

**ORTHODOX AND ALTERNATIVE STRATEGIES TO CONTROL  
POSTHARVEST DECAY IN TABLE GRAPES**

BY

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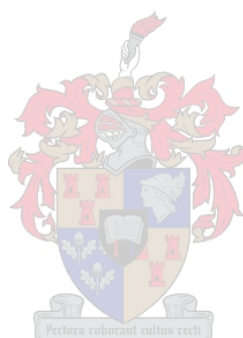
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## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

Signature



Date

## SUMMARY

More and more markets develop around the world for South African grapes and it becomes a challenge to store grapes for longer and reach the market with superior quality. The most destructive decay fungus, *Botrytis cinerea* can cause huge economic losses and successful postharvest control in the table grape industry relies on SO<sub>2</sub>. This gas not only controls the fungus but also causes losses due to phytotoxicity. SO<sub>2</sub> also creates allergic reactions amongst certain people. In modern times the focus is on food safety and governments consequently impose certain regulations and restrictions to restrict the use of chemicals and ensure “cleaner” produce. The objective of this study was to find a steriliser to reduce *B. cinerea* inoculum on the berry surface prior to storage, to be used in conjunction with the current method of control – the SO<sub>2</sub> generator pad.

Very high rainfall and hot weather contribute to increased disease pressure and latent infections during the growing season. Table grape cultivars differ in their sensitivity to decay, caused by *B. cinerea*, and damage by SO<sub>2</sub> and there is a fine margin between effective decay control and damage due to SO<sub>2</sub> phytotoxicity. The Chilean device, Dosigas, was used for in-carton fumigations on grapes. Dosigas injects accurate doses into a carton of grapes packed with a dual phase SO<sub>2</sub> generator. The use of in-carton SO<sub>2</sub> fumigation on cultivars less sensitive to decay, such as Red Globe, did not account for any reduction in decay that is of commercial value. The sensitivity to SO<sub>2</sub> was evident with damage that is aggravated by increasing SO<sub>2</sub> concentration. This cultivar had less decay in perforated liners, viz. 2mm- or micro-perforated, but the addition of in-carton fumigation did not account for any differences. Thompson Seedless, a cultivar sensitive to decay and split, had very high decay levels but in-carton fumigation caused drastic reductions. The addition of in-carton fumigation reduced decay in all types of liners. However, high SO<sub>2</sub> concentrations induced berry splits that created an entrance port for *B. cinerea* spores or more damage by the SO<sub>2</sub> gas.

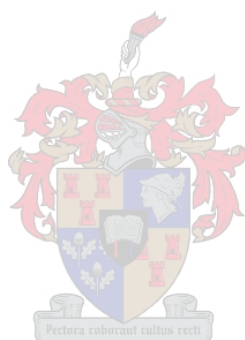
ClO<sub>2</sub> is widely used to eliminate fungal spores in dump tanks of apple and pear packhouses. This chemical has ‘Generally Regarded as Safe’ (GRAS) status and ClO<sub>2</sub> gas was used in this study since liquids have negative effects on postharvest quality and because of greater penetration ability. Exposure to extremely high ClO<sub>2</sub> concentrations damages the plant tissue and this predisposes the tissue to *B. cinerea* infections or damage by SO<sub>2</sub>. Very low decay levels on Red Globe and Dauphine and the absence of infection spreading from one berry to

another indicated that prepack ClO<sub>2</sub> sterilisers decreased the viability of fungal spores on the berry surface. Prepack fumigation with ClO<sub>2</sub> caused no reaction of SO<sub>2</sub> with stems, which were drier and had fungal growth on them. Fumigation with ClO<sub>2</sub> diminishes negative effects such as split and shattering of grapes with high pulp temperatures. Excessive release of SO<sub>2</sub> was, however, enhanced by packing warm grapes (> 30°C) in non-perforated liners. Neither the prepack ClO<sub>2</sub> fumigation nor SO<sub>2</sub> was capable to reduce decay levels on grapes harvested during these conditions. ClO<sub>2</sub> is a highly reactive chemical and due to this characteristic were grapes exposed to various ClO<sub>2</sub> concentrations since no provision was made to ensure accurate concentrations on the grapes during the study.

Significant interactions occurred between ClO<sub>2</sub> concentration and exposure time for decay and SO<sub>2</sub> damage on Red Globe and Dauphine. Visual observations of the grape appearance indicated the presence of sodium chloride (NaCl) crystals on all cultivars treated with 75 µg/mL ClO<sub>2</sub>. Grapes with different pulp temperatures were fumigated with ClO<sub>2</sub>. Despite the differences in decay levels on Dauphine, the highest (35°C) and lowest (15°C) pulp temperatures did not differ significantly. High pulp temperatures, however, caused SO<sub>2</sub> damage. On Red Globe, 30°C pulp temperatures had the highest decay levels and levels decreased with very high pulp temperatures (35°C). Despite the negative effect of high pulp temperatures (35°C) on decay and SO<sub>2</sub> damage, the combination of ClO<sub>2</sub> with high pulp temperatures may eradicate *B. cinerea* on the berry surface.

Investigation of the potential of 'Generally Regarded as Safe' (GRAS) chemicals as sterilisers were compared with a fungicide and led to the conclusion that: Teldor, Sporekill and Citrofresh were effective in reducing *B. cinerea* infection on grapevine leaves with no significant differences between the application methods. On inoculated Red Globe grapes, Teldor, Sporekill and ClO<sub>2</sub> were equally effective and reduced decay levels to 8.9%, 11.0% and 12.7%, respectively, compared with the water sprayed control (27.1%). This cultivar is not very sensitive to decay and fungal infections that spread from one berry to another are not a common observation. No difference was observed between spray and dip application of these compounds. Optimal coverage of fruit surfaces during sterilising treatments is an essential requirement for efficacy. Liquid dip-treatments, however, have negative effects on quality and storage potential, which might not be experienced with spray application, although sufficient spray coverage should still be ensured. A spraying method is conflicting with current commercial packing practices in South Africa and installation of spraying

machines in pre-cooling rooms might eliminate the problems with long drying periods and logistical problems in the packhouse. Postharvest spray application of these GRAS chemicals therefore offers an alternative to SO<sub>2</sub> fumigation and should be investigated further.



## OPSOMMING

### **Ortodokse en alternatiewe strategieë vir na-oes bederfbeheer op tafeldruie**

Meer en meer markte ontwikkel vir Suid Afrikaanse druiwe in die wêreld en dit word 'n uitdaging om druiwe vir langer op te berg om markte met uitmuntende gehalte te bereik. Die mees vernietigende bederf swam, *Botrytis cinerea*, kan groot ekonomiese verliese veroorsaak en suksesvolle beheer berus op SO<sub>2</sub>. Hierdie gas beheer nie net die swam nie, maar veroorsaak ook groot verliese as gevolg van fitotoksiteit en kan allergiese reaksies by sommige mense veroorsaak. Huidiglik is die fokus op voedselveiligheid en regerings stel gevolglik sekere regulasies en beperkings in om "skoner" produkte te verseker. Die doel van hierdie studie was om 'n steriliseerder te vind wat die *B. cinerea* inokulum op die korreloppervlak voor opberging verminder en wat saam met die huidige metode van beheer, SO<sub>2</sub> gasvel, gebruik kan word.

Baie hoë reënval en warm weer dra by tot toenemende siektedruk en latente infeksies gedurende die groeiseisoen. Tafeldruifkultivars verskil in hul sensitiwiteit vir bederf, veroorsaak deur *B. cinerea*, en skade deur SO<sub>2</sub> en daar is 'n nou grens tussen effektiewe bederfbeheer en skade as gevolg van SO<sub>2</sub> fitotoksiteit. Binne-karton SO<sub>2</sub> beroking is met die Chileense toerustingstuk, Dosigas, gedoen wat die oppervlak van die druiwe steriliseer. Dosigas spuit akkurate dosisse in 'n karton druiwe wat met 'n dubbelfase SO<sub>2</sub> gasvel gepak is. Die gebruik van binne-karton SO<sub>2</sub>-beroking op kultivars wat minder sensitief is vir bederf, soos Red Globe, het nie enige vermindering in bederfvlakke teweeg gebring wat van kommersiële waarde was nie. Die sensitiwiteit vir SO<sub>2</sub> was duidelik, met skade wat vererger word deur toenemende SO<sub>2</sub> konsentrasies. Hierdie kultivar het minder bederf gehad in geperforeerde sakke, nl. 2 mm of mikro geperforeerde sakke, maar die toediening van binne-karton SO<sub>2</sub>-beroking het nie enige verskille teweeg gebring nie. Thompson Seedless, 'n kultivar wat sensitief is vir bederf en bars, het baie hoë vlakke van bederf gehad, maar binne-karton SO<sub>2</sub>-beroking het drastiese vermindering tot gevolg gehad. Die toediening van binne-karton SO<sub>2</sub>-beroking het 'n vermindering in bederf in alle tipe sakke tot gevolg gehad. Hoë SO<sub>2</sub> konsentrasies het egter korrelbars geïnduseer wat ingangsplekke vir *B. cinerea* of meer skade deur SO<sub>2</sub> gas geskep het.

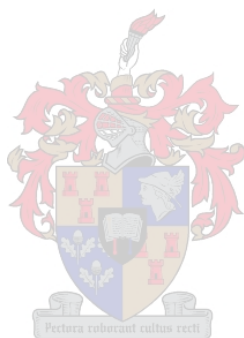
ClO<sub>2</sub> word wyd verspreid in die dompeltenke van appel- en peer pakstore gebruik om swamspore te vernietig. Dié chemiese middel het 'Generally Regarded as Safe' (GRAS) status en ClO<sub>2</sub> gas is in hierdie studie gebruik aangesien vloeistowwe 'n negatiewe effek op

naoes kwaliteit het en omdat die gas 'n groter indringbaarheidsmoontlikheid het. Blootstelling aan baie hoë ClO<sub>2</sub> gas beskadig die plantweefsels en dit stel weefsel bloot aan *B. cinerea* infeksies of beskadigings deur SO<sub>2</sub>. Baie lae bederfvlakke op Red Globe en Dauphine en die afwesigheid van infeksies wat van een korrel na 'n ander versprei, dui aan dat voor-pak ClO<sub>2</sub> steriliseerders die ontkiemingsvermoë van swamspore verlaag het. Voor-pak beroking met ClO<sub>2</sub> het geen reaksie van SO<sub>2</sub> met die stingels veroorsaak nie en dit was droër met swamgroei daarop. Beroking met ClO<sub>2</sub> verlaag die negatiewe effekte soos korrelbars en loskorrels van druiwe met hoë pulp temperature. Oormatige vrystelling van SO<sub>2</sub> was egter bevorder deur warm druiwe (> 30°C) in nie-geperforeerde sakke te verpak. Nie die voor-verpakkings ClO<sub>2</sub> beroking óf die SO<sub>2</sub> was instaat om bederfvlakke op druiwe wat in hierdie kondisies geoes was, te verlaag nie. ClO<sub>2</sub> is 'n hoogs reaktiewe chemiese middel en asgevolg van hierdie eienskap was druiwe aan 'n verskeidenheid ClO<sub>2</sub> konsentrasies blootgestel omdat geen voorsiening gemaak was om akkurate konsentrasies op die druiwe in hierdie studie te verseker nie.

Betekenisvolle interaksies het tussen ClO<sub>2</sub> konsentrasie en blootstellingstyd vir bederf en SO<sub>2</sub> skade van Red Globe en Dauphine voorgekom. Visuele waarnemings van die druifvoorkoms dui aan dat natriumchloried-(NaCl) kristalle op alle kultivars voorkom wat met 75 µg/mL ClO<sub>2</sub> behandel was. Druiwe van verskillende pulptemperature is met ClO<sub>2</sub> berook. Ten spyte van verskille in bederfvlakke van Dauphine, was die hoogste (35°C) en laagste (15°C) pulptemperature nie betekenisvol verskillend nie. Hoë pulptemperature het egter SO<sub>2</sub> skade veroorsaak. In Red Globe het 30°C pulptemperature die hoogste bederfvlakke gehad en by baie hoë temperature (35°C) het vlakke weer afgeneem. Afgesien van die negatiewe effek van hoë pulp temperature (35°C) op verrotting en SO<sub>2</sub> skade mag die ClO<sub>2</sub> in kombinasie met hoë pulptemperatuur kombinasie *B. cinerea* op die korreloppervlak vernietig.

