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Development of a Sulphur Dioxide Fumigation Protocol for the Ontario Vitis labrusca 'Sovereign Coronation' Table Grape Industry

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Introduction

In order to compete with local produce as well as imported grapes, the fresh grape industry in Ontario is interested in developing methods to extend the postharvest storability of Vitis labrusca 'Sovereign Coronation' table grapes. One of the biggest challenges faced in the preservation of this crop during long-term storage, is how to control the Botrytis development, while reducing associated weight loss due to berry and stem dessication. In 2014, Vineland Research and Innovation Center employed sulpher dioxide-generating pads, to demonstrate that the use of sulphur dioxide with Ontario-grown 'Sovereign Coronation' grapes is an effective method to extend postharvest storage duration. The ability to increase storage viability of 'Sovereign Coronation' grapes generates opportunity to market the product later into the season, reduce losses, and provides presence of local produce on store shelves into October or November, thus increasing sales and the profit margin.

Use of Sulphur Dioxide

Adequate controls of *Botrytis* cannot be accomplished with rapid cooling alone (Crisosto and Smilanick, nd). Efficient control of Botrytis is achieved with the use of sulphur dioxide (SO_2) and if grapes are not treated, gray mould can lead to substantial losses (Teles et al., 2014). Prior to the use of SO_2 to control gray mould, long-term storage of table grapes was essentially impossible to achieve (Nelson, 1985). Effective control of Botrytis is accomplished through standard practices involving weekly applications of SO_2 gas through fumigation in chambers, following an initial harvest fumigation treatment (Luvisi et al. 1992), as well as through continuous release SO_2-generating pads placed in the packaging boxes, or through a combination of both methods (Crisosto and Smilanick, nd; Maldonado, 2013). Recommendations for the use of SO_2 in the fumigation of table grapes was first published in 1925 in the U.S.A. (Luvisi et al., 1992). Extensive research and recommendations exist for its usage, primarily with respect to the dominant Vitis vinifera varieties present in California.

Grapes which are intended for domestic market in the U.S.A. do not use SO_2-generating pads and rely on SO_2 fumigation as standard practice (Crisosto and Smilanick, nd; Maldonado, 2013). Pads are typically employed for international shipments or in situations where regular fumigation is not feasible. In the case of fumigation, typically an event occurs directly after harvest (typically 2,500 to 3,000 ppm for 20 mins), followed by a weekly fumigation, which continues until the grapes are shipped to their domestic destination (Maldonado, 2013). The rate of SO_2 required to kill Botrytis spores and mycelium is calculated as a cumulative concentration, which is a function of the concentration and length of exposure, and is called a "CT product" (Crisosto and Smilanick, nd). A minimum CT of 100 ppm-hour is required to kill Botrytis mycelium and spores at 0°C (Crisosto and Smilanick, nd). A CT product can be calculated as a function of the average SO_2 concentration (ppm), multiplied by the fumigation time (hours) (Nelson, 1985; Luvisi et al., 1992).

The limiting factor to the concentration of SO_2 is that of phytotoxicity to the grapes, which typically manifests in the form of hairline cracks, bleaching of berries, sunken areas (Crisosto et al., 1994; Teles et al., 2014) and rachis damage (Baiano et al., 2007). In particular, Vitis labrusca varieties can be highly sensitive to symptoms of phytotoxicity upon exposure to high levels of SO_2 (Carlos Crisosto, personal communication, September 26, 2013).

Sulphur pads were an appropriate preliminary approach used in 2014 to judge the potential for the successful postharvest use of SO_2 in Ontario-grown 'Sovereign Coronation'. Sulphur pad treatments did not show any berry bleaching due to SO_2 phytotoxicity. In order to further improve the storability of Ontario-grown 'Sovereign Coronation', weekly fumigation of bulk grapes during storage requires investigation.

For an effective treatment of SO_2 gas to occur, a dosage amount which includes a high enough concentration to control Botrytis and stem browning, while not inducting phytotoxic effects to the grapes, must be properly balanced. Fumigation would allow for a tighter control of the SO_2 concentration, in order to achieve these goals. Although established fumigation system designs exist in the U.S.A. and other countries, the use of SO_2 fumigation in Ontario must be adapted to the local industry, and specifically to the 'Sovereign Coronation' variety.

