Australia

Final Report

Red Blue Beetle Table Grape Disinfestation Research

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TG13002

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Abbreviations

R&D	Research & development
DEDJTR Vic	Department of Economic Development, Jobs, Transport and Resources, Victoria
HIA	Horticulture Innovation Australia
ATGA	Australian Table Grape Association Inc
Qfly, QFF	Queensland fruit fly (*Bactrocera tryoni*)
Medfly, MF	Mediterranean fruit fly (*Ceratitus capitata*)

Media summary

Australian table grape exports have increased substantially with new markets in China, Japan and South Korea now accessible. However, accidental contamination of table grape export consignments with live insects is a major quarantine issue which may threaten the viability of these new and important markets. Overseas research has shown that exposure to cold and sulphur dioxide (SO_2) can kill some insects. Sea freight export of table grapes to major markets such as China requires at least 4 weeks cold storage during transit and all cartons are treated with a SO_2 generator sheet to control rots. In this experiment, a recorded contaminant of table grape exports, long tailed mealy bug and a common lady bird beetle were placed in cartons of Thompson Seedless table grapes, with or without a SO_2 generator sheet and stored for up to 6 weeks at 1^{o}C to simulate sea freight export.

Grape quality was acceptable after 6 weeks of storage in cartons with SO_2. There was no significant difference between treatments with respect to berry soluble solids concentration, total acidity, berry colour or stem browning. However, with increased storage time grapes became more yellow and stems browner. Grapes stored without SO_2 were unsaleable after 4 and 6 weeks storage due to excessive rots. Treatment with SO_2 reduced percentage of botrytis rot by bunch weight from 25% to 3.9% after 6 weeks storage.

After 2 weeks storage all insects were dead, regardless of whether they had been treated with SO_2 or not, and this was confirmed in subsequent removals. Total mortality of both insect species is a promising result as sea transport to overseas markets generally takes at least 4 weeks. Further trials to test insects of concern to export markets and other insects that have been found in export table grape consignments are recommended.

Technical summary

The survival of two insect species, long tailed mealy bug (*Pseudococcus longispinus*) and a lady bird beetle (*Chilocorus* sp) was tested under conditions similar to those used during sea freight export to China and other export markets. Insects, 5 mealy bugs or a single beetle were transferred to grape berries inside small vented containers which were then placed inside 10kg cartons of export grade table grapes (*Vitis Vinifera*, L cv Thompson seedless). A single container of 5 mealy bugs or 9 containers with a single beetle were included within each of 6 replicate cartons with or without an Uvasys® green dual release SO_2 generating sheet (Grapetek Pty Ltd, Cape Town South Africa; a.i. 970 g/kg Sodium metabisulphite) which was placed on top of the grapes inside the plastic carton liner. Cartons were then stored for up to 6 weeks at 1°C and SO_2 levels inside cartons were measured 2 or 3 times per week. Cartons were removed at two week intervals and the survival of insects assessed. Grape quality with respect to soluble solids concentration (SSC), total acidity (TA), visual quality and rots was measured at each removal.

All insects were dead after 2 weeks in both storage treatments. This was confirmed in subsequent removals. SO_2 levels in cartons varied between the bottom and top with levels at the top adjacent to the generator sheet substantially higher than levels recorded in the bottom of the carton liners. Levels in the top of cartons reached 83.0 µL/L on day 1 which dropped rapidly to 4.3 µL/L on day 2 and remained at around this level for the remainder of the storage period. Levels in the bottom of cartons rose to 17.5 µL/L on day 1 and then dropped rapidly to 1.1 µL/L on day 2. The level then dropped below the detectable concentration for the remainder of the storage period and was recorded as zero.

After 4 and 6 weeks storage the quality of grapes in the cartons with a SO_2 generator sheet were rated as fair to good quality while those in cartons without SO_2 were unsaleable due to botrytis rot. After 6 weeks storage percentage of botrytis rot by bunch weight was 25% in grapes without SO_2 compared to 3.9% in grapes stored with SO_2. Approximately 20% of berries by weight had slight to moderate bleaching on their stem end after 6 weeks storage in grape bunches stored with SO_2. There was no significant effect of storage treatment or storage time on SSC or sugar to acid ratio, grape berry colour or rachis browning. However grape berries became more yellow and rachis browning increased significantly with storage time.

Under the conditions described here, cold storage for two weeks at 1°C provided an effective disinfestation treatment for long tailed mealy bug and *Chilocorus* beetle in cartons of Thompson Seedless table grapes. Further research is recommended to test the survival of insects of concern to export markets as well as other recorded contaminants of grape export consignments.

Introduction

Contamination of table grape export consignments to China with live insects is threatening the survival of this new and important export market. Insects of concern to China are Mediterranean Fruit Fly (*Ceratitis capitata*), Queensland Fruit Fly (*Bactrocera tryoni*), Lesser Queensland fruit fly (*Bactrocera neohumeralis*), Black Peach Aphid (*Brachycaudus persicae*), Fuller's Rose Weevil (*Asynonychus cervinus*), Garden Weevil (*Phlyctinus callosus*), Light Brown Apple Moth (*Epiphyas postvittana*), Plague thrips (*Thrips imagines*), Codling moth (*Cydia pomonella*) and Pear scale (*Diaspidiotus pyri*). However, a number of other insect contaminants have been found in table grape consignments packed for export to China. These include the red and blue pollen beetle (*Dicranolaius bellulus* Guérin-Méneville), long tailed mealy bug (*Pseudococcus longispinus*), common or European earwig (*Forficula auricularia* Linnaeus), Argentine ant (*Linepithema humile* Mayr) and carpophilus beetle (*Carpophilus* spp). It is a matter of urgency to develop disinfestation protocols to ensure no live insects survive the journey to China. The objective is to develop a disinfestation protocol to avoid or minimise the risk of contamination before storage and to determine if low temperatures and sulphur dioxide (SO_2) can kill insects during storage and transportation.