Die ondersoek na die vermoë van 'Generally Regarded as Safe' (GRAS) chemikalieë as steriliseerders is met 'n swamdoder vergelyk en het die volgende resultate gelever: Teldor, Sporekill en Citrofresh was effektief om *B. cinerea* op wingerdblare te verminder met geen betekenisvolle verskille tussen die toedieningsmetodes. Op geïnkuleerde Red Globe tafeldruiwe, het Teldor, Sporekill en ClO<sub>2</sub> gelyke effektiwiteit gehad en het dit bederfvlakke tot (onderskeidelik) 8.9%, 11.0% and 12.7% verminder in vergelyking met die waterkontrole (27.1%). Hierdie kultivar is nie baie sensitief vir verrotting nie en swaminfeksies wat van een korrel na 'n volgende versprei kom nie algemeen voor nie. Geen verskille is opgemerk tussen

die spuit- en doop-toediening van hierdie middels nie. Optimale bedekking van die vrugoppervlakke gedurende steriliseringsbehandelings is 'n noodsaaklike vereiste vir effektiwiteit. Doop-behandelings het 'n negatiewe effek op kwaliteit en opbergpotensiaal gehad, wat nie by spuit toedienings ondervind word nie, alhoewel voldoende spuit bedekking nog steeds verseker moet word. 'n Spuitmetode is teenstrydig met huidige verpakkingspraktyke in Suid-Afrika en die installering van spuitmasjiene in voorverkoelingskamers kan hierdie probleem van lang drogingsperiodes en logistieke probleme in die pakstoor uitskakel. Naoes spuit toedienings van GRAS chemikalieë bied dus 'n alternatief vir SO<sub>2</sub> beroking en moet verder ondersoek word.





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## TABLE OF CONTENTS

<b>A.</b>	<b>Orthodox and Alternative Strategies to Control Postharvest Decay on Table Grapes .....</b>	<b>1</b>
1.	<b>INTRODUCTION.....</b>	<b>1</b>
1.1	United States of America .....	1
1.2	Chile .....	2
1.3	South Africa .....	2
2.	<b>POSTHARVEST DECAY.....</b>	<b>2</b>
2.1	<i>Botrytis cinerea</i> .....	3
2.2	Other decay fungi .....	6
3.	<b>POSTHARVEST CONTROL STRATEGIES .....</b>	<b>6</b>
3.1	Sulphur dioxide .....	7
3.2	Chlorine.....	11
3.3	Other.....	13
4.	<b>CONCLUSION.....</b>	<b>18</b>
5.	<b>REFERENCES.....</b>	<b>19</b>
<b>B.</b>	<b>Article I: .....</b>	<b>35</b>
	<b>The use of in-carton SO<sub>2</sub> fumigation to control postharvest decay on table grapes. ....</b>	<b>35</b>
1.	<b>INTRODUCTION.....</b>	<b>36</b>
2.	<b>MATERIALS AND METHODS.....</b>	<b>38</b>
	Grapes.....	38
	Inoculation.....	38
	Packaging .....	38
	In-carton fumigation.....	39
	Quality evaluation .....	39
	Statistical analysis .....	40
3.	<b>RESULTS.....</b>	<b>40</b>
	Trial 1 – Optimum concentration .....	40
	Trial 2 – Different liners.....	42
	Trial 3 – Packing after rain.....	44
4.	<b>DISCUSSION .....</b>	<b>45</b>
5.	<b>REFERENCES.....</b>	<b>48</b>
<b>C.</b>	<b>Article II.....</b>	<b>64</b>
	<b>Control of <i>Botrytis cinerea</i> in table grapes using a prepack chlorine dioxide fumigation in conjunction with SO<sub>2</sub> generating pads.....</b>	<b>64</b>
1.	<b>INTRODUCTION.....</b>	<b>65</b>
2.	<b>MATERIALS AND METHODS.....</b>	<b>67</b>
	Grapes.....	67
	Inoculation.....	67

CIO <sub>2</sub> Fumigation .....	68
Trial 1 – Optimum CIO <sub>2</sub> concentration and exposure time.....	68
Trial 2 – Different pulp temperatures.....	68
Trial 3 – Optimum CIO <sub>2</sub> concentration and exposure time for adverse weather conditions .....	69
Packing.....	69
Statistical analyses.....	69
<b>3. RESULTS.....</b>	<b>70</b>
Trial 1 – Optimum concentration and exposure time.....	70
Trial 2 – Pulp temperature.....	71
Trial 3 – Optimum concentration and exposure time for grapes harvested in adverse weather conditions.....	72
<b>4. DISCUSSION .....</b>	<b>73</b>
<b>5. REFERENCES.....</b>	<b>76</b>
<b>D. Article III .....</b>	<b>95</b>
<b>Potential prepack treatments for surface sterilisation of table grapes .....</b>	<b>95</b>
<b>1. INTRODUCTION.....</b>	<b>96</b>
<b>2. MATERIALS AND METHODS.....</b>	<b>99</b>
Chemical treatments.....	99
Inoculation.....	100
Leaves.....	100
Grapes.....	101
Statistical analyses.....	101
<b>3. RESULTS.....</b>	<b>102</b>
Leaves.....	102
Grapes.....	102
<b>4. DISCUSSION .....</b>	<b>103</b>
<b>5. REFERENCES.....</b>	<b>104</b>
<b>GENERAL DISCUSSION AND CONCLUSION.....</b>	<b>118</b>
<b>APPENDICES .....</b>	<b>121</b>

## A. Literature Review

### Orthodox and Alternative Strategies to Control Postharvest Decay on Table Grapes

#### 1. INTRODUCTION

Grapes have been cultivated for thousands of years and many adaptations have been made to improve the quality and appearance of the product. With new markets developing around the world, it becomes a challenge to store the grapes for longer to reach the markets with superior quality. Consumers consider high quality fruit to be those with nice appearance, high nutritional value and good taste (Crisosto & Crisosto, 2002; Crisosto *et al.*, 2002 b). This is why technology developed over the years to preserve the grapes for longer (Morris *et al.*, 1992). Consumer demands for reduced chemical use are also growing and this created a need for alternative forms of pest and disease control (Retamales *et al.*, 2003; Whiteman & Stewart, 1998).

##### 1.1 United States of America

Most of the United State's grapes are produced in California (Couey & Uota, 1961; Crisosto *et al.*, 2002 a; Nelson & Ahmedullah, 1973, 1976; Palou *et al.*, 2002 b; Pirog, 2000). The Coachella and San Joaquin valleys are the most important production regions in America (Pirog, 2000). Thompson Seedless, Flame Seedless, Red Globe, Ruby Seedless, Perlette and Sugraone are the most important cultivated varieties in the country (Pirog, 2000). The most important markets for Californian grapes are the United States and Canada with grapes being imported from Chile, Mexico and South Africa in the winter (FDA-USDA, 1998; Pirog, 2000). In California, grapes are packed in the field into 9 – 10 kg wooden boxes (Cappellini *et al.*, 1986; Crisosto *et al.*, 2001 b; Karabulut *et al.*, 2003; Perkins-Veazie *et al.*, 1992) or polystyrene foam and fibreboard boxes (Harvey *et al.*, 1988). From the field, grapes are directly placed in the pre-cooler where they are fumigated with SO<sub>2</sub> gas and kept at 0°C and high relative humidity (Couey & Uota, 1961; Crisosto *et al.*, 2001 b; Crisosto *et al.*, 2002 a; Nelson & Ahmedullah, 1973). Californian grapes are normally transported via rail or truck, which takes ± 10 days, but grapes are stored for long periods before transport to reach the desired markets (Nelson & Ahmedullah, 1973).

## 1.2 Chile

Table grapes are Chile's most important export crop. Their success can be ascribed to the shift in consumer preferences for grapes all year round, abundant natural resources that are suitable for fruit production and a government that enhances access to changing markets (USDA, 1992). North America and Europe are Chile's biggest markets for exported fruit and South Africa, Australia, New Zealand and Argentina compete with it for the specific markets (USDA, 1992). Asian markets such as Singapore and Taiwan are growing markets for Chile's fruit. Their most important exported cultivars are Flame and Thompson Seedless, Ribier, Red Globe and a sharp increase in Crimson Seedless (Stein & McEachern, 2004). Grapes are packed in boxes with sulphur dioxide (SO<sub>2</sub>) generator pads, palletised and pre-cooled to ±2°C. Refrigerated trucks are the only means of transport of grapes within Chile. It takes 2½ -3 days after harvest for grapes to be ready to load on the ship for sea transport (Pirog, 2000).

## 1.3 South Africa

The most important table grape producing areas in South Africa are the Orange River Valley, Berg River Valley and the Hex River Valley. The Orange River Valley is Chile's biggest competitor for the early imports to the northern hemisphere. Grapes are harvested in the early morning when temperatures are below 25°C and packed in a pack house in a 4.5 or 8.2 kg carton. Bunches are packed in a polyethylene liner with a SO<sub>2</sub> generator pad. Adequate SO<sub>2</sub> concentrations range between seven and 20 µL/L with high concentrations immediately after packing and lower concentrations during long term storage (Anon, 2002 b). The protocol is to get grape pulp temperature to 10°C within 12 hours after packing and then to the desired – 0.5°C, which is also the transport temperature within 48 hours after this (Anon, 2000).

## 2. POSTHARVEST DECAY

Decay fungi account for huge economic losses as they negatively affect quality and eventually lead to grapes that are not saleable. Decay fungi include *Alternaria*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Botrytis cinerea* of which the last mentioned is the most important (Ferreira & Venter 1996; Tournas & Katsodas, 2005; Avissar & Pesis, 1991).

## 2.1 *Botrytis cinerea*

*Botrytis cinerea* is a necrotrophic fungus that actively kills plant cells and subsequently lives on the dead tissue (Elmer & Michailides, 2004; Kaile *et al.*, 1991; Karabulut *et al.*, 2005; Ten Have, 2000). It is the common cause of bunch rot of table grapes (Combrink & Ginsburg, 1972; Karabulut *et al.*, 2003; McClellan *et al.*, 1973; Palou *et al.*, 2002 a; Peiser & Yang, 1985; Thompson & Latorre, 1999; Witbooi *et al.*, 2000 a) and is responsible for huge economic losses during storage and transport of the harvested crop (Leroux *et al.*, 1999; Nigro *et al.*, 1998; Ten Have, 2000). Botrytis rot in grapes is also commonly known as grey mould or slip skin (Combrink & Ginsburg, 1972; Ten Have; 2000).

### 2.1.1 *Infection and conducive conditions*

Postharvest decay can be traced to infections that occur either between flowering and fruit maturity or during harvesting and handling, storage and marketing (Coertze & Holz, 2002; Jarvis, 1980). Infection of grape berries often occurs at bloom and is followed by a latent period (Keller *et al.*, 2003; McClellan *et al.*, 1973; Verhoeff, 1980). Entrance is obtained through the stylar end of the grape flower (McClellan *et al.*, 1973), which is, however, conflicting with results from South African researchers who found the stylar end to be free from fungi (Coertze & Holz, 1999; Holz *et al.*, 2003, 2004).

The young (immature) berry exhibits high resistance against the fungus, based on their cuticle structure and tannin-like blockers of fungal enzymes in the cell walls of the berry skin (Johnston & Williamson, 1992; Keller *et al.*, 2003; Verhoeff, 1980). Berries are relatively resistant to infection during the early stages of development, but susceptibility increases from véraison onward or with an increase in total soluble solids content (Coertze & Holz, 1999; Tenberge, 2004; Verhoeff, 1980; Zahavi *et al.*, 2000). The fungus germinates on the skin of the berry and then penetrates to attack the underlying tissue (Combrink & Ginsburg, 1972; Kamoen, 1992; Verhoeff, 1980). At this stage the first symptom, “slip skin”, appears (Droby & Lichter, 2004). Any pressure that is applied to the berry will cause the skin to be separated from the underlying tissue (Mouton, 1991). This is also accompanied by a red-brown discolouration that is not easily identified on red and black cultivars. After this, white to grey fungal growth appears on the berry surface and soon whole bunches are covered (Coertze & Holz, 2002; Johnston & Williamson, 1992; Mouton, 1991). The decaying berry infects neighbouring berries and infection can spread over an entire bunch (Droby & Lichter, 2004).