Objective

The objective of this project is to develop an SO_2 fumigation protocol and method, which can be adopted to the Ontario table grape industry. This method must be adapted specifically to the 'Sovereign Coronation' table grape variety. The duration of this project covers a one-year time span, encompassing the 2016 harvest season.

Materials and Methods

Experimental setup

'Sovereign Coronation' table grapes were harvested at commercial maturity on August 31 2016. Grapes were harvested into clamshell packages, and placed in cardboard boxes. After harvest, the grapes were transported to the Vineland Research and Innovation Centre. The grapes where then transferred into reusable plastic containers (RPC's) in order to avoid SO_2 absorption by the cardboard during the SO_2 treatment. In each of the RPC's, half of the grapes were placed as "loose bunches" and the other half were left in clamshells. The purpose of this separation was to validate any difference between the two storage methods during the fumigation process (Figure 1).

Figure 1: Grapes were transfered from cardboard boxes to RPC's, half as loose bunches, and half in clamshells

The plastic containers were then stacked into single stacks of five smart crates, for each respective treatment. The stacks were constructed in a specific way to be conducive to a predetermined fumigation method. The stacks were forced-air cooled and stored at their recommended optimal temperature and relative humidity (-1°C to 0°C and 90%-95% RH). (Figure 2).

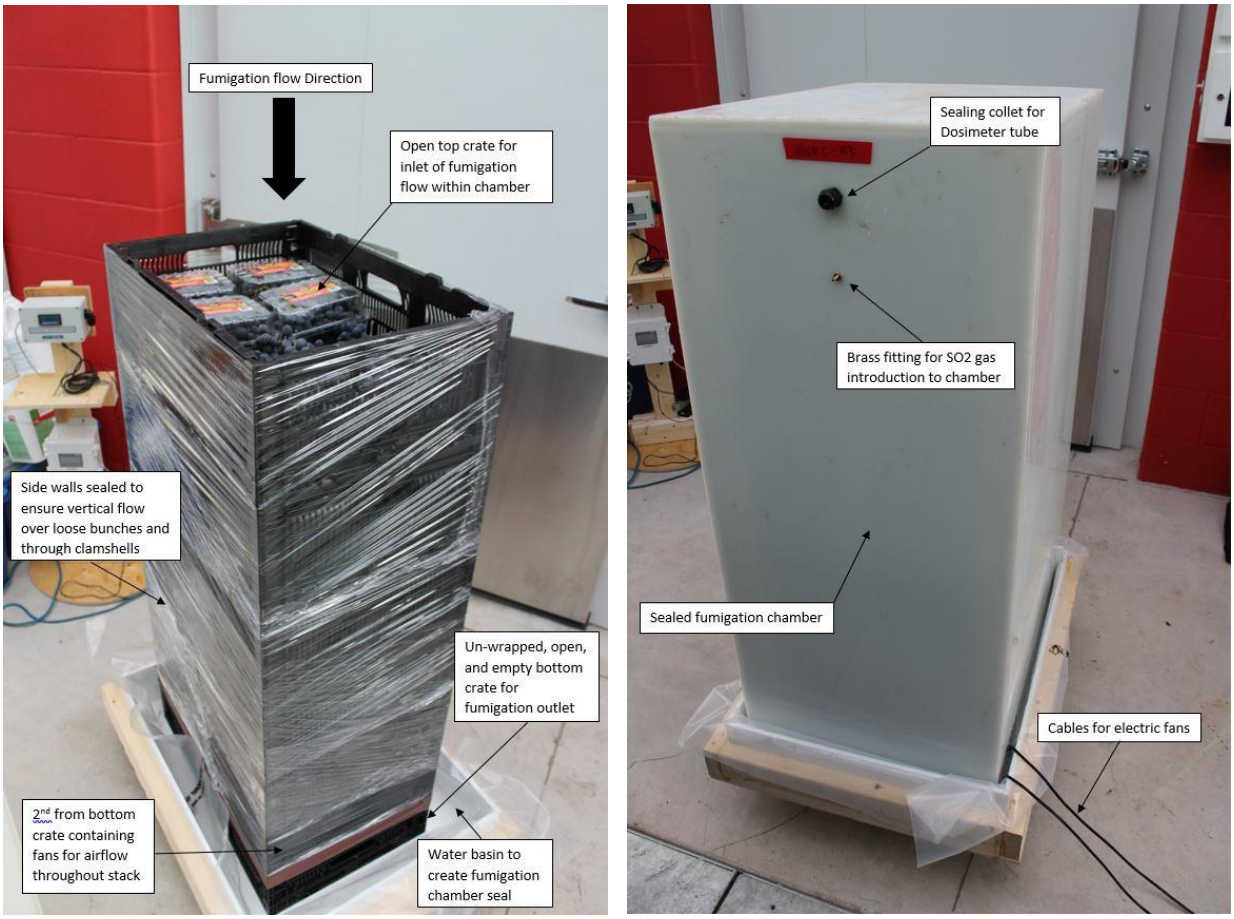

Figure 2: (Left) Fumigation stack contruction with chamber hood removed, (Right) Complete fumigation chamber with hood.

Fumigation Protocol

Previous research has proven that the amount of SO_2 required to kill Botrytis spores is 100 ppm-hours (Luvisi et al. 1992). There are two methods, which are typically utilized in postharvest grape fumigation. The traditional method consists of introducing a large amount of SO_2 into the storage room for a short period of time, and then ventilating the room in order to get the combination concentration-time required. The second method is called the 'total utilization' method. In this experiment, the total utilization method was used. The SO_2 injected is balanced by the amount of SO_2 absorbed by fruit, boxes, and the room itself. With this method, nearly all of the SO_2 will naturally dissipate by the end of the treatment cycle of a set duration, and the SO_2 concentration in the room air is usually quite low.