Initially the red and blue beetle was the target insect for this research as it was the main insect contaminant of consignments in the first season of exports to China. Little is known about the ecology and biology of this common, native beetle. It builds up to large numbers at certain times of the year and is known to feed on pollen and is a generalist predator of many insect pests (Yen and Tomkins, 2015; Tomkins and Yen 2015). In the year of this study it was not a major issue and could not be found in large enough numbers to conduct disinfestation trials. After consultation with ATGA and table grape exporters, long tailed mealy bug (*Pseudococcus longispinus*) was identified as a major contamination issue so it was one of the pests chosen for this study. In place of red and blue beetle, a lady bird beetle, *Chilocorus* sp. was also tested.

There have been a small number of studies reported that have examined the effect of low temperature and SO_2 on survival of mealy bug. A review of postharvest treatments for disinfestation of fruits in South Africa claims that exposure to 0^oC for 3 weeks controls Pseudococcidae or members of the mealy bug family but provides scant evidence to support this claim (Pryke and Pringle 2008). A study in California showed that storage of ruby seedless grapes infested with grape mealybug (*Pseudococcus maritimus* Ehrhorn) at 0^oC and with around 1 µL/L SO_2 resulted in greater than 92% mortality (Yokohama et al 2001). New Zealand research demonstrated that storage of Royal Gala apples infested with the obscure mealy bug (*Pseudococcus viburni* Signoret) at 0^oC resulted in 100% mortality after 6 weeks (Hoy and Whiting, 1997). These results indicate that low temperature with and without SO_2 can be effective in killing mealy bug but the efficacy appears to vary between mealy bug species. Furthermore, there do not appear to be any published studies on the effect of low temperature and SO_2 on long tailed mealy bug.

Materials and Methods

Grapes

Cartons (10 kg) of white table grapes (*Vitis Vinifera*, L cv Thompson seedless) were obtained directly from a specialist grape wholesaler. Fruit was harvested by hand at commercial maturity and packed directly into 1 kg bunch bags with 10 bunch bags packed into each carton. Cartons were then transported within 3 h of harvest to a forced air cooling facility and stored at 2^{o}C overnight before transport under refrigeration to the wholesaler. The next morning the fruit were delivered under refrigeration to the laboratory where they were stored at 1^{o}C until used. On arrival fruit were in field fresh condition with green stems and firm berries with an attractive waxy bloom.

Insects

Long tailed mealy bug was reared in a commercial facility (Biocontrol Services at Langwarrin South, Victoria). Colonies of mealy bug still on their food host plants were delivered to the laboratory on the morning before the experiment commenced. Using a camel hair brush, five live adult or late instar females were transferred under a dissecting microscope to a small bunch of grapes comprising two berries still attached their rachis and a length of stem. To ensure mealy bugs were not injured, each insect was lightly tickled with the brush until it withdrew its mouthparts and moved. It was then carefully picked up on the brush and transferred to the grape bunchlet. When all mealy bugs were transferred, the bunchlet was placed in a vented container and kept at room temperature overnight. Each bunchlet was checked under a dissecting microscope the next morning to ensure that the mealy bugs were alive and feeding before they were placed inside a bunch bag in a carton.

Ladybird beetles (*Chilocorus* sp.) were reared in a commercial facility (Bugs for Bugs Pty Ltd, Mundubbera , Queensland). Adult beetles were shipped to the laboratory by overnight courier the day before the experiment commenced. Beetles were shipped with adequate food and liquid for the journey and arrived healthy and active. On arrival, single ladybirds were transferred to a vented container with a halved grape berry as a moisture and food source if needed. The next morning containers were checked to ensure the beetles were still alive and active then each of nine containers were placed in the remaining 9 bunch bags in each experimental carton.

Cartons

Containers of insects were inserted among the grapes within the bunch bags in each experimental carton. Each carton contained one bag with a container holding 5 mealy bugs and the remaining 9 bunch bags per carton contained a vented box with single ladybird beetle. A Uvasys® green dual release SO_2 generating sheet (Grapetek Pty Ltd, Cape Town South Africa; a.i. 970 g/kg Sodium metabisulphite) was placed immediately on top of the bunch bags in half the cartons. All cartons contained a low density polyethylene carton liner which was closed and securely folded over the contents of the carton after the insects and/or a SO_2 sheet were added.

Cool store

All cartons were stored on racks in a randomised complete block design in a cool room set at 1^{o}C ± 0.5^{o}C and 80% ± 5% RH and removed for examination after 2, 4 and 6 weeks storage. Six replicate cartons with or without the SO_2 generator sheet were removed at each examination time. Tiny Tag Ultra 2 data loggers (Hastings Data Loggers, Port Macquarie, NSW) were used to record room temperature and humidity.

Insect assessments
On removal, containers of insects were remove from cartons and examined under a dissecting microscope. All insects were tickled with a camel hair brush and whether they moved or not and there general appearance was recorded. All containers were kept at room temperature overnight and were re-examined in the same manner the following day. If insects did not show any response when stimulated they were assumed to be dead.

Grape assessments
On the day after removal from storage, 4 bunch bags were removed from each carton at random and the grapes assessed for visual quality, rots, soluble solids concentration (SSC) and titratable acid (TA). Grapes were then stored at 18^{o}C for 7 days to simulate distribution and marketing at ambient temperatures and reassessed for visual quality and rots.

Visual quality of berries from cartons with or without SO_2 was compared with respect to berry colour and stem (rachis) browning (Table 1). Grape bunches from within each bunch bag were weighed, laid out on a white plastic tray and scored for rachis browning using a 1 to 5 scoring scale where 1 = green rachis and 5 = completely brown rachis (Fig. 1). When bunch bags contained multiple bunches, each bunch was scored separately and the average rachis browning score determined for that bunch bag.