The formation of latent infections in young berries is very common for this fungus but it has different routes/pathways to penetrate and establish within the host (Heale, 1992; Holz *et al.*, 2004). Holz *et al.* (2004) found the attachment zone of the berry-pedicel to be an important site for development of the fungus. Direct penetration on the berry skin was observed by several researchers (Holz *et al.*, 2004; Kamoen, 1992; Leone, 1992; Verhoeff, 1980).

This fungus may penetrate the host through specialised host structures, natural openings and through wounds (Blakeman, 1980; Kamoen, 1992; Keller *et al.*, 2003). Wounds are very important entry sites for the fungus and are caused by insects, frost, hail, windblown sand, sunburn and splitting due to sharp increases in turgor pressure of berries (Coertze & Holz, 1999; Jarvis, 1980). Other infection pathways include stigmata, pedicels and natural openings (Coertze & Holz, 2001; Holz *et al.*, 2004; Kamoen, 1992; Keller *et al.*, 2003; Verhoeff, 1980). Two scenarios can be identified with wounds, viz. a) the wound is formed and newly arrived conidia should be close to it to cause an infection, or b) previously deposited conidia (latent) that would infect the newly formed wound (Holz *et al.*, 2004). In both cases this should be accompanied by favourable weather conditions and/or the presence of free water (Blakeman, 1980; Coertze & Holz, 2001; Holz *et al.*, 2004; Jarvis, 1980). These conditions, however, seldom prevail simultaneously and damage caused by insects is an important contributing factor (Coertze & Holz, 2001; Holz *et al.*, 2004; Jarvis, 1980). An increase in nutrients in the berry from véraison onwards results in regular visits from insects such as the fruit fly (Engelbrecht, 2002), which creates wounds, which are excellent entrance ports for the fungus.

The cuticle forms the first barrier and consists of cutin and a polyester of hydroxylated fatty acids that is covered by a waxy layer of fatty alcohols (Comménil *et al.*, 1995; Kamoen, 1992; Tenberge, 2004). *Botrytis* pathogens are equipped with a set of enzymes and/or metabolites that enable the pathogen to invade host tissue, kill the cells and convert the host tissue into fungal mass (Comménil *et al.*, 1995; Leone, 1992). The fungus produces an extracellular lipase that is capable of hydrolysing the components of the cutin, which are the long chain fatty acids (Comménil *et al.*, 1995; Leone, 1992; Viterbo *et al.*, 1992). When the cuticle is breached an intermediate swelling is formed in the cell wall just beneath the cuticle and cell wall degrading enzymes may diffuse from the pathogen into the host at this stage (Kamoen, 1992; Kars & van Kan, 2004; Tenberge, 2004). *Botrytis* will kill the underlying epidermal cells before they are invaded by hyphae (Kars & van Kan, 2004).

Rigorous handling during packing and at ports and insufficient temperature and humidity control are important factors that are conducive to development of latent infections after harvest (Nelson & Ahmedullah, 1976; Sommer, 1985). Relative humidity and the presence of free water are often thought of as the most important single factors influencing infection (Blakeman, 1980). A wound is not necessary for infection when wet and/or high relative humidity conditions prevail (Comménil *et al.*, 1999; Holz, 1999; Keller *et al.*, 2003; Kupferman, 1999; Latorre *et al.*, 1997; Peacock & Smilanick, 1998; Thompson & Latorre, 1999; Ten Have, 2000). It takes 1-3 hours during wet conditions or with >93% relative humidity for spores to germinate, but free water may reduce the time needed for infection (Blakeman, 1980; Holz, 1999). Temperatures below 0°C delay development of the fungus but do not kill it (Sommer, 1985; Elad *et al.*, 1992). Active host defence mechanisms are inhibited at these low temperatures, and the tissue is predisposed to infection (Coertze & Holz, 2001). Blakeman (1980) reported the optimum temperature for *Botrytis* germination and infection to be between 20 and 25°C.

Inoculum sources: Any part of the fungus can serve as a survival structure and it varies from sclerotia, chlamydospores, and conidia to mycelium (Holz *et al.*, 2004; Jarvis, 1980). Conidia are considered to be the most important propagules of the fungus (Epton & Richmond, 1980; Holz *et al.*, 2004; Tenberge, 2004) and therefore they are considered to be the primary inoculum for infection and dispersal (Coertze & Holz, 2001; Holz *et al.*, 2004). Conidia are dispersed by wind, water and insects (Coertze & Holz, 1999, 2001; Jarvis, 1980; Keller *et al.*, 2003) and direct sunlight, more specifically UV light, causes mortality (Holz *et al.*, 2004). Conidia that land on host tissue will germinate and penetrate the tissue (Kars & van Kan, 2004). Adhesion in water to the berry surface is very strong and here the conidia will settle in the centre of a droplet (Coertze & Holz, 1999; Holz *et al.*, 2004). This will settle close to and around stomata that will serve as a site of entry (Holz, 1999; Holz *et al.*, 2004).

Germination is identified by a swelling and the presence of a germ tube and it is a process that requires water and in some cases sugars and amino acids (Epton & Richmond, 1980; Heale, 1992; Holz *et al.*, 2004; Tenberge, 2004; Verhoeff, 1980). Infection from conidia requires free water (Jarvis, 1980).



Sclerotia, which are formed in adverse weather conditions can survive extreme environmental conditions and can be considered as the most important survival structures (Coley-Smith, 1980; Holz *et al.*, 2004). The formation of sclerotia is influenced by temperature, light, pH and nutrition but the formation of many hyphal branches is a prerequisite (Coley-Smith, 1980). Sclerotia will germinate to produce either mycelium or conidia (Beever & Weeds, 2004). Germination is favoured by lower temperatures and occurs in autumn and/or spring (Coley-Smith, 1980).

Chlamydoconidia are formed during the transformation of vegetative mycelium. It will germinate in moist conditions with sufficient nutrients (Holz *et al.*, 2004). Another source of inoculum is infected berries that dropped during the pre-harvest stages, which provide conidial and mycelial inoculum for late season infections (Coertze & Holz, 1999).

## 2.2 Other decay fungi

*Penicillium sp.*, *Alternaria* and *Rhizopus nigricans* are decay causing fungi that can limit the postharvest storage life of table grapes (Avissar & Pesis, 1991). Gao *et al.* (2003) identified these to be of a lesser problem in comparison to *Botrytis*. *Alternaria* is identified by tan-coloured lesions that turn brown and develop fungal growth on the berry. This fungus does not spread from one berry to another (Ferreira & Venter, 1996). Very little information is currently available about the primary effect of *Alternaria* and *Rhizopus*, but these are also part of the complex sour rot on table grapes. Both of these fungi infect table grapes but do not develop during low temperature storage (Crisosto *et al.*, 2002 b). *Penicillium sp.* normally occurs on the surface of damaged berries and do not infect healthy berries (Crisosto *et al.*, 2002 b; Ferreira & Venter, 1996).

## 3. POSTHARVEST CONTROL STRATEGIES

Control of decay during cold storage is very important as growth of the *B. cinerea* fungus is delayed but not stopped during cold storage (Droby & Lichter, 2004). With control strategies, preservation of the quality, original colour, and stem condition are of utmost importance. Infection occurs preharvest and control is typically achieved through fungicide applications (Plotto *et al.*, 2003; Schena *et al.*, 1999). Reduced efficacy of pre-harvest fungicides, inability of them to control postharvest diseases, the risk it holds for consumers and environmental

pollution encourage the need for alternative control strategies (Lima *et al.*, 1998; Plotto *et al.*, 2003; Zhang *et al.*, 2007).

### ***3.1 Sulphur dioxide***

In America, SO<sub>2</sub> has been used for more than 70 years to reduce the occurrence of grey mould on table grapes (Chervin *et al.*, 2005; Crisosto *et al.*, 2002 a; László *et al.*, 1981; Palou *et al.*, 2002 a; Smilanick *et al.*, 1990). This gas is highly corrosive to metal and extremely irritating to people, but the contribution of this gas to air pollution is, however, minimal (Nelson & Ahmedullah, 1973; Smilanick & Henson, 1992; Zahavi *et al.*, 2000). SO<sub>2</sub> gas was previously on the list of Generally Regarded as Safe (GRAS) chemicals of the United States Food and Drug Administration (Austin *et al.*, 1997; Zahavi *et al.*, 2000). The gas is commercialised as a liquid or compressed gas and is neither combustible nor explosive.

#### *3.1.1. Mechanism of action*

SO<sub>2</sub> damages fungal membranes and inhibits various enzymes (Droby & Lichter, 2004). The hydrated form of SO<sub>2</sub>, which is sulphurous acid, is the active agent (Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002 a). The hydration of SO<sub>2</sub> and the diffusion of the sulphurous acid to the sensitive sites on the berry skin are affected by temperature (Couey & Uota, 1961; Nelson & Ahmedullah, 1972; Smilanick & Henson, 1992). Sodium metabisulphite releases SO<sub>2</sub> when it reacts with water (Zoffoli *et al.*, 1999) and polyethylene bags (packaging) stabilise the emission of SO<sub>2</sub> (Kokkalos, 1986; Mustonen, 1992).

#### *3.1.2 Application methods*

Two methods of applying the SO<sub>2</sub> gas have been adopted throughout the world: the use of regular fumigations, or the use of in-package SO<sub>2</sub> generator pads, or combinations of these (Karabulut, 2003; Lagunas-Solar *et al.*, 1992; Mustonen, 1992). Room fumigation is a common practice in California and is implemented by exposing the grapes to large doses before storage, which serves as a surface steriliser (Austin *et al.*, 1997; Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2001 a; Crisosto *et al.*, 2002 a; Droby & Lichter, 2004; Nelson & Ahmedullah, 1973; Palou *et al.*, 2002 a). This, however, does not kill inocula within the tissue and is followed by weekly fumigations during cold storage (Austin *et al.*, 1997; Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002 a; Droby & Lichter, 2004; Mustonen, 1992; Nelson & Ahmedullah, 1973). In the total

utilisation system, used by the American industry, the initial fumigation happens simultaneously with pre-cooling (Couey & Uota, 1961; Crisosto *et al.*, 2002 a; Franck *et al.*, 2005; Palou *et al.*, 2002 a). Gas enters the room from a cylinder, using a needle valve to ensure an even gas flow. The amount of SO<sub>2</sub> gas that reaches the grapes is proportional to the air speed (Combrink & Ginsburg, 1972; Combrink & Truter, 1979; Crisosto *et al.*, 2002 a). Commercial applications use 5000 µL/L SO<sub>2</sub> for 20 to 30 minutes for the initial fumigation and 2500 to 5000 µL/L SO<sub>2</sub> for 30 minutes for the weekly fumigations (Combrink & Truter, 1979; Franck *et al.*, 2005; Lagunas-Solar *et al.*, 1992; Smilanick *et al.*, 1990; Smilanick & Henson, 1992; Zoffoli *et al.*, 1999). Fumigation during shipment or transport is impossible and grapes are packed with in-package SO<sub>2</sub> generator pads (Crisosto *et al.*, 2002 a; Palou *et al.*, 2002 a; Zoffoli *et al.*, 1999). Previously, sodium metabisulphite solution was sealed in a polyethylene sachet and a number of sachets were placed on the grapes (Combrink & Ginsburg, 1972; Combrink & Truter, 1979). However, sachets sometimes broke inside the containers and/or shifted around and caused localised fruit injury and were not economical to manufacture. The Californian pad was then designed that used sodium metabisulphite powder glued between two paper strips, which formed a pad that was cut to the size of the container (Mustonen, 1992; Palou *et al.*, 2002 a; Zoffoli *et al.*, 1999). The most common SO<sub>2</sub> pads contain between 5.0 g and 8.4 g sodium metabisulphite (Mustonen, 1992).