The experiment was comprised of three separate treatments as follows: An untreated control to serve as a comparator (no SO_2), fumigation at a CT product of 100 ppm-hour (low concentration), and fumigation at a CT product of 500 ppm-hour (high concentration). Each fumigation treatmentment was comprised of three replicates; therefore, the experiment contained nine stacks in total (*Figure 2*).

Figure 2: Layout of nine stacks within the cold room, three control (black stacks), three low concentration chambers, and three high concentration chambers.

Individual fumigation treatments consisted of weekly injections of the SO_2 gas into the airtight fumigation chambers. This operation was carried out by utilizing a pressurized SO_2 gas cylinder, connected to a pressure regulator. Syringes were employed to obtain the desired volumes of gas from the regulator, and then injected into the individual chambers in a controlled manner. (Figure 3).

Figure 3: Injection of SO_2 gas using a syringe for precise control of gas input.

SO2 Fumigation Volume

In order to determine an appropriate fumigant dosage to achieve the desired contact time of SO_2 within the fumigation chamber, one must consider that there are several dynamic variables involved. The most important consideration is that SO_2 will deplete with time. The rate at which this occurs is dependent upon the various materials in contact with the fumigant, the exposed surface area of these materials, and the relationship that this has to the internal volume of the chamber itself. In addition to this, successive treatments need to be adjusted to account for the saturation of previous fumigant within the materials in the chamber. This is especially significant in relation to the grapes, and contained water in this particular case. In order to address these challenges, various formulae were used to establish a baseline volume of pure SO_2 gas, which was calculated to achieve a result close to the desired ppm-hr targets outlined for this experiment. From this baseline, we were able to adjust the volumes per treatment, in an effort to achieve the desired result.

Dosimeter tubes were utilized to measure the SO_2 concentration-time in each of the treated chambers, to gage the contact time achieved in each particular treatment of fumigant. Since each fumigation chamber employed its own forced air system, it was known that the fumigant would be dispersed evenly, and that exposure of the broken tip through the sidewall of the chamber would return a result reflectant of the consistent concentration found within. (See Figure 5).