Bunch colour was scored using a scoring scale based on Chesterfield & Smith (1990) where 1 = dark green, 2 = light green (optimal colour), 3 = green to yellow, 4 = light yellow and 5 = dark yellow. A score of 2 represents 'light green' bunch colour, and is generally considered as the minimum colour required at harvest to meet consumer preferences. Bunches scored as 4 or 5 were likely to have been highly exposed to sunlight in the vineyard resulting in advanced maturity or sunburn.

Table 1. Description of ratings used to score quality attributes of individual berries.

Quality attribute	Ratings and Description				
	1 (none)	2 (trace)	3 (slight)	4 (moderate)	5 (severe)
Berry surface browning	None	< 5 berries per bunch bag	5 to 10 berries per bunch bag	11 to 30 berries per bunch bag	> 30 berries per bunch bag
Berry bleaching	None	< 5% surface bleaching	5 to 25% surface bleaching	26 to 50% surface bleaching	> 50% surface bleaching
Berry shrivel	None	Shrivel around pedicel only	Shrivel < 10% of berry surface	Shrivel on 10-25% of berry surface	Shrivel on > 25% of berry surface
Berry colour (Yellowing)	Dark green (berries firm and immature)	Light green	Green to Yellow (more light green than yellow)	Light yellow (more yellow than green)	Yellow (majority of berries yellow)
Stem (rachis) browning	All green	Most pedicels showing browning	Pedicels brown and laterals partially brown	Pedicels and all laterals showing browning	Whole rachis severely browned

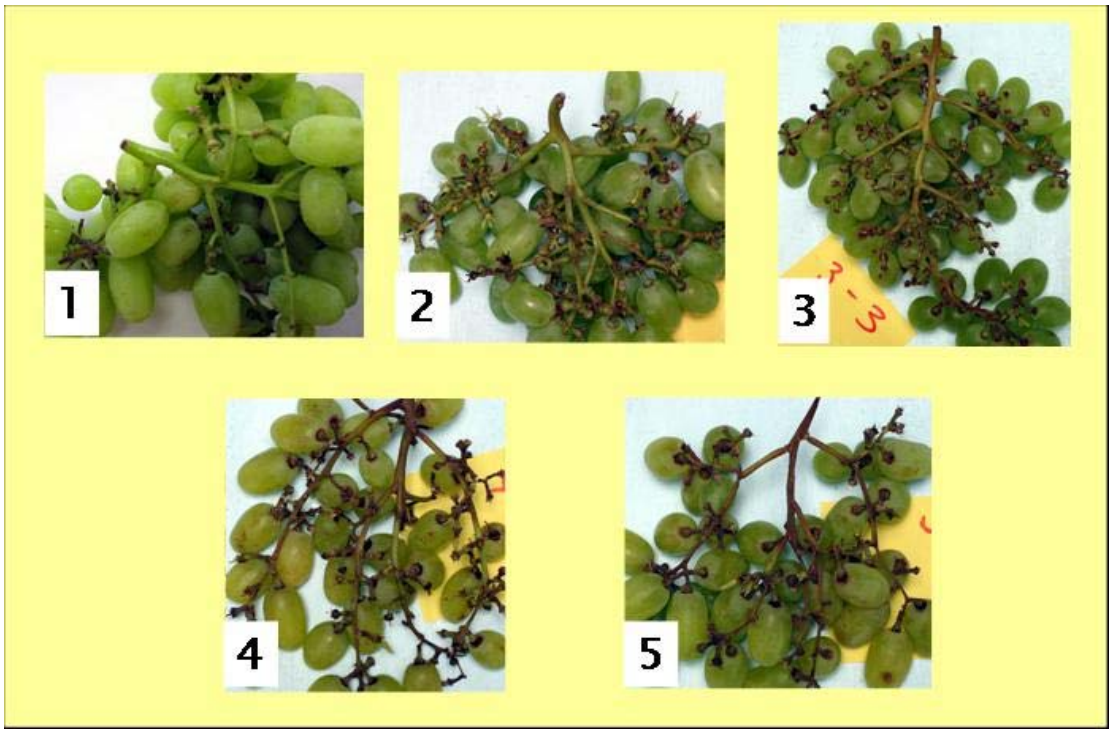

Figure 1. Visual rating scales and descriptions used for scoring of bunch rachis browning

Number of berries with symptoms of botrytis rot was recorded for each replicate bunch bag both after removal from cool storage, and after storage at 18°C for 7 days. Infected berries were weighed and the percent weight of berries with botrytis within each bunch bag was calculated.

After berries with rots were recorded, 10 berries were removed at random from each bag for soluble solids concentration (SSC) and titratable acidity (TA) analyses. Berries were crushed using a mortar and pestle and the free juice decanted. Juice was analysed for SSC expressed as ^{o}Brix using an Atago® pocket refractometer (Atago, Tokyo, Japan) calibrated with distilled water.

Titratable acidity of the juice was measured using an Accuvin Titratable Acidity Quick Test™ (Accuvin LLC, Napa, CA USA) and expressed as g/L tartaric acid equivalents. TA measurements were conducted on a composite 8 ml juice sample collected from four bunch bags per replicate carton by placing 91 uL of juice into an Accuvin Eppendorf tube as supplied and comparing the resulting colour change to the colour chart provided. The TA range measured by the kit is 4.0 to 11.0 g/L. Each Eppendorf tube was shaken for 30 sec to ensure complete colour change before assessment.

Sugar to acid ratio (i.e. SSC to TA ratio) was calculated for each bunch bag using individual SSC measurements and average TA measurements based on four bunch bags per replicate carton:

$$\text{Sugar to acid ratio} = \frac{\text{SSC} \times 10}{\text{TA}}$$

Sulphur dioxide measurement
In-carton atmospheres were sampled regularly during storage through a septum attached to the LDPE carton liner using a 50 ml disposable plastic syringe. Samples were taken from the top of the carton near the SO_2 sheet and from the bottom of the carton. The sample was then injected into the sample port of a DragerSensor®XS EC SO_2 Pac III gas detector (Draeger Safety Inc, Pittsburgh, PA USA). The gas detector was calibrated before each use with an Air Liquide Calgaz containing 5 µL/L SO_2 with the balance nitrogen (Air Liquide, Sunshine, Victoria). SO_2 concentration in the bags is expressed as µL/L.