Dual-phase SO<sub>2</sub> generator pads make provision for the quick and slow release of SO<sub>2</sub> gas. The high concentration from the first stage (quick release) is released for a short time, which immediately kills *Botrytis* spores that are present on the surface. After a few days at 0°C, the second phase (slow release) starts to produce a low level of SO<sub>2</sub> (Mustonen, 1992; Nelson, 1983). The dual-phase SO<sub>2</sub> is widely used since 1968 for grapes that are transported and stored for ±3 months (Nelson, 1983; Nelson & Ahmedullah, 1972). The single, quick release generator is available for grapes transported and stored for periods shorter than two weeks. Modern SO<sub>2</sub> generator pads consist of two sheets of polyethylene-coated Kraft paper that seals in sodium metabisulphite granules for the second stage. A third sheet of uncoated Kraft paper is glued to the one side to form these pockets with additional sodium metabisulphite for the first stage (Gentry & Nelson, 1968). These generator pads are used in Chile in combination with perforated polyethylene liners. This technology was developed in California and is currently used worldwide (Palou *et al.*, 2002 a). The desirable concentration for in-package SO<sub>2</sub> generator was first established at 75 µL/L, but in 1932 it was found that the effective SO<sub>2</sub> concentration is 20 µL/L. In 1940, it was concluded that 10 to 20 µL/L is

the most effective concentration without any adverse effects. For South African conditions grapes are stored with a 20  $\mu\text{L/L}$   $\text{SO}_2$  concentration (Anon, 2002 b). The continuous release of lower  $\text{SO}_2$  concentrations provides control during longterm storage.

Kokkalos (1986) found the use of sodium metabisulfite pads on the bottom and top of the grapes to be highly effective for these conditions. In other cases grapes are packed with a two-stage  $\text{SO}_2$  generator and do not receive any initial fumigation (Crisosto *et al.*, 2002 a). Different combinations of the single-phase and the dual-phase pad are used throughout the world. Grapes packed with these generators should be quickly cooled and any temperature fluctuation will result in excessive release of the gas that might cause damage (Couey & Uota, 1961; Karabulut *et al.*, 2003). For Californian handling conditions, the best packaging was the use of a 0.3 to 1.2 % vented polyethylene liner with a slow release  $\text{SO}_2$  generator after an initial high dose fumigation (Palou *et al.*, 2002 a).

No fumigations are necessary when an in-package  $\text{SO}_2$  generator pad is used. High initial concentrations are, however, necessary to kill inoculum on the surface (Kokkalos, 1986). Crisosto *et al.* (1994) acknowledged the advantage of an initial fumigant in combination with a generator pad and polyethylene liner, but also recognised the limitation of it under current Californian conditions. This is an even bigger problem under South African and Chilean packing/storage conditions. The development of Dosigas, a Chilean device, came from the efficacy of these high initial dose fumigations. Dosigas is an alternative for fumigation rooms and it can inject extremely small and accurate doses of  $\text{SO}_2$  gas into a packed container of grapes. It is an application that is done after grapes are packed in the desired export carton (Anon, 2002 a).

### 3.1.3 Constraints

Packaging material varies from country to country and ranges from wood/Kraft veneer, polystyrene, fibreboard containers and perforated polyethylene bags which all have a different level of absorption of the gas (Crisosto *et al.*, 2002 a; Smilanick & Henson, 1992). The absorption of gas by the packaging, the cold store and the high level of residues was the reason for the shift towards a total utilisation system. In South Africa and Chile, room fumigation is not a common practice and the initial high concentration is dependent on the release of  $\text{SO}_2$  from a dual phase  $\text{SO}_2$  generator pad, which on the other hand is dependent on

the moisture content in the polyethylene liner, whether vented or unvented (Combrink & Truter, 1979; Franck *et al.*, 2005; Kokkalos, 1986; Palou *et al.*, 2002 a).

Spanish, Chilean and South African grapes are all packed with in-package SO<sub>2</sub> generators and fluctuation in temperature has a great influence on the efficacy of treatment, as well as the damage done to the grapes (Kokkalos, 1986; Nelson & Ahmedullah, 1973). A rise in temperature results in free moisture in the carton that triggers the excessive release of SO<sub>2</sub> gas from the generator pad. A rise in temperature will also result in rapid fungal growth (Combrink & Ginsburg, 1972; Nelson & Ahmedullah, 1973; Witbooi *et al.*, 2000 a).

SO<sub>2</sub> is highly soluble in water. High relative humidity and water condensation, caused by temperature fluctuation, are important factors influencing SO<sub>2</sub> residues and toxicity (Couey & Uota, 1961; Gentry & Nelson, 1968; Nelson & Ahmedullah, 1973; Palou *et al.*, 2002 a). Sulphite residues and phytotoxicity that include bleaching and hairline splitting on the berry surface are the main problems with the SO<sub>2</sub> generator pads (Crisosto *et al.*, 1994; Palou *et al.*, 2002 a; Smilanick & Henson, 1992). Single and dual-phase generator pads differ in problems and design. For the first stage, the interval after closure is critical before SO<sub>2</sub> can be generated. Exposure to the gas should extend over one to two days to kill the surface spores. The rate and duration of SO<sub>2</sub> generation are important parameters for the second stage. The delay before the second phase starts is not as critical as the constant dose of low levels of SO<sub>2</sub> (Nelson & Ahmedullah, 1972).

Special care is necessary to avoid high concentrations of SO<sub>2</sub>, expressed as bleaching (Droby & Lichter, 2004). There must be a barrier that will limit water vapour reacting with sodium metabisulphite or regulating the release of SO<sub>2</sub> from the generator pad onto the grapes (Nelson & Ahmedullah, 1972). Bleaching occurs around the pedicel-end or around microscopic holes or cracks in the cuticle (Crisosto *et al.*, 1994; Droby & Lichter, 2004). Long storage periods with continuous fumigations should be avoided to minimise SO<sub>2</sub> residues on sensitive cultivars. Grapes packed in unvented containers with dual-phase generators may also cause damage after one month of storage (Nelson & Ahmedullah, 1973). It is also important to maintain storage temperature between 0 to 1°C (Smilanick *et al.*, 1990). This emphasises the importance of rapid cooling after harvest to reduce damage caused by SO<sub>2</sub> (Berry & Aked, 1996; Nelson & Ahmedullah, 1972; Witbooi *et al.*, 2000 b). The maximum residue limit for SO<sub>2</sub> has been established at 10 µL/L by the Environmental

Protection Agency in America (Austin *et al.*, 1997; Crisosto *et al.*, 1994; Crisosto *et al.*, 2002 a; EPA, 1999; Palou *et al.*, 2002 a; Zoffoli *et al.*, 1999). Grapes must then be kept/stored at these low temperatures to limit moisture loss, thereby reducing excessive SO<sub>2</sub> release and phytotoxicity and to slow the development of the fungus (Berry & Aked, 1996).

Bleaching of grapes, as a result of SO<sub>2</sub> toxicity, increases with an increase in exposure time and SO<sub>2</sub> concentration. This is also true when temperature and relative humidity increase (Gao *et al.*, 2003; Palou *et al.*, 2002 a). Temperature fluctuation will cause condensation in the carton that will enhance the hydration of sodium metabisulfite and the excessive release of SO<sub>2</sub> (Palou *et al.*, 2002 a). Another problem is the increasing concern of residues on freshly consumed produce. Apart from the phytotoxicity and residues, it is still the aim to deliver high quality and decay-free grapes to the market. Phytotoxicity is associated with bleaching of the berry and hairline splits/cracks (Crisosto *et al.*, 2002 a; Gao *et al.*, 2003; Mustonen, 1992; Nelson, 1982; Palou *et al.*, 2002 a; Zhang *et al.*, 2003). Some cultivars are more prone to decay than others and some cultivars are more sensitive to phytotoxicity than others. Sensitive cultivars have an epidermis with a concave microstructure and with severe damage/bleaching, the surface appears concave as a result of a broken wax layer (Crisosto *et al.*, 1994; Zhang *et al.*, 2003). Crisosto *et al.* (2002 a) went into further detail by explaining how the skin will rupture and cell sap exudes. Permeability and turgor pressure of the underlying tissue increase and sulphite binds to the anthocyanins of the peel resulting in the bleaching spots (Palou *et al.*, 2002 a; Zhang *et al.*, 2003). This is a beneficial factor for the stems during long term storage, as it remains the lush green colour (Mustonen, 1992). Maturity of the crop at harvest, handling and storage are the major factors that influence the storage life of the crop. Less mature grapes absorb more SO<sub>2</sub> and a scar on a damaged berry creates an entrance port for latent infections of the fungus (Mustonen, 1992; Palou *et al.*, 2002 a).

### **3.2 Chlorine**

The concerns with sulphite residues on table grapes, bleaching damage and poor decay control have driven the search for alternatives to SO<sub>2</sub>. Table grapes can withstand extremely low temperatures and -0.5°C is recommended for storage. That low temperature is of high value for prevention of fungal decay but it is only sufficient to slow down the development of the fungus (Droby & Lichter, 2004).



Humphrey Davy discovered chlorine ( $\text{Cl}_2$ ) in 1810 and it is a green-yellow gas with a pungent smell whereas the liquid state is yellow in colour (Saroaha, 2005).  $\text{Cl}_2$  is an effective and economical biocide and recognised as safe in many countries (EPA, 1999; Zoffoli *et al.*, 1999).  $\text{ClO}_2$  is a selective bleaching agent and both  $\text{Cl}_2$  and  $\text{ClO}_2$  are used to treat water worldwide but they also have extensive use in the production of paper, dyestuffs, textiles, petroleum products, medicines, antiseptics, insecticides, food solvents, paints and plastics. Because of these properties,  $\text{Cl}_2$  shows potential to control post harvest decay in a modified atmosphere for table grapes (Anon., 2005; Apel, 2002; Huang, 2001; Junli *et al.*, 1997; Prusky *et al.*, 2001). It is a strong oxidising agent and has the ability to effectively kill microorganisms (Apel, 2002; Gagnon *et al.*, 2005; Han *et al.*, 1999; Lee *et al.*, 2004; Roberts & Reymond, 1994).

### 3.2.1 Mechanism of action

$\text{Cl}_2$  is highly reactive and it is quickly hydrolysed to hypochlorous acid. Zoffoli *et al.* (1999) evaluated  $\text{Cl}_2$  generators that consist of a salt mixture, calcium hypo chloride (27%), sodium chloride (27%), citric acid (48%) and calcium chloride (2%). Complete inhibition of *B. cinerea* is obtained with a 10-minute exposure to 18  $\mu\text{g}/\text{mL}$  (Roberts & Raymond, 1994; Zoffoli *et al.*, 1999). Spotts & Peters (1980) found that a 50  $\mu\text{L}/\text{L}$   $\text{Cl}_2$  steriliser completely inhibited germination of *B. cinerea* in a packhouse. Prolonged protection is obtained by the high oxidation potential of the hypochlorous acid. The  $\text{Cl}_2$  generators open the possibility of replacing the  $\text{SO}_2$  generator pad because it has no adverse effects on the quality of the fruit (Zoffoli *et al.*, 1999).

$\text{ClO}_2$  is highly unstable and onsite production of the gas is essential to ensure efficiency (Apel, 2002).  $\text{ClO}_2$  causes an interruption of cellular processes when the organic material acts with the  $\text{ClO}_2$  (EPA, 1999). It directly reacts with the cell wall of the microorganisms and can even kill the organism when it is inactive (Junli *et al.*, 1997; Anon, 2004; Steynberg *et al.*, 1993). Disinfection occurs in two steps and chlorate and chlorine are sometimes formed, but both are oxidising agents, which dissociate to form sodium chloride (Dąbrowska *et al.*, 2003; Anon, 2004). The disinfection process entails a disruption of protein synthesis and permeability of the outer membrane.

### 3.2.2 Constraints

The disadvantages of Cl<sub>2</sub> as a disinfectant include its corrosive nature to metal equipment and its efficacy that is affected by pH (Prusky *et al.*, 2001; Roberts & Reymond, 1994; Spotts & Peters, 1980). The efficiency of ClO<sub>2</sub> decreases with a decrease in temperature, while pH has little or no effect (Apel, 2002; EPA, 1999). Organic substances that change the pH of a solution cause a decrease in the fungistatic activity of Cl<sub>2</sub> (Prusky *et al.*, 2001). There is the possibility that free Cl<sub>2</sub> can indirectly influence the stability of ClO<sub>2</sub> by reacting with organic substances (Hofmann *et al.*, 2004). The liquid state is more stable and also less reactive to organic material (Anon., 2005; Apel, 2002; Beuchat, 2004; Han *et al.*, 1999, 2000 a, 2000 b; Prusky *et al.*, 2001; Roberts & Reymond, 1994; Spotts & Peters, 1980). Gas has greater penetration ability than liquid and gaseous ClO<sub>2</sub> may be more effective (Han *et al.*, 2000 a, 2000 b).