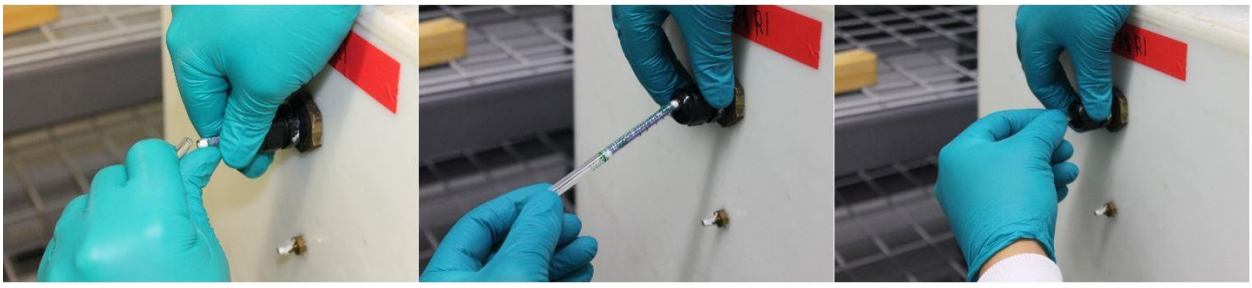

Figure 5: Placement of a Dosimeter tube (From L to R) break off tip, insert open end into chamber, tighten collet. Remove and record reading at time interval recommended by the dosimeter manufacturer.

Quality Analysis

Before initiation of storage, the pre-treatment weight was recorded from a SmartCrateTM for each treatment. These same SmartCratesTM were re-weighed at each subsequent evaluation event described above. Weights were measured using a scale accurate to 0.0005 kg with a 30 kg capacity (Ranger OHAU-RC30LS; OhausTM, USA) and weight loss was expressed as a percentage of original weight.

A weekly evaluation was also performed on 10 clusters from each treatment for quality analysis including desiccation rating, and SO2 damage and decay ratings following established methods (Lichter et al., 2008). An index rating of 1 to 5 was used to score desiccation and SO2 damage. Desiccation ratings were 1 = rachis and pedicels green and full as at harvest; 2 = slight browning; 3 = browning of rachis and pedicels but no shriveling; 4 = browning and some shriveling; and 5 = both rachis and pedicels dry and brown (Lichter et al., 2008). Clusters with a rating above 3 were considered unmarketable. SO2 ratings were based on the total number of berries that exhibited bleaching: 1 = no apparent bleaching; 2 = two to five berries; 3 = six to ten berries; 4 = 11 to 20 berries; and 5 = over 20 bleached berries per 10 bunches (Lichter et al., 2008). Decay was rated by scoring the percent of healthy bunches out of the 10 bunches selected per treatment. Healthy bunches were defined as having only one or no decayed berries (Lichter et al., 2008). A score of 1 was given for a healthy cluster and a score of 0 for one that is non-healthy. The average on the 10 clusters was established and the percentage of healthy clusters were obtained

Statistical Analysis

An ANOVA analysis was performed on mean values of each quality parameter using XL STAT, version 2013: Microsoft Corporation. Treatment effects reported were significant according to a t-test. Significant differences between results were compared using the Least Significant Difference (LSD) with an interval of confidence of 95% ($t<0.05$).

Results

The weekly SO2 volume injected and the resulting concentration-time is presented below, in Table 1 and Table 2. As observed from the tables, the volume of SO2 injected was reajusted weekly to reach a concentration-time that was "close enough" to the target. During the first few weeks the volume of SO2 required to reach the desired concentration level increased since the grapes, packaging material, and the storage box were absorbing more SO2. Then, as the experiment progressed, the experimental set up was saturated with SO2 gas, resulting in less SO2 absorption. From week eight, the volume required to reach our target stabilized.