Experimental design
The experiment was a randomised complete block design. Treatments were +/- SO_2 and three removal times (2, 4 and 6 weeks). There were 6 replicates (10 kg cartons) of each treatment. Representative bunch bags (4) were removed from each replicate carton for grape quality assessments. Each replicate carton contained a vented container with 5 long tailed mealy bug and 9 vented containers with a single lady bird beetle.

Statistical analysis
There was no need to analyse insect mortality data as all insects from all treatments were dead at the first removal.

For grape quality attributes, a two-way analysis of variance (ANOVA) was conducted using GenStat statistical software (Version 14, Lawes Agricultural Trust, 2011) to determine the main effect of SO_2 treatment and storage period on bunch quality, and any interaction effects between treatment and storage period. Means were compared using 95% confidence intervals, or Fisher's Least Significant Difference (LSD) test with statistical differences between means determined at the 5% significance level ($\alpha = 0.05$).

Results and Discussion

Insect survival
At both the two and four week removals all insects from boxes with or without SO_2 appeared dead or moribund when viewed under a dissecting microscope and did not respond when stimulated with a camel hair brush. Insects were kept at room temperature and re-examined after 24 h and the same result was obtained. Nearly all beetles were lying on their backs with their legs retracted. Approximately 50% of the mealy bugs were still attached to the stems or berries and at first glance appeared to be alive. However, even after vigorous stimulation with the camel hair brush they did not withdraw their stylets and move off as healthy mealy bugs do, confirming that all insects from both storage treatments were dead.

Previous studies with mealy bug have shown that exposure to low temperature alone or with SO_2 results in high mortality over time. In general, exposure to 0^oC for about 3 weeks is sufficient to kill members of the Pseudococcidae or mealy bug family (Pryke and Pringle, 2008). However studies with individual species have shown that they vary in susceptibility to low temperatures. In a study by Yokohama et al (2001) less than 8% of grape mealybug, (*Pseudococcus maritimus* Ehrhorn) infesting clusters of ruby seedless grapes survived 8 weeks storage at 0^oC with a sulphur pad and SO_2 increased mortality compared with low temperature alone. Hoy and Whiting (1997) found that 6 weeks at 0^oC resulted in 100% mortality of the obscure mealy bug *Pseudococcus viburni* Signoret (formerly *Pseudococcus affinis* Maskell) on Royal Gala Apples; this obscure mealy bug is closely related to the grape mealy bug (*P. maritimus*). Based on this study, long tailed mealy bug is highly susceptible to low temperature with 100% mortality observed after storage at 0^oC for two weeks.

Storage for two weeks at 1°C also resulted in 100% mortality of the lady bird beetles (*Chilocorus* sp.).

In-package SO_2 levels

In-package SO_2 levels increased rapidly in day one. The mean level in the top of the cartons was 83.0 µL/L on day 1 which dropped rapidly to 4.3 µL/L on day 2 and remained at around this level for the remainder of the storage period ranging between 2.4 and 5.4 µL/L. Levels in the bottom of cartons rose to 17.5 µL/L on day 1 and then dropped rapidly to 1.1 µL/L on day 2. The level then dropped below the detectable concentration for the remainder of the storage period and was recorded as zero.

The Uvasys® green dual release SO_2 generating sheet released SO_2 according to specification. That is, a rapid release initially followed by a controlled, low level release for the remainder of the storage period (Fig. 2). This provided significant protection against rots particularly during the first 6 weeks of storage compared to the grapes stored without an SO_2 sheet (Fig. 3).