## 3.3 Other

### 3.3.1 Vapour Heat

Water vapour heat applied in the range of 50 to 55°C for 12 to 32 minutes showed potential to sterilise table grapes (Droby & Lichter, 2004). Lydakakis and Aked (2003) found the vapour heat treatment to be effective as an alternative to SO<sub>2</sub> on condition that there is no recontamination. The effect on quality attributes has, however, not been quantified.

### 3.3.2 Ethanol

Ethanol is an approved disinfectant and sanitiser and occurs in many food products. *Conidia* are highly sensitive to ethanol (Chervin *et al.*, 2003; Droby & Lichter, 2004). The authors furthermore found that dipping of grape bunches offered protection against decay during short-term storage. Concentrations of 30 to 50% were effective to control decay and the substance showed huge potential for organic growers (Gabler *et al.*, 2005). Chervin *et al.* (2003) expressed the disadvantages of a liquid postharvest treatment as it causes osmotic damage and the authors found ethanol vapours to be effective to improve storage. Ethanol treatment also causes some quality defects, such as dry stems, and can therefore not be considered as an alternative for long-term storage but may be used in conjunction with SO<sub>2</sub> (Chervin *et al.*, 2003).



### 3.3.3 Biocontrol agents

Microbial biocontrol agents have shown great potential as an alternative to fungicides (Lima *et al.*, 1998; Zhang *et al.*, 2007). Effective biocontrol agents for the control of postharvest decay include *Trichoderma harzianum*, *Aureobasidium pullulans*, *Pythium periplocum* and *Metschnikowia fruticola* (Abdelghani *et al.*, 2004; Harman *et al.*, 1996; Latorre *et al.*, 1997; Malathrakis & Kritsotaki, 1992; Paul, 1999; Schena *et al.*, 1999). Many bacteria and yeast such as *Bacillus subtilis*, *Kloeckera apiculata*, *Candida guilliermondii* and *Cryptococcus laurentii* were found to be effective in controlling *Botrytis*-incited diseases (McLaughlin, 1992; Stotz *et al.*, 2004; Zhang *et al.*, 2007). Lima *et al.* (1998) confirmed the efficacy of *Cryptococcus laurentii* and added *Rhodoturula glutinis* to be equally effective against *Botrytis* on table grapes and other commodities.

Yeasts are applied as postharvest dips, and despite the efficacy, it negatively affected quality by removing the bloom on the berries (Zahavi *et al.*, 2000). Yeasts react by competing for nutrients, physical interaction with hyphae, production of cell wall lytic enzymes and inducing host resistance (Lima *et al.*, 1998; Zhang *et al.*, 2007). The activity of yeast is very sensitive to temperature changes (Kulakiotu *et al.*, 2004). For antagonistic organisms to be effective, it is important to be established on the berry surface before the decay fungus. Technically this means that the yeast must be applied pre-harvest, a stage where nutrients are low and temperatures vary in the field (Schena *et al.*, 1999). This is extremely difficult with *Botrytis* as this fungus can be latent on plant parts (Ben-Arie *et al.*, 1991; Latorre *et al.*, 1997; Moyls *et al.*, 1996).

Kolakiotu *et al.* (2004) identified the potential of grape volatiles as an antifungal agent. In the studies done by these authors, volatiles from Isabella grapes suppressed *Botrytis*. It is a fact, however, that no single biocontrol agent is effective to control diseases. An ideal strategy would involve a combination of agents that will eradicate the fungus and ensure prolonged protection during storage (Lima *et al.*, 1998; Schena *et al.*, 1999; Singh *et al.*, 2003; Zhang *et al.*, 2007).

### 3.3.4 Plant extracts

Plant extracts are generally considered to be more acceptable and less hazardous to consumers than synthetic compounds. Singh *et al.* (2002) described essential oils as GRAS substances. Essential oils are plant extracts and showed potential as an anti-fungal agent during *in vitro* studies (Isman, 2000; Kulakiotu *et al.*, 2004; Plotto *et al.*, 2003). They are obtained from either steam distillation or ethanol extraction of the plant foliage (Sousa *et al.*, 2002). The anti-fungal activity is associated with monoterpenic phenols such as thymol, carvacrol and eugenol (Isman, 2000; Singh *et al.*, 2002; Sousa *et al.*, 2002). Phenol components affect the phospholipid bilayer and permeability increases (Singh *et al.*, 2002). Isman (2000) warns about the phytotoxicity of it, as high concentrations tend to be most effective. The effects on invertebrates and natural enemies of pests are not well documented. *In vitro* studies by Plotto *et al.* (2003) had 100% control of *Botrytis* by thyme, lemongrass cilantro and spearmint oils. Volatiles from *Thymus vulgaris* effectively control *Botrytis* on grapes for 12 weeks during storage (Kulakiotu *et al.*, 2004). The anti-fungal properties of tea tree oil showed potential with concentrations between 100 to 500  $\mu\text{L/L}$  that prevent postharvest decay on table grapes (Jobling, 2002). It is, however, difficult to apply in a commercial set-up, as the oils are fungistatic but act transiently. This means that the oil will stop the growth of the fungus when it is exposed to the oil, but the fungus will continue growth as soon as the oil is removed (Jobling, 2002). The oil evaporates from the plant surface and continuous application of oils is necessary to ensure coverage and protection against the fungus.

### 3.3.5 Controlled atmosphere storage

Controlled atmosphere (CA) refers to a decrease in oxygen and an increase in carbon dioxide concentrations during storage with precise management of it. CA storage with 5-10%  $\text{CO}_2$ , or 10%  $\text{CO}$  has potential as an alternative to the use of chemicals. Yahia *et al.* (1983) found that  $\text{CO}_2$  concentrations higher than 15% were effective to control *B. cinerea* but pre-maturely harvested grapes developed off-flavours after treatment and storage (Retamales *et al.*, 2003). Crisosto *et al.* (2002 b) found that  $\text{CO}_2$  concentrations above 15% on Thompson Seedless grapes was effective against grey mould, but had a negative effect on quality by inducing off flavours, causing stems to dry and berries to brown (Ahumada *et al.*, 1996). By combining these high  $\text{CO}_2$  concentrations with a 3, 6 or 12%  $\text{O}_2$  concentration, *B. cinerea* was inhibited and no adverse effects were observed (Crisosto *et al.*, 2002 b; Fourie *et al.*, 2005). CA storage with elevated  $\text{CO}_2$  concentrations is successfully used to control postharvest diseases

on several other commodities such as apples and pears (Crisosto *et al.*, 2002 b; Kader, 2003). Extensive research has been done to determine the exact CO<sub>2</sub> and O<sub>2</sub> concentration for every cultivar. The gases in the storage room constantly change due to respiratory activity and gas leakages and constant monitoring and adjustment are necessary (Fourie *et al.*, 2005; Niemann, 2005; Salveit, 2003). Ahumada *et al.* (1996) observed excellent decay control of Thompson Seedless grapes during short term (< two months) storage but longer storage resulted in fungal growth on stems (Crisosto *et al.*, 2002 b). The use of CA storage alone as an alternative to the use of SO<sub>2</sub>, is a risk as removal from storage leaves grapes unprotected and susceptible to *B. cinerea* infections and it may have a negative effect on quality (Ahumada *et al.*, 1996; Crisosto *et al.*, 2002 b; Retamales *et al.*, 2003). These periods include the time after packing, during transport and from the market to the retail shelf.

Modified atmosphere (MA) packaging is becoming a popular method to extend shelf life of fruits (Moyle *et al.*, 1996). MA packaging with 15% O<sub>2</sub> and 10% CO<sub>2</sub> is a cheap and easy technique and might be useful as an alternative to SO<sub>2</sub> (Artés-Hernández *et al.*, 2004; Crisosto *et al.*, 2002 b). This packaging avoids decay development and maintains visual quality, flavour and eating quality (by not changing the sugar and acid content) (Artés-Hernández *et al.*, 2004). It is dependant on the release of CO<sub>2</sub> from the bunch and respiration of the product alters the immediate atmosphere (Droby & Lichter, 2004; Moyle *et al.*, 1996). However, it takes ± 21 days before the above-mentioned levels of O<sub>2</sub> and CO<sub>2</sub> are reached (Artés-Hernández *et al.*, 2004; Moyle *et al.*, 1996) and during this time the existing atmosphere has almost no effect on *B. cinerea* development. A break in packaging and respiration of the product change the immediate environment, which is another limitation of MA packaging (Moyle *et al.*, 1996; Zoffoli *et al.*, 1999). High relative humidity that occurs within a packed carton promotes stem condition but also induces fungal growth and may cause splitting of sensitive cultivars (Fourie *et al.*, 2005). Due to the limited efficacy of MA packaging on its own, the use was mainly reported in conjunction with substances such as acetic acid, chlorine and volatiles (Droby & Lichter, 2004; Moyle *et al.*, 1996).

### 3.3.6 Ozone (O<sub>3</sub>)

Ozone is a disinfectant that is able to inactivate more pathogenic microorganisms than conventional disinfectants (Gabler & Smilanick, 2001; Von Gunten, 2003). Ozone is the most efficient disinfectant that is applied in the treatment of drinking water as it destroys

viruses, bacteria, fungi and protozoa (Buchan *et al.*, 2005). Resveratrol is a phytoalexin with antioxidant activity that inhibits the *Botrytis* fungus and application of ozone to grapes elevated the level of resveratrol (Von Gunten, 2003). The required exposure time, however, is very long and this might lead to the formation of undesired by-products such as bromate (Von Gunten, 2003). Bromate is carcinogenic and removal of this by-product is non-economical. Infection that spreads from one berry to the next, and eventually a whole bunch, is inhibited by continuous exposure to 0.3  $\mu\text{L/L}$  ozone (Droby & Lichter, 2004; Palou *et al.*, 2002 b). Gabler & Smilanick (2001) found a 10  $\mu\text{L/L}$  ozone concentration to be effective against *Botrytis* but berry condition influenced its efficacy.

### 3.3.7 Acetic acid

Acetic acid is commonly found in many food products and there is no limit on the daily intake for humans (Sholberg *et al.*, 1996). Sholberg *et al.* (1996) evaluated acetic acid to control decay on table grapes and found it to be as effective as  $\text{SO}_2$  fumigation and did not produce any deleterious quality effects or toxic residues. Moyls *et al.* (1996) also found that acetic acid treated grapes were free from decay for up to two months of storage. The inhibitory action of acetic acid vapours against *Botrytis* was observed by Kulakiotu *et al.* (2004) on different fruit types including grapes. The effect on quality attributes was, however, not quantified. The low cost of acetic acid, availability and ability to act at low concentrations make it an ideal alternative for  $\text{SO}_2$  (Moyls *et al.*, 1996). Even though acetic acid is commonly found in food products, there is a need to determine the effect on the grape composition (total soluble solids- and acid content) and methods to accurately determine the concentrations during application (Moyls *et al.*, 1996; Sholberg *et al.*, 1996).

### 3.3.8 Hydrogen peroxide

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is widely used to sterilise plastic and stainless steel surfaces. Sholberg *et al.* (1996) found that hydrogen peroxide and acetaldehyde also showed potential to reduce germination of *Botrytis* on table grapes (Rij & Forney, 1995). Studies by Rij & Forney (1995) showed that an 11 minute exposure to 0.27 mg/L  $\text{H}_2\text{O}_2$  killed almost all *Botrytis* spores. However,  $\text{H}_2\text{O}_2$  dries the pedicels and internal tissue, which is an unacceptable adverse effect (Rij & Forney, 1995; Sholberg *et al.*, 1996).  $\text{H}_2\text{O}_2$  vapours are removed by high relative humidity or free water. The removal of the vapours degrades the cuticle, which will cause water loss (Rij & Forney, 1995).