Table 1 - Low Concentration Values

LOW CONCENTRATION INJECTION				
DATE	**TREATMENT (WEEK)**	**SO2 VOLUME (L)**	**RESULTING SO_2 Concentration-Time (PPM-HR)**	**DETERMINATION**
Sep-01	0	0.22	47	LOW
Sep-08	1	0.37	63	LOW
Sep-15	2	0.37	50	LOW
Sep-22	3	0.37	APPROX 100 ± 10%	ACCEPTABLE
Sep-29	4	0.37	APPROX 100 ± 10%	ACCEPTABLE
Oct-06	5	0.37	FULL SCALE	HIGH
Oct-13	6	0.28	FULL SCALE	HIGH
Oct-20	7	0.15	FULL SCALE	HIGH
Oct-27	8	0.1	28	LOW
Nov-03	9	0.15	NO INDICATION	LOW
Nov-10	10	0.2	APPROX 100 ± 10%	ACCEPTABLE
Nov-17	11	0.2	APPROX 100 ± 10%	ACCEPTABLE
Nov-24	12	0.2	APPROX 100 ± 10%	ACCEPTABLE
Dec-01	13	0.2	APPROX 100 ± 10%	ACCEPTABLE
Dec-08	14	0.2	APPROX 100 ± 10%	ACCEPTABLE

Table 2 – High Concentration Values

HIGH CONCENTRATION INJECTION				
DATE	**TREATMENT (WEEK)**	**SO2 VOLUME (L)**	**RESULTANT AVG READING (PPM-HR)**	**DETERMINATION**
Sep-01	0	1.1	233	LOW
Sep-08	1	1.2	600	ACCEPTABLE
Sep-15	2	1.2	383	LOW
Sep-22	3	1.2	APPROX 500 ± 10%	ACCEPTABLE
Sep-29	4	1.2	APPROX 500 ± 10%	ACCEPTABLE
Oct-06	5	1.2	FULL SCALE	HIGH
Oct-13	6	0.91	FULL SCALE	HIGH
Oct-20	7	0.6	FULL SCALE	HIGH
Oct-27	8	0.45	500	ACCEPTABLE
Nov-03	9	0.35	300	LOW
Nov-10	10	0.4	APPROX 500 ± 10%	ACCEPTABLE
Nov-17	11	0.4	APPROX 500 ± 10%	ACCEPTABLE
Nov-24	12	0.4	APPROX 500 ± 10%	ACCEPTABLE
Dec-01	13	0.4	APPROX 500 ± 10%	ACCEPTABLE
Dec-08	14	0.4	APPROX 500 ± 10%	ACCEPTABLE

Weight Loss

Weight loss in crops during cold storage is mainly the result of loss of water. Grape berries are covered with a thick wax coating called a cuticle, which aids in prevention of water loss. The rachis (the stem axis which bears the grapes) does not have the same level of cuticle protection. In addition, stem or rachis respiration rate is about 15 times higher than the rate of berry respiration (Crisosto and Smilanick, nd). As such, water loss occurs first from the rachis and subsequently from the berries. Grape berries do not show water loss symptoms until after damage to the rachis is substantial (Soylemezoglu, 2001). The weight loss in table grapes that occurs during storage or handling results mainly in stem browning, berry shatter, and wilting and shriveling of the fruit (Crisosto *et al*, 2001). To maintain a good grape quality during storage, it is essential to minimize weight loss to an accepted level in order to minimize the detrimental effect. In general, a weight loss of over 5% to 6% is required before shrinkage is evident in berries (Nelson, 1985; Soylemezoglu, 2001), although berries may begin to lose noticeable turgor at around 3% weight loss (Soylemezoglu, 2001). The low critical threshold value for water loss resulting in rachis browning varies depending on the variety of table grape. Previous studies have shown values from 2.0% to 2.5% for the low critical threshold and up to 3.3% to 4.1% for the appearance of severe stem browning, dependent on the variety tested (Crisosto et al., 2001).

Table 3 represent the weight loss data in time for all the treatments and packaging. As expected, the average weight loss shows an increasing trend over time; however, the overall weight loss at the end of the 15 weeks of storage was very low for all treatments with a highest loss of 2.34%. At week 8, we started to see some mold on the control grapes. The grapes from the control treatment were removed from storage at week 10 and this is the reason why no value was recorded at week 15.