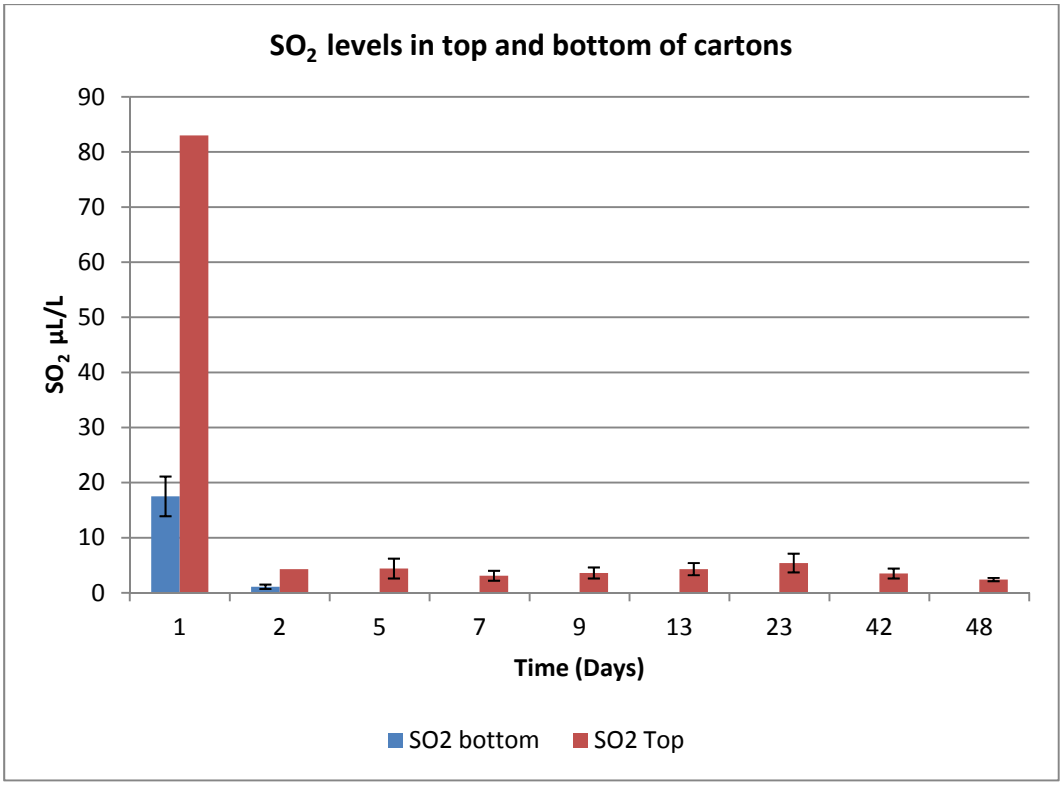

Figure 2. In-package SO_2 levels in the top (Red bars) and bottom (Blue bars) of cartons of Thompson Seedless table grapes during 48 days storage at 1°C. Each carton contained a single A Uvasys® green dual release SO_2 generating sheet (Grapetek Pty Ltd, Cape Town South Africa; a.i. 970 g/kg Sodium metabisulphite) which was placed immediately on top of the grapes inside the carton liner.

(A) (B)

Figure 3. Grape quality after 6 weeks storage at 1°C both with (A) and without (B) an Uvasys® green dual release SO_2 generating sheet. Note high incidence of rots in carton without SO_2 generator sheet.

Grape Quality

There was no significant effect of storage treatment or storage time on SSC or sugar to acid ratio (Fig. 4 and Fig. 5). The grapes were harvested quite early in the season for Thompson Seedless and their sugar acid ratio ranged from 17.1 to 20.3. This is quite low and the grapes were of poor to fair eating quality. Thomson seedless grapes with a sugar acid ratio of less than or equal to 20 are considered poor eating quality (Lopresti and Tomkins, 2015). Consequently, the grapes used here should have been harvested at a later date, when the sugar acid ratio exceeds 22 to provide fruit that meet consumer expectations for eating quality.

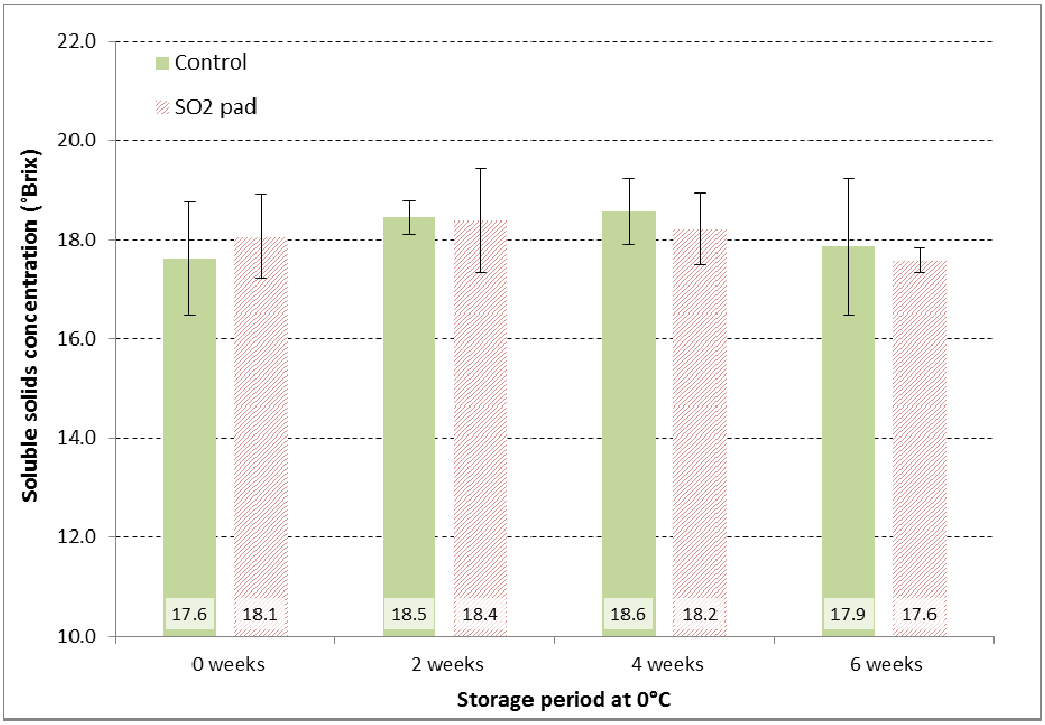

Figure 4. Effect of SO_2 treatment and storage period on SSC in 'Thompson Seedless' grapes stored at 1°C. Bars represent the 95% confidence intervals for mean SSC. Treatment, storage period and, treatment x storage period, *p*-values were not significant at α = 0.05.