### 3.3.9 Acetaldehyde

Acetaldehyde is a natural component found in fruits and it accumulates during the ripening stage. As for the use of acetaldehyde to control postharvest decay, promising results were obtained but it had a negative effect on the composition of the berry ( $^{\circ}$ Brix, titratable acid and pH) and left grapes with a slight off-flavour (Sholberg *et al.*, 1996). Avissar & Pesis (1991) found acetaldehyde vapours to be effective to control *Botrytis* on table grapes after a two-week storage period. This phenomenon was also observed by Kulakiotu *et al.* (2004). These vapours had no effect on the composition and flavour of the grapes. Concentrations of 1% tend to cause browning of stems and berries and it evaporates easily or is metabolised to ethanol (Avissar & Pesis, 1991). This method can only be considered as an alternative to SO<sub>2</sub> during short-term storage.

## 4. CONCLUSION

Several researchers agree that SO<sub>2</sub> is currently the most efficient method to control postharvest decay. The severity of a disease and the inoculum levels that stays viable from one season to another is influenced by many factors that cannot be easily controlled. Postharvest decay involves development from pre-harvest infection together with new infections. The importance of sanitation throughout the harvesting and packing process cannot be overemphasised. Management of temperature during the postharvest life is the most important factor to control the disease, and all other factors are supplementary to this. Current methods of decay control by SO<sub>2</sub> have their drawbacks and alternatives such as acetic acid, ethanol, ozone, and biological control methods are promising. Studies currently focus on methods of sterilisation, which cannot control latent *Botrytis* infections. The focus should move to disinfectant or continuous sterilisation throughout the storage life of the fruit. It is, however, important to get these methods on a commercial and sustainable level. Until this is realised, we can only adapt and modify current methods with SO<sub>2</sub>. It is the SO<sub>2</sub> that provided control for almost a century.

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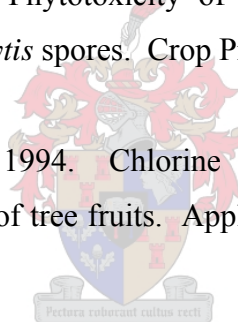
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## B. Article I

### **The use of in-carton SO<sub>2</sub> fumigation to control postharvest decay on table grapes.**

**Keywords:** Dosigas, SO<sub>2</sub> fumigation, *Botrytis cinerea*, decay, split, polyethylene liners, moisture, stem condition, table grapes

#### **Abstract**

*Botrytis cinerea* is an important fungus causing decay on table grapes, causing huge economic losses. Consumer demands and the current focus on food safety urge the transition to alternative forms of decay control, namely non-chemical methods but also alternative methods of application that can reduce some of the deleterious effects of SO<sub>2</sub> such as bleaching. The effects that an in-carton SO<sub>2</sub> fumigation had on the quality of table grapes were evaluated in two consecutive seasons. Grapes were packed during adverse weather conditions and different types of liners were used to quantify the effect. The SO<sub>2</sub> was applied with a Dosigas applicator after packing, and grapes were stored for six weeks at -0.5°C and a shelf life of one week at 15°C. Decay on Red Globe and Thompson Seedless grapes was reduced when the optimum concentration was tested, but increasing SO<sub>2</sub> concentration increased SO<sub>2</sub> phytotoxicity to unacceptable levels and caused an unpleasant appearance on Red Globe grapes. In-carton SO<sub>2</sub> fumigation aggravated the problem with berry splits of Thompson Seedless. It did not result in any difference amongst liner treatments of Red Globe grapes but lower decay levels were observed with the non- and micro perforated liners. Decay levels on Thompson Seedless were very high despite of effective reductions by in-carton SO<sub>2</sub> fumigation. The in-carton application of SO<sub>2</sub> to Barlinka harvested in adverse weather conditions resulted in decay reductions but with Dauphine high SO<sub>2</sub> concentrations enhanced decay development.



## 1. INTRODUCTION

New markets develop around the world and it becomes a challenge to store grapes for longer to reach the markets with superior quality. Consumer demands for reduced chemical use are also growing and this created a need for alternative forms of pest and disease control (Retamales *et al.*, 2003; Whiteman & Stewart, 1998). It takes time for a non-chemical method to be commercially acceptable and reduced and modified methods of SO<sub>2</sub> application poses as an interim solution. *Botrytis cinerea* is a necrotrophic fungus that actively kills plant cells and subsequently lives on the dead tissue (Elmer & Michailides, 2004; Kaile *et al.*, 1991; Karabulut *et al.*, 2005; Ten Have, 2000). It is the common cause of bunch rot of table grapes (Combrink & Ginsburg, 1972; Karabulut *et al.*, 2003; McClellan *et al.*, 1973; Palou *et al.*, 2002; Peiser & Yang, 1985; Thompson & Latorre, 1999; Witbooi *et al.*, 2000 a) and is also commonly known as grey mould or slip skin (Combrink & Ginsburg, 1972; Ten Have, 2000). It is responsible for huge economic losses during storage and transport of the harvested crop (Leroux *et al.*, 1999; Nigro *et al.*, 1998; Ten Have, 2000).

This fungus may penetrate the host through specialised host structures, natural openings and through wounds (Keller *et al.*, 2003). The fungus germinates on the skin of the berry and then penetrates to attack the underlying tissue (Combrink & Ginsburg, 1972). At this stage the first symptom, “slip skin”, appears (Droby & Lichter, 2004). Relative humidity and the presence of free water are often thought of as the most important single factors influencing infection (Blakeman, 1980). A wound is not necessary for infection when wet and/or high relative humidity conditions prevail (Comménil *et al.*, 1999; Holz, 1999; Keller *et al.*, 2003; Kupferman, 1999; Latorre *et al.*, 1997; Peacock & Smilanick, 1998; Thompson & Latorre, 1999; Ten Have, 2000). Sommer (1985) reported that temperatures below 0°C delay development of the fungus but do not kill it.

In America, SO<sub>2</sub> has been used for more than 70 years to reduce the occurrence of grey mould on table grapes (Chervin *et al.*, 2005; Crisosto *et al.*, 2002; László *et al.*, 1981; Palou *et al.*, 2002; Smilanick *et al.*, 1990). This gas is highly corrosive to metal and extremely irritating to people, but the contribution of this gas to air pollution is, however, minimal (Nelson & Ahmedullah, 1973; Smilanick & Henson, 1992; Zahavi *et al.*, 2000). SO<sub>2</sub> gas was previously on the list of Generally Regarded as Safe (GRAS) chemicals of the United States Food and Drug Administration (Austin *et al.*, 1997; Zahavi *et al.*, 2000). The gas is commercialised as a liquid or compressed gas and is neither combustible nor explosive.

Room fumigation with SO<sub>2</sub> is a common practice in California and is implemented by exposing the grapes to large doses before storage, which serves as a surface steriliser (Austin *et al.*, 1997; Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002; Droby & Lichter, 2004; Nelson & Ahmedullah, 1973; Palou *et al.*, 2002). This, however, does not kill inocula within the tissue and is followed by weekly fumigations during cold storage (Austin *et al.*, 1997; Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002; Droby & Lichter, 2004; Mustonen, 1992; Nelson & Ahmedullah, 1973). Fumigation during shipment or transport is impossible and grapes are packed with in-package SO<sub>2</sub> generator pads (Crisosto *et al.*, 2002; Palou *et al.*, 2002; Zoffoli *et al.*, 1999). These pads contain sodium metabisulphite that releases SO<sub>2</sub> in a controlled manner upon reaction with water vapour.

In South Africa and Chile, room fumigation is not a common practice and the initial high concentration is dependent on the release of SO<sub>2</sub> from a dual phase SO<sub>2</sub> generator pad, which on the other hand is dependent on the moisture content in the polyethylene liner, whether vented or unvented (Combrink & Truter, 1979; Franck *et al.*, 2005; Kokkalos, 1986; Palou *et al.*, 2002). Spanish, Chilean and South African grapes are all packed with in-package SO<sub>2</sub> generators and fluctuation in temperature has a great influence on the efficacy of treatment, as well as the damage done to the grapes (Kokkalos, 1986; Nelson & Ahmedullah, 1973). A rise in temperature will result in free moisture in the carton and this triggers the excessive release of SO<sub>2</sub> gas from the generator pad. The hydrated form of SO<sub>2</sub>, which is sulphurous acid, is the active agent (Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002). Sulphurous acid diffuse to the sensitive sites on the berry skin and causes damage (Couey & Uota, 1961; Nelson & Ahmedullah, 1972; Smilanick & Henson, 1992).

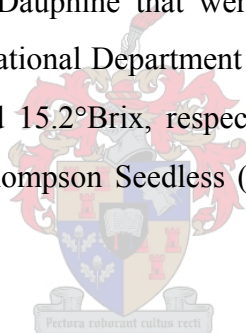
High relative humidity and water condensation, caused by temperature fluctuation, are important factors influencing residues and toxicity (Couey & Uota, 1961; Gentry & Nelson, 1968; Nelson & Ahmedullah, 1973; Palou *et al.*, 2002). Sulphite residues and phytotoxicity that include bleaching and hairline splitting on the berry surface are the main problems with the SO<sub>2</sub> generator pads (Crisosto *et al.*, 1994; Smilanick & Henson, 1992; Palou *et al.*, 2002). Long storage periods with continuous fumigations should be avoided to minimise SO<sub>2</sub> residues on sensitive cultivars.

The development of Dosigas, a Chilean device, came from the efficacy of these high initial dose fumigations. Dosigas is an alternative for fumigation rooms and it can inject extremely low and accurate doses of SO<sub>2</sub> gas into a packed container of grapes. The objectives of this study were 1.) to determine the optimum dose of initial in-carton SO<sub>2</sub> fumigation to control post harvest decay on table grapes without any adverse effects, 2.) to quantify the effect of in-carton fumigation when grapes are packed with different liners and 3.) determine the effect of in-carton fumigation when packing after rain.

## 2. MATERIALS AND METHODS

### *Grapes*

These experiments were carried out on farms in the Western Cape, South Africa during 2003 and 2004. In 2003 trials were conducted on Bonheur, Sunred Seedless, Red Globe, Thompson Seedless, Barlinka and Dauphine that were harvested according to the export standards and requirements of the National Department of Agriculture (17.0°Brix, 20.5°Brix, 17.9°Brix, 16.0°Brix, 16.8°Brix and 15.2°Brix, respectively). The trials were repeated in 2004 on Red Globe (16.9°Brix), Thompson Seedless (19.0°Brix), Barlinka (17.0°Brix) and Dauphine (17.7°Brix).



### *Inoculation*

*B. cinerea* was isolated from naturally infected table grape berries (Kulakiotu, 2004) and grown on potato dextrose agar at 22°C (Karabulut *et al.*, 2005; Roberts & Reymond, 1994; Zahavi *et al.*, 2000). A spore suspension was prepared by washing cultures with distilled water to remove the spores (Karabulut *et al.*, 2005; Lee *et al.*, 2004; Lydakis & Aked, 2003; Spotts & Peters, 1980; Zahavi *et al.*, 2000). Grapes were inoculated by spraying them with the spore suspension.

### *Packaging*

Grapes were packed in 4.5 kg supervent cartons and into polyethylene carry bags (Mustonen, 1992). A piece of tissue paper was placed within the liner, which was big enough to cover the

grapes after packing (Nelson & Ahmedullah, 1973). The tissue paper was folded to cover the grapes. A UVAS Quality SO<sub>2</sub> sheet (970 mg. kg<sup>-1</sup> Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>) was placed between 2 pieces of tissue paper and placed on top of the grapes and then covered by the other tissue paper.

### ***In-carton fumigation***

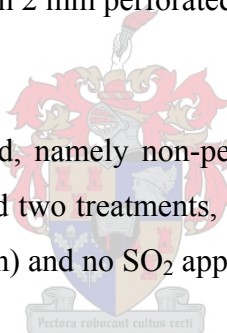
The liner was closed and the desired dose of SO<sub>2</sub> was applied using the Dosigas apparatus (Proquivi, Santiago, Chile). The gun probe was inserted through the liner and under the tissue paper. By pressing the gun firmly against the side of the carton, the device triggered the release of the pre-determined dose of SO<sub>2</sub> gas.

#### ***Trial 1 – Optimum concentration***

Three different doses were applied namely 4.5 ml/kg, 6.0 ml/kg and 7.5 ml/kg (Austin *et al.*, 1997; Gao *et al.*, 2003). No SO<sub>2</sub> fumigation was applied for the treatment that served as the control. All treatments were packed in 2 mm perforated liners (Franck *et al.*, 2005).