The weight loss difference was minimal between the two packaging technique: clamshell and loose bunch in the plastic container. As presented in Figure 4, there was no statistical difference between all the treatments except for the clamshell with the control, with an average weight loss of 1.1%. Even if there is a significant difference, the maximum difference is less than 0.6%. The decision of the packaging method should not be based on the weight loss because we did not observe enough difference between loose bunches and clam shell.

Table 3 - Average weight loss per treatment

Time (week)	Control		Low Concentration		High Concentration	
	Loose bunch	Clam shell	Loose bunch	Clam shell	Loose bunch	Clam shell
0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
4	1.05%	0.95%	1.37%	1.72%	1.23%	1.48%
6	1.98%	1.26%	1.26%	1.84%	1.42%	1.55%
8	2.22%	1.33%	1.78%	1.92%	1.61%	1.59%
10	2.49%	1.53%	1.97%	2.03%	1.80%	1.71%
15	-	-	2.23%	2.34%	2.15%	2.05%

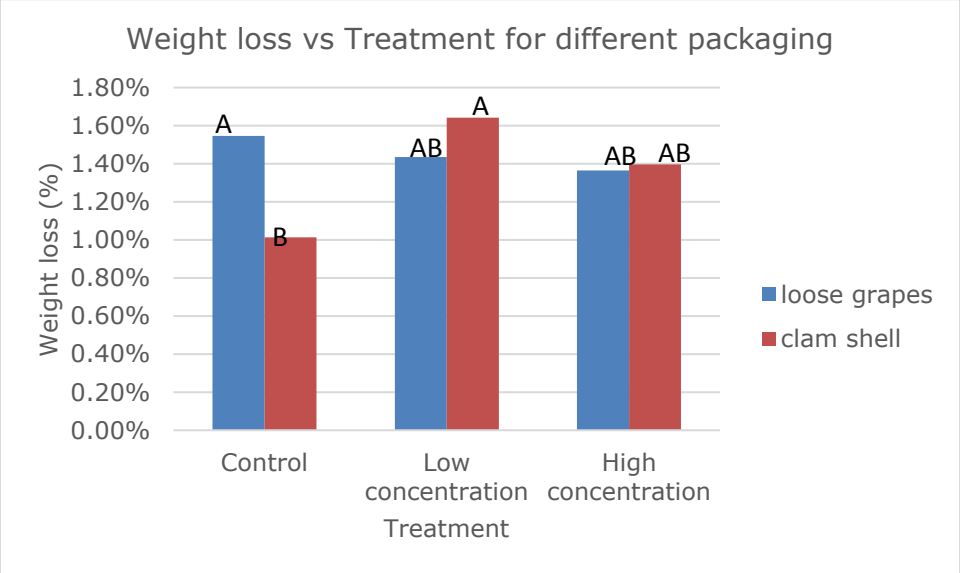

Figure 4 - Weight loss vs. Treatment for different packaging

Rachis Browning and Desication

An important quality indicator in table grapes is the colour and turgor of the stem. The green colour and freshness of the rachis is a good indication to decide if whether or not a cluster is marketable. In a previous study, SO_2 has been shown to retard the browning of the rachis in table grapes (Nelson, 1983).

For this experiment, a standard desiccation colour index rating of 1 to 5 was used to score rachis condition after each storage period and a rating above 3 was considered unmarketable (Lichter et al., 2008). Even after 8 weeks of storage for the control and 15 weeks for both low and high SO_2 concentration, the colour index rating was below 3 regardless of the treatment (Table 4). There was a significant difference between the treatments with the high concentration resulting in a higher rachis browing (Table 5).

It is not clear as to why the control and the low concentration treatment outperformed the high concentration SO_2 treatment with respect to rachis browning. Phytotoxicity to SO2 can manifest as rachis damage (Baiano et al., 2007), so it is possible that this could explain the rachis browning scores for the high SO2 concentration.