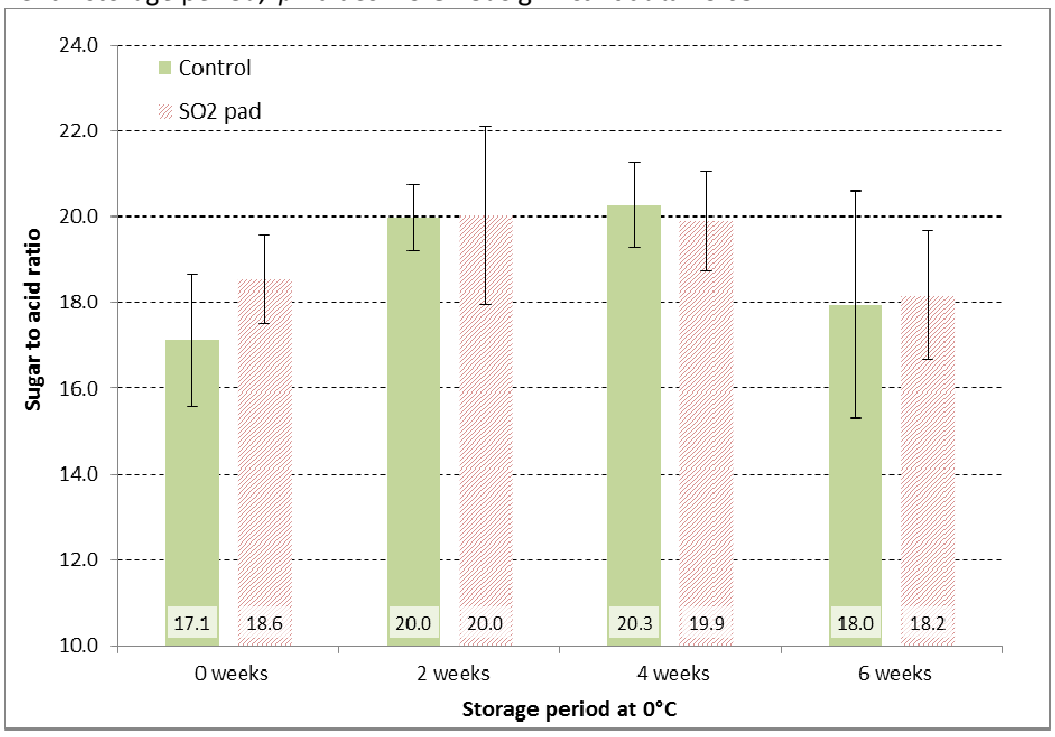

Figure 5. Effect of SO_2 treatment and storage period on sugar to acid ratio in 'Thompson Seedless' grapes stored at 1°C. Bars represent the 95% confidence intervals for mean sugar to acid ratio. Treatment, storage period and, treatment x storage period, *p*-values were not significant at α = 0.05.

Storage treatment did not have a significant effect on bunch colour but bunches were significantly more yellow after 4 weeks storage (Fig. 6). There was no significant effect of SO_2 treatment on rachis browning but stems became significantly browner with increased storage (Fig. 7). Although considered still marketable rachis browning could be a major problem if storage is prolonged further.

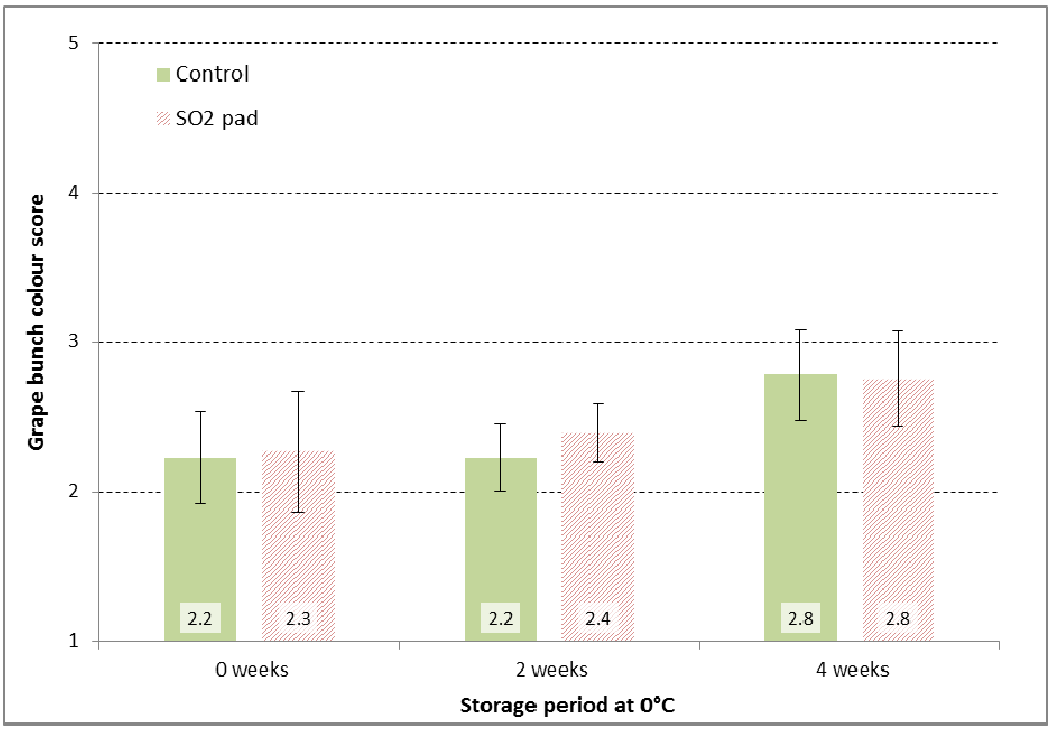

Figure 6. Effect of SO_2 treatment and storage period on grape bunch colour score in 'Thompson Seedless' grapes stored at 1°C. Bars represent the 95% confidence intervals for mean sugar to acid ratio. Treatment and, treatment x storage period, *p*-values were not significant at $\alpha = 0.05$. Effect of storage period was significant ($P = 0.007$).