#### ***Trial 2 – Different liner types***

Three different liner types were used, namely non-perforated, 2 mm perforated and micro perforated liners. Each liner type had two treatments, which consisted of an SO<sub>2</sub> application of 6.0 mL/kg (27.0 mL SO<sub>2</sub> gas/carton) and no SO<sub>2</sub> application.



#### ***Trial 3 – Packing after rain***

In 2003 this trial was conducted after ±17 mm of rain and in the 2004 season after 11.5 mm rain. Three different doses were applied namely 4.5 ml/kg, 6.0 ml/kg and 7.5 ml/kg (Austin *et al.*, 1997; Gao *et al.*, 2003). No SO<sub>2</sub> was applied for the treatment that served as the control. All treatments were packed in 2 mm perforated liners (Franck *et al.*, 2005).

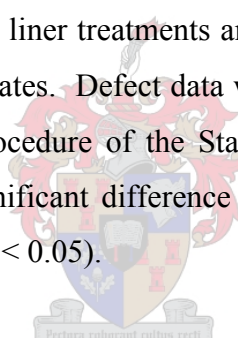
### ***Quality evaluation***

After packing and SO<sub>2</sub> application, grapes were transported to cold rooms where they were forced air cooled and then stored. Evaluations were conducted after six weeks at -0.5°C (85 to 90% relative humidity) and again after a shelf life simulation of seven days at 15°C (Crisosto *et al.*, 1994). Fruit were evaluated for dry stems on a scale of one to five where one is green and five is brown (Crisosto *et al.*, 1994; Perkins-Veazie, 1992; Sholberg *et al.*, 1996;

Witbooi *et al.*, 2000 b), for the presence of moisture on a scale of one to three where one is dry, two condensation and three presence of free moisture (Sholberg *et al.*, 1996; Witbooi *et al.*, 2000 b). Loose berries (shatter) and berries with decay and split were removed, weighed and expressed as a percentage of the nett mass of the grape sample (Franck *et al.*, 2005; Witbooi *et al.*, 2000 b). A random sample of berries (100 berries) was sampled per replicate, juiced, and filtered to get a clear sample. Total soluble solids (TSS) were determined by measuring the refractive index with an Atago DBX-55 digital refractometer (Atago Bussan Co. Ltd, Japan) and the acidity was measured by titration with a 719S automatic titrator (Metrohm, South Africa) using 0.1N NaOH to pH end point of 8.2.

### ***Statistical analysis***

The experimental design of trial one and three was a complete randomised design with four concentration treatments. Trial two had a complete randomised experimental design with a 3 × 2 factorial treatment design with 3 liner treatments and 2 sub-treatments (SO<sub>2</sub> application). All trials had six single carton replicates. Defect data were subjected to analyses of variance using the General Linear means procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1999). The least significant difference was calculated at the 5% confidence level to compare treatment means ( $P < 0.05$ ).



## **3. RESULTS**

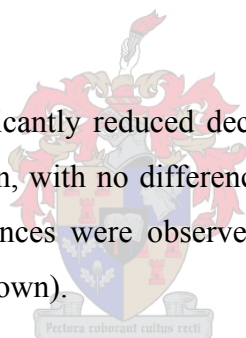
### ***Trial 1 – Optimum concentration***

Analysis of variance of the defect data for the quality parameters (decay, shatter and split) of Red Globe table grapes in the 2003 season indicated no significant difference between the different treatments. This was also evident for decay development and split berries during the 2004 season (Table 1). In both seasons SO<sub>2</sub> damage varied between the different treatments. Although, not significant, results indicated that the level of SO<sub>2</sub> damage seems to increase with an increase in fumigation concentration in 2004 (Table 1). The 4.5 mL/kg fumigation concentration did not differ significantly from non-fumigated fruit, but these levels were very high. Disease tolerance for decay caused by *B. cinerea* is 0.5% and levels above this were considered to be high with very high levels above 5.0%. In 2003, decay development never reached levels higher than 1.0% on SO<sub>2</sub> fumigated grapes, whereas high decay levels occurred

for all treatments during the 2004 season (Table 1). During the 2004 season, high SO<sub>2</sub> damage was accompanied by high split berry levels and shattering. SO<sub>2</sub> damage was localised and physical damage caused by the SO<sub>2</sub> applicator was visible. Damaged berries mean that underlying tissue was exposed and, consequently absorbed more gas (SO<sub>2</sub>), which explains the high SO<sub>2</sub> damage on this cultivar.

With Thompson Seedless, analysis of variance of defect data indicated significant differences between the treatments of all quality parameters during the 2003 season (Table 2). These significant differences were not evident during the 2004 season. Extremely high decay levels were recorded on non-fumigated fruit during the 2003 season, which differed significantly from fumigated fruit (Table 2). In the specific season (2003), the drastic reduction in decay levels in fumigated fruit was accompanied by higher levels of SO<sub>2</sub> damage that differed significantly from non-fumigated fruit (Table 2). Berry split increased with the addition of the SO<sub>2</sub> fumigation, although the lowest fumigation concentration did not increase split significantly above control levels.

In Bonheur, SO<sub>2</sub> fumigations significantly reduced decay ( $P = 0.0011$ ) and increased berry split ( $P = 0.0073$ ) in the 2003 season, with no differences between SO<sub>2</sub> concentrations (data not shown). No significant differences were observed in any of the quality variables of Sunred Seedless in 2003 (data not shown).



TSS was not affected by the fumigation treatments and no significant differences were observed in Bonheur, Sunred Seedless (data not shown), Red Globe and Thompson Seedless during the 2003 season (data not shown). In both seasons, the acidity of Red Globe and Thompson Seedless differed significantly amongst the treatments whilst the other cultivars were unaffected. In 2003, Red Globe and Thompson Seedless grapes fumigated with 7.5 mL/kg SO<sub>2</sub> had the lowest acid content, differing significantly from other treatments. The same result was evident in Thompson Seedless grapes during the 2004 season (data not shown). During the 2004 season, this was not evident in Red Globe and the lowest acid content was observed on control grapes, which did not differ significantly from 4.5 mL/kg SO<sub>2</sub> fumigated grapes but from the 6.0 mL/kg and 7.5 mL/kg SO<sub>2</sub> fumigated grapes (data not shown).



There was a significant difference in stem condition and moisture of the different treatments of Bonheur during the 2003 season. Stem condition was the best on grapes fumigated with 7.5 mL/kg SO<sub>2</sub> and the driest stems were found on control grapes (data not shown). During the 2003 season, the fumigation treatment had a significant effect on the stem condition of Red Globe, Thompson Seedless and Sunred Seedless grapes, but results varied between the different SO<sub>2</sub> concentrations (data not shown). Thompson Seedless and Bonheur grapes fumigated with 7.5 mL/kg had significantly more moisture in the carton when compared to control grapes and Sunred Seedless and Red Globe did not have these differences (data not shown). This was also evident with Red Globe during the 2004 season, but significant differences were observed for both stem condition and moisture of Thompson Seedless ( $P < 0.0001$  for both; data not shown). Condensation and free water occurred in the cartons treated with SO<sub>2</sub> while grapes from the control were dry.

### ***Trial 2 – Different liners***

Analysis of variance of data for SO<sub>2</sub> damage on Red Globe showed a significant interaction between type of liner and SO<sub>2</sub> fumigation (Table 3) during the 2003 season, which was not evident during the 2004 season (Table 4). SO<sub>2</sub> damage was low during the 2003 season (Table 3), but high levels were recorded during the 2004 season (Table 4). These high levels of damage were not attributed to the fumigation with SO<sub>2</sub> as it showed a non significant difference to treatments not receiving fumigation (Table 4). In both seasons decay levels stayed below 2% but a significant interaction between type of liner and SO<sub>2</sub> fumigation was observed during the 2004 season ( $P = 0.040$ ; Table 5). This interaction was not evident in 2003 and the lowest decay levels were observed on grapes packed in non- and micro perforated liners (Table 6), which differed significantly from the 2 mm perforated liner. The fumigation with SO<sub>2</sub> did not result in any significant difference in decay levels (Table 4). A significant interaction between SO<sub>2</sub> fumigation and liner type was observed for decay ( $P = 0.005$ ) and SO<sub>2</sub> damage ( $P = 0.002$ ) of Bonheur during the 2003 season (data not shown). Decay levels of  $\pm 50\%$  (data not shown) were recorded on grapes packed in the 2 mm- and micro perforated liners. Decay levels of Sunred Seedless also showed this significant interaction ( $P = 0.0041$ ) but none of the treatments had decay levels higher than 1.5% (data not shown). High levels of SO<sub>2</sub> damage were observed on Sunred Seedless grapes packed in the non perforated liner (23.0%), which differed significantly from the 2 mm- and micro perforated liners (12.2% and 15.5% respectively; data not shown).



There were no significant interactions between type of liner and SO<sub>2</sub> fumigation for all quality variables of Thompson Seedless grapes in both the 2003 and 2004 season (Tables 7 & 8). In 2003, very high decay levels were recorded on grapes that did not receive SO<sub>2</sub> fumigation, which differed significantly from SO<sub>2</sub> fumigated grapes (Table 7). Despite the efficacy of the SO<sub>2</sub> fumigation to reduce the presence of decay, levels were still unacceptable. None of the liners (non-, micro- and 2 mm perforated) showed a significant difference in the occurrence of decay (Table 7). These high decay levels were not evident during the 2004 season and the fumigation with SO<sub>2</sub> did not cause a significant reduction (Table 8). Comparing the different liners proved that decay occurrence was the highest on grapes packed in the 2 mm perforated liners. This cultivar is prone to split and visual observations indicated that these cracks resulted in development of either decay or SO<sub>2</sub> damage, by creating entry sites for latent *B. cinerea* spores or the gas. Severe damage caused by SO<sub>2</sub> phytotoxicity was observed during the 2004 season with no significant difference between SO<sub>2</sub> fumigated and non-fumigated grapes (Table 8).

During both seasons there was no significant interaction between type of liner and SO<sub>2</sub> fumigation for TSS and acid content of Red Globe and Thompson Seedless grapes and no differences were observed between the different treatments (data not shown). This was also observed with Sunred Seedless during the 2003 season (data not shown). Data for TSS and acidity of Bonheur showed a positive interaction between type of liner and SO<sub>2</sub> application, with the lowest acidity on grapes packed in the micro perforated liners and fumigated with SO<sub>2</sub> (0.56%). In the 2004 season, the TSS and acidity of Thompson Seedless and Red Globe were not significantly affected by SO<sub>2</sub> fumigations (data not shown).

There was no significant interaction between type of liner and SO<sub>2</sub> fumigation for stem condition and moisture content of Bonheur and Red Globe grapes during the 2003 season, which was also evident with moisture of Red Globe during 2004 (data not shown). For Red Globe, condensation occurred in the non perforated liner accompanied by green stems that differed significantly from the 2 mm- and micro-perforated liner (data not shown). During the 2004 season stem condition had a significant interaction between type of liner and SO<sub>2</sub> fumigation ( $P < 0.0001$ ). SO<sub>2</sub> fumigation did not have any effect on the stem condition of Sunred Seedless in 2003 but free moisture was present in the non perforated liner. Data for stem condition of Red Globe in 2004, showed a positive interaction between type of liner and

SO<sub>2</sub> fumigation and driest stems occurred on grapes packed in the micro perforated liner with no SO<sub>2</sub> fumigation. Thompson Seedless had slightly drier stems with SO<sub>2</sub> fumigated treatments and free moisture was present in all treatments (data not shown).

### ***Trial 3 – Packing after rain***

In 2003, SO<sub>2</sub> fumigation at all concentrations significantly reduced decay in Barlinka grapes packed after adverse weather conditions (Table 9). SO<sub>2</sub> damage was significantly higher for all fumigation treatments compared to the control, although at relatively low levels. In 2004, the highest SO<sub>2</sub> concentration accounted for significantly higher incidence of decay. Severe damage caused by SO<sub>2</sub> was observed during this season (2004) but levels were not significantly influenced by fumigation (Table 9).

In 2003, fumigation at all concentrations significantly reduced decay on Dauphine (Table 10), but did not affect shatter, berry split or SO<sub>2</sub> damage. In 2004, SO<sub>2</sub> damage was considerably higher than in 2003, but fumigation had no influence on any of the quality or defect variables (Table 10).