Table 4: Average colour index recorded at different week of storage

Week	Colour index	Significance			
15	2.970	A			
10	2.711		B		
8	2.533			C	
6	2.411			C	
4	2.411			C	
2	1.000				D

Table 5: Average colour index for the different treatment

Treatment	Colour index	Significance		
High conc.	2.767	A		
Low conc.	2.206		B	
Control	2.046			C

Healthy Cluster

Healthy clusters were measured by scoring the percent of healthy clusters after each storage period. The amount of decay was most pronounced in the control treatment, where the storage treatment was concluded by week 10 due to mould development noticed at week 8. From *Table 6*, we can observe that the percentage of healthy clusters decreased significantly at week 10 of storage. The high and low concentration treatment were stored up to 15 weeks where the decay began to accelerate.

Table 6: The overall percentage of healhy cluster over time

Week	Healthy Cluster	Significance	
2	100.0%	A	
4	100.0%	A	
6	100.0%	A	
8	94.4%	A	
10	83.3%		B
15	78.6%		B

The low concentration treatment had the highest percentage of healthy clusters followed by the high concentration and the control, the control being significantly different from the sulfur treated grapes (Table 7).

Table 7: The average percentage of healthy cluster for the different treatment

Treatment	Healthy Cluster	Significance	
Low conc	0.978	A	
High conc	0.939	A	
Control	0.865		B

Sulphur Dioxide Damage

Sulphur dioxide damage ratings were based on a visual observation of berries, which exhibited bleaching caused by SO_2. No bleaching was observed in either of the SO2 treatments at any of the evaluation time points.

Conclusion

A fumigation protocol was developed for the SO_2 fumigation of 'Sovereign Coronation' table grapes, which can be used in a scaled fashion, and be adapted to the Ontario table grape industry. By applying the same principles set forward in this report, producers have the tools available to implement this Postharvest treatment.

Through adaptation of the total utilization method described above and in the literature, paired with the sealed chamber method, equipment for fumigating palletized loads of table grapes could be adapted in a scaled capacity by producers who wish to fumigate their own product.

When utilized in tandem with a controlled cold room held at ideal conditions, this treatment and set-up was able to extend postharvest viability of table grapes from 8 (control) to 15 weeks (low and high treatments).

Upon completion of this study, certain key impacts must be addressed in relation to the Postharvest storability of 'Sovereign Coronation' table grapes. First, it should be highlighted that proper postharvest handling, packaging, and environmental control of cold storage facilities must come paramount to any other treatments. This fundamental understanding was highlighted in the results of this project in several ways. First, the grapes received appeared to be in good condition, with minimal mechanical damage to berries observed. These findings suggest that proper picking and packaging practices were utilized. Additionally, harvested grapes were placed immediatley into cold storage, where forced air-cooling was employed to bring internal product temperatures down expediently, thus reducing the weight loss and the detrimental effects associated with it. During the harvesting operation, even a short cooling delay at high air temperatures, results in premature stem browning caused by weight loss. Low critical cluster water-loss threshold values combined with weight loss that can occur during harvesting operations enhance the need to minimize cooling delays. In addition, cold room humidity was held above 95% for the duration of storage. In turn, the untreated control was able to attain an 8-week storage life and maintain its marketability.

In the case of both low and high concetration treatments, sovereign coronation grapes were able to achieve 15 weeks of postharvest storage before being deemed unmarketable. This finding reinforces previous research, which suggests that a minimum 100 ppm-hr contact time is necessary to kill Botritis mycelium. In addition, it was observed that in the case of a high SO_2 concentration treatment; stem browning appeared sooner than in the case of the low concentration treatment. It is important to note however, that stem browning was the main visual reaction to the sulfur, and was present in both treatments, progressing over time with each successive treatment. No additional cracking, bleaching, or other phytotoxic effects were observed (See Figure 7, Figure 8 and Figure 9).

Appendix A:

Figure 7: (From L to R) Control visual evaluation at weeks 4, 8 and 10

Figure 8: (From L to R) Low concentration visual evaluation at week 4, 8 and 10

Figure 9: (From L to R) High concentration visual evaluation at weeks 4, 8 and 10

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