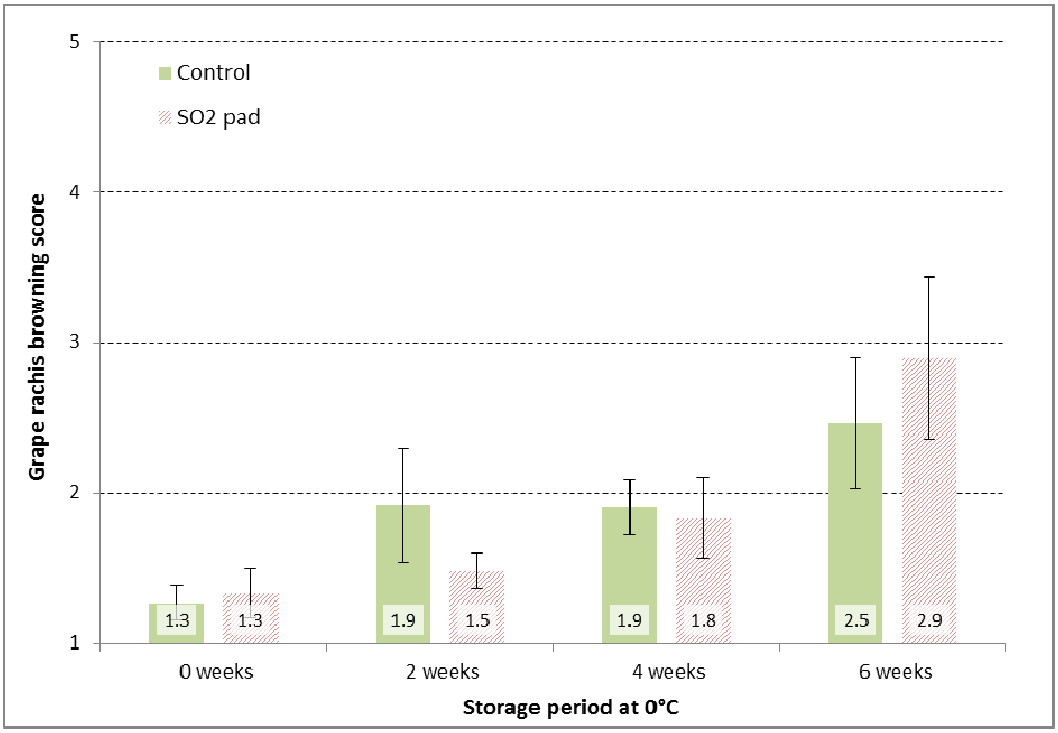

Figure 7. Effect of SO_2 treatment and storage period on rachis browning score in 'Thompson Seedless' grapes stored at 1°C. Bars represent the 95% confidence intervals for mean browning score. Treatment and, treatment x storage period, *p*-values were not significant at $\alpha = 0.05$. Effect of storage period was significant ($P < 0.001$).

Bleaching of berries increased with storage time and was only evident in cartons with a SO_2 generator sheet (Fig. 8). In general, bleaching severity was low with 19% of berries by weight affected after 6 weeks storage. Bleaching was considered far more acceptable than rots. After storage for 6 weeks 25% of berries in cartons without SO_2 sheets had rots while only 3.9% were affected in cartons with a SO_2 sheet (Fig. 9). After another 7 days at 18°C this had increased to 44.3% and 17.0%, respectively (Fig. 10). This clearly demonstrates the need for SO_2 sheets to control Botrytis rot of Thompson Seedless table grapes during storage under the conditions described here.

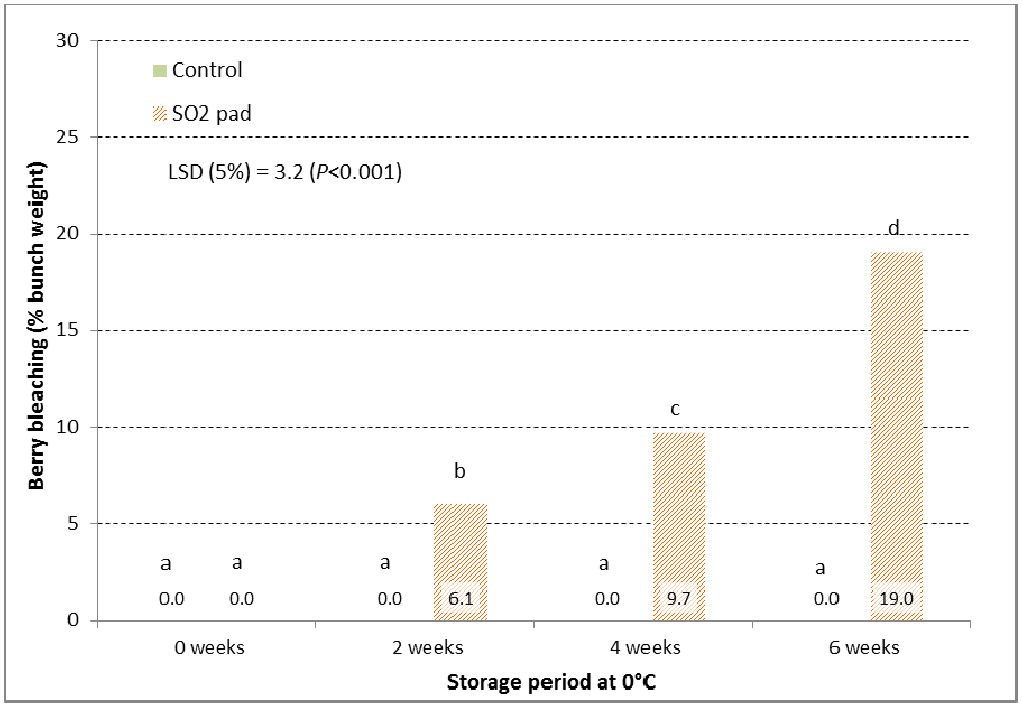

Figure 8. Effect of SO_2 treatment and storage period on berry bleaching in 'Thompson Seedless' grapes stored at 1°C. Means followed by different letters are significantly different at $P \leq 0.05$.

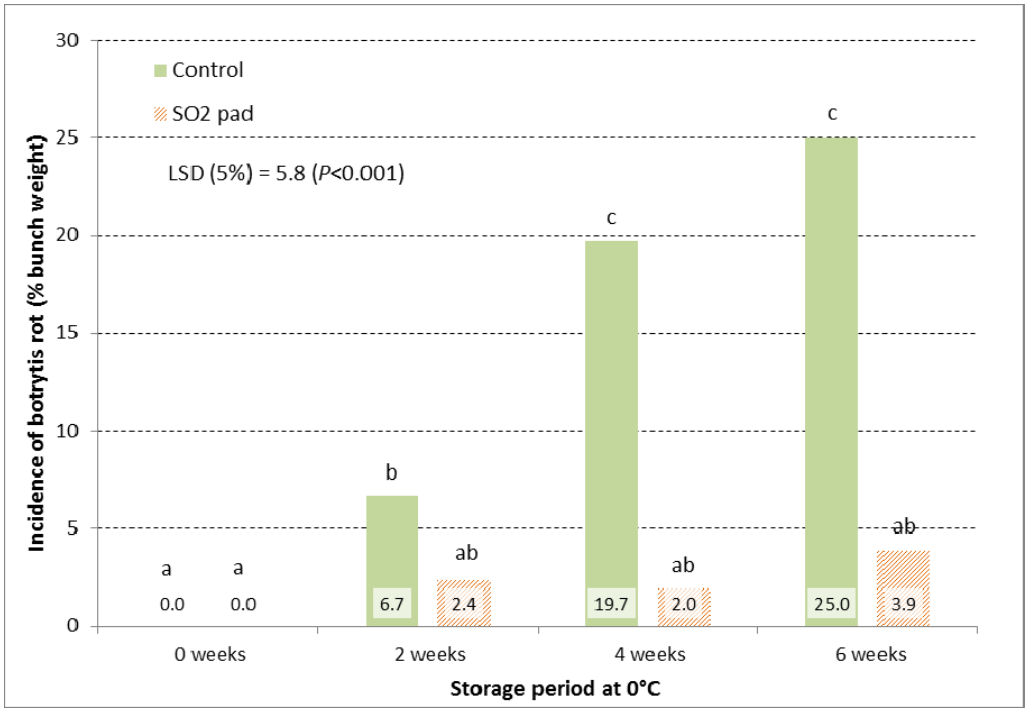

Figure 9. Effect of SO_2 treatment and storage period on incidence of botrytis rot in 'Thompson Seedless' grapes stored at 1°C. Means followed by different letters are significantly different at $P \leq 0.05$.

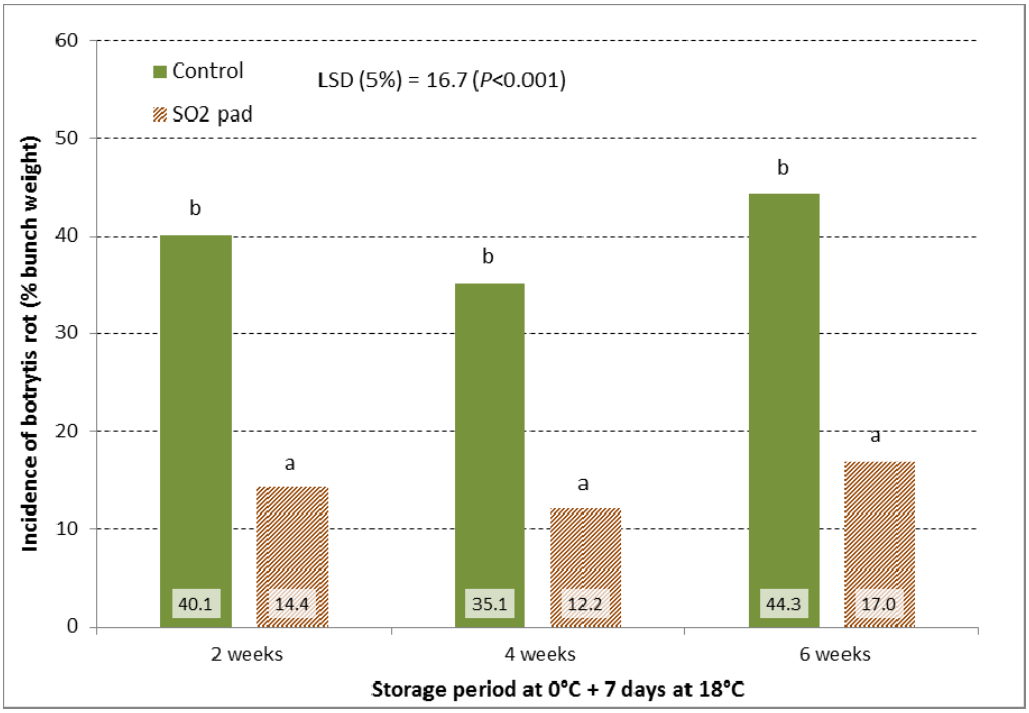

Figure 10. Effect of SO_2 treatment and storage period on incidence of botrytis rot in 'Thompson Seedless' grapes stored at 1°C for 2, 4 and 6 weeks and at 18°C for 7 days. Means followed by different letters are significantly different at $P \leq 0.05$.

Technology Transfer

Project outputs have been reported in the Vine Magazine (Tomkins and Yen, 2015) and a review on the ecology and biology of red and blue beetle completed (Yen and Tomkins, 2015). Another article is under preparation and will be published in The Vine magazine in late 2015.

Project progress and results have been regularly reported verbally to CEO ATGA and major table grape exporters and growers.

Conclusions and Recommendations

Under the conditions described here 2 weeks storage at $1^{o}C \pm 0.5^{o}C$ resulted in 100% mortality of long tailed mealy bugs and *Chilocorus* lady bird beetles placed in cartons of export grade Thompson seedless table grapes. This indicates that these insects would not survive in consignments of table grapes exported by sea from Australia to China. Storage and transport of grapes to China takes around 4 to 5 weeks.

Larger scale tests to validate this result and tests with other potential insect contaminants of export grape consignments are recommended. The experiments will need to be designed to meet Chinese quarantine requirements for demonstration of disinfestation.

Eating quality of the grapes used here was considered poor as the SSC to TA ratio was ≤ 20. It appeared the grapes were immature and harvested before optimum eating quality was obtained. However, visual quality of the grapes remained acceptable for at least 6 weeks but botrytis rot was a major problem in grapes stored without a SO_2 generator sheet. The SO_2 sheets performed according to specification with a large release of SO_2 in the first day followed by a low level release for the remainder of the storage period. Under the conditions described here, the use of a SO_2 generator sheet in cartons to reduce losses due to Botrytis rot is highly recommended.

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