Fumigation did not have any significant effect on the TSS and acid content of Barlinka and Dauphine grapes during the 2003 season (data not shown). This was also evident in the TSS content of Barlinka during the 2004 season, but a significant difference occurred amongst the acid content of the different treatments ( $P = 0.0317$ ). Lowest acidity was recorded on grapes fumigated with 7.5 mL/kg SO<sub>2</sub> that only differed significantly from the 4.5 mL/kg SO<sub>2</sub> fumigated grapes (data not shown). Dauphine grapes showed no significant difference amongst the treatments for TSS and acid content during the 2004 season (data not shown).

Significant differences amongst treatments were observed for stem condition and moisture of Barlinka grapes during the 2003 and 2004 season (data not shown). Grapes that did not receive SO<sub>2</sub> fumigation had no free moisture in the carton after the storage period and differed significantly from the 4.5 mL/kg SO<sub>2</sub> fumigated grapes, where condensation occurred during the 2004 season (data not shown). In the 2003 season, 7.5 mL/kg SO<sub>2</sub> fumigated Dauphine grapes had significantly less moisture in the carton compared with other treatments and also had the best stem condition, but this was not significantly different from control grapes. This

was also evident with stem condition in the 2004 season but moisture was not affected by SO<sub>2</sub> fumigation (data not shown).

#### 4. DISCUSSION

Very high rainfall and hot weather contributed to increased disease pressure and latent infections during the growing season and prior to harvest in 2003, whereas conditions conducive for disease development only occurred after the harvesting in 2004. This factor contributed to the inconsistency of results obtained during the two seasons.

SO<sub>2</sub> damage is first observed as a bleached spot with a discolouration of the berry colour (Zhang *et al.*, 2003; Crisosto *et al.*, 2002). This bleached spot later appears as a sunken (concave) area or hairline split that will lose water because of the damaged epidermal tissue (Crisosto *et al.*, 1994; Palou *et al.*, 2002; Zhang *et al.*, 2003). With a green cultivar such as Thompson Seedless, the bleached and sunken area occurs round the pedicel end of the berry whereas Red Globe, previously identified as one of the most sensitive cultivars to SO<sub>2</sub> (Gao *et al.*, 2003; Zhang *et al.*, 2003), absorbs most of the gas through the berry and not through the stem. This was also the major cause of berry shatter in Thompson Seedless that was not evident with Red Globe. The area of absorption of SO<sub>2</sub> gas was also the reason for green rachises that were not retained for the storage period in Red Globe. High SO<sub>2</sub> concentrations form H<sub>2</sub>SO<sub>4</sub> that injure the waxy cuticle and expose underlying tissue. This damage results in Thompson Seedless absorbing more water that burst the epidermis and expose berry juice for more severe damage by *B. cinerea* (Mustonen, 1992; Palou *et al.*, 2002). With Red Globe, the absence of the cuticle caused the mesocarp with colour pigments to absorb more water (Zhang *et al.*, 2003) that had a diluted and bleached effect (Smilanick *et al.*, 1990).

Zhang *et al.* (2003) identified Red Globe as the most sensitive cultivar to SO<sub>2</sub> and this can be ascribed to differences in the structure of the epidermal tissue. The epidermal structure of different cultivars is the factor that determines either sensitivity to SO<sub>2</sub> gas or to the *Botrytis cinerea* fungus. The sensitivity of Red Globe and Thompson Seedless to SO<sub>2</sub> gas was confirmed in this study and damage on Thompson Seedless aggravated losses due to the decay. Several researchers have mentioned advantages of high initial fumigation concentrations but packaging hampers this effect (Zhang *et al.*, 2003). With in-carton fumigation these high concentrations can be obtained but the sensitivity to SO<sub>2</sub> and the

absorption ability of the packaging material should be taken into account when determining the optimum in-carton fumigation concentration. In this study, the lowest in-carton fumigation concentration (4.5 mL/kg) appeared to be too high for a SO<sub>2</sub> sensitive cultivar such as Red Globe.

Large berries and tight bunches limit the space in a packed carton of grapes and the careless insertion of the Dosigas applicator can damage the berries. In these trials, combination of wounds and very high SO<sub>2</sub> concentrations accelerated SO<sub>2</sub> phytotoxicity and *B. cinerea* infection during storage. These infections settle in the wounds and the initial high concentration fumigation (Crisosto *et al.*, 1994; Palou *et al.*, 2002) acts as a surface steriliser and is not capable of eradicating established infections. High initial fumigation concentrations have been found by several researchers (Crisosto *et al.*, 1994; Palou *et al.*, 2002) to reduce *B. cinerea* development, as did the results of this study. Higher concentrations resulted in lower disease incidence but also aggravated SO<sub>2</sub> damage (Kokkalos, 1986).

The use of SO<sub>2</sub> fumigation on Red Globe and Thompson Seedless did not account for a reduction in decay levels that was of commercial value. Not only did these grapes have an unacceptable appearance after storage but the berries also absorb large quantities of SO<sub>2</sub> and human consumption could have detrimental health effects. Despite the emphasis on high initial SO<sub>2</sub> concentrations, it is important to quantify the amount that is absorbed by packaging and by the berries and lost through packaging (Harvey *et al.*, 1988). As such, the minimum SO<sub>2</sub> concentration must be quantified for the different packaging combinations in South Africa.

Polyethylene liners are used to stabilise emission of gas from SO<sub>2</sub> generators in the carton (Mustonen, 1992; Witbooi *et al.*, 2000 b). Hereby it also reduces water loss and the problems with dry/brown rachises (Kokkalos, 1986). SO<sub>2</sub> fumigation reduced the incidence of decay on Red Globe and Thompson Seedless in all different liners that were tested. In a non-perforated liner, high flesh temperature of the grapes triggers the rapid release of SO<sub>2</sub> from generator pads (Witbooi *et al.*, 2000 b) and combined with SO<sub>2</sub> fumigation resulted in excessive SO<sub>2</sub> concentrations. Perforated liners combined with SO<sub>2</sub> fumigation and forced air cooling contributed to the loss of SO<sub>2</sub> in the immediate environment of the grapes (Palou *et al.*, 2002).

The formation of sulphurous acid in the presence of water (Witbooi *et al.*, 2000 b) and high disease pressure makes packing grapes after rain a high-risk activity with huge financial implications. The low decay levels recorded on grapes harvested during adverse weather conditions makes in-carton SO fumigation an ideal option to continue harvesting and packing during these conditions. Removal of field heat, harvesting grapes at lower flesh temperatures (Witbooi *et al.*, 2000 b; Nelson & Ahmedullah, 1973), limiting temperature fluctuation and the use of slow release SO<sub>2</sub> generators would reduce/eliminate phytotoxicity significantly (Palou *et al.*, 2002; Nelson & Ahmedullah, 1973).

High initial fumigation concentrations sterilise the berry surface but with latent infections continuous sterilisation is needed, thus requiring a SO<sub>2</sub> generator. The initial fumigation concentration and type of SO<sub>2</sub> generator should be adjusted according to disease pressure prior to harvest and the type of liner that will be used. An advantage of in-carton SO<sub>2</sub> fumigation is that very high concentrations are obtained without the corrosive effect to metal or irritation and discomfort to humans. Modified cartons for easy insertion of the Dosigas applicator and bunches that are not packed tightly will reduce the problems of damage during application. The in-carton fumigation was applied as an alternative method of applying SO<sub>2</sub> and the purpose was to sterilise the berry surface of *B. cinerea* spores. The use of SO<sub>2</sub> has to be reduced and the focus should shift towards disinfection to remove the SO<sub>2</sub> generator pad. Further study is anticipated to determine the minimum SO<sub>2</sub> concentration that kills *B. cinerea* spores to disinfect (rather than sterilise) the berry surface. Consumer focus is on food safety and SO<sub>2</sub> fumigation is therefore only a solution for the interim and alternatives with natural/organic products are required.

High initial SO<sub>2</sub> concentrations obtained by means of in-carton fumigation are effective in reducing decay caused by *B. cinerea*. The selection of a type of liner should be done in accordance with the sensitivity of the cultivar to decay and SO<sub>2</sub> damage. The use of non perforated liners with in-carton fumigation should not be considered for cultivars sensitive to either decay or SO<sub>2</sub>. Reduced decay incidence also occurred with harvesting in adverse weather conditions. None of the SO<sub>2</sub> concentrations can be identified as the optimum and further study is anticipated to test lower concentrations and the effect on cultivars sensitive to decay and SO<sub>2</sub> damage.

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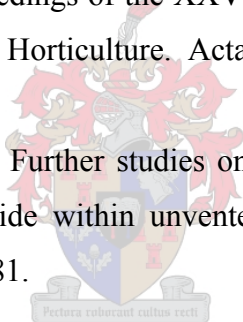
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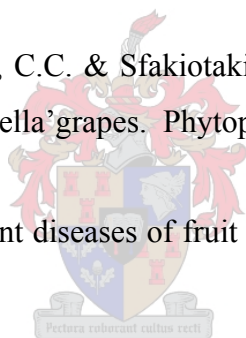
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Table 1

Effect of different concentrations of in-carton SO<sub>2</sub> fumigation on the postharvest quality of Red Globe table grapes evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C in the 2003 and 2004 seasons.

	% Decay	% Shatter	% Split	% SO <sub>2</sub> Damage
<b>2003 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation</b>	1.3	1.2	0.2	6.0b <sup>1</sup>
<b>4.5 mL/kg</b>	1.0	1.1	1.1	12.2a
<b>6.0 mL/kg</b>	0.7	1.1	0.9	6.9b
<b>7.5 mL/kg</b>	0.7	1.0	0.7	8.0b
<b>LSD (0.05)</b>				3.832
<b>P-Value</b>	0.323	0.890	0.065	0.011
<b>2004 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation</b>	8.5	0.6b	1.4	28.0b
<b>4.5 mL/kg</b>	14.0	0.4b	2.0	34.1b
<b>6.0 mL/kg</b>	7.8	0.2b	0.5	55.3a
<b>7.5 mL/kg</b>	8.5	1.6a	2.2	60.4a
<b>LSD (0.05)</b>		0.959		20.734
<b>P-Value</b>	0.656	0.046	0.112	0.005

<sup>1</sup>Means with the same letter in a column are not significantly different (P < 0.05)

Table 2

Effect of different concentrations of in-carton SO<sub>2</sub> fumigation on the postharvest quality of Thompson Seedless table grapes evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C in the 2003 and 2004 seasons.

	%	%	%	% SO <sub>2</sub>
	Decay	Shatter	Split	Damage
<b>2003 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation</b>	73.3a <sup>1</sup>	1.0b	6.0b	1.1b
<b>4.5 mL/kg</b>	18.0b	4.6a	13.9ab	7.3a
<b>6.0 mL/kg</b>	31.5b	4.1a	17.9a	6.6a
<b>7.5 mL/kg</b>	28.2b	4.7a	18.1a	7.5a
<b>LSD (0.05)</b>	27.102	1.739	9.472	4.063
<b>P-Value</b>	0.001	0.0002	0.047	0.007
<b>2004 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation</b>	4.1	2.2	2.9	7.9
<b>4.5 mL/kg</b>	1.2	1.7	4.0	11.7
<b>6.0 mL/kg</b>	1.1	1.6	2.4	12.7
<b>7.5 mL/kg</b>	0.9	1.5	2.5	10.4
<b>P-Value</b>	0.112	0.118	0.324	0.210

<sup>1</sup>Means with the same letter in a column are not significantly different (P < 0.05)

Table 3

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on the SO<sub>2</sub> damage of Red Globe table grapes packed in non-, micro- and 2 mm perforated liners and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C in the 2003 season.

% SO <sub>2</sub> Damage		
	No SO <sub>2</sub> fumigation	SO <sub>2</sub> fumigation
<b>Non perforated</b>	3.2bc <sup>1</sup>	7.0a
<b>Micro perforated</b>	2.8bc	4.2b
<b>2 mm perforated</b>	2.5c	3.9bc
<b>LSD (0.05)</b>	1.521	
<b>P-value (liner type)</b>	0.001	
<b>P-value (SO<sub>2</sub> fumigation)</b>	<0.0001	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.047	

<sup>1</sup>Means with the same letter in a column are not significantly different (P < 0.05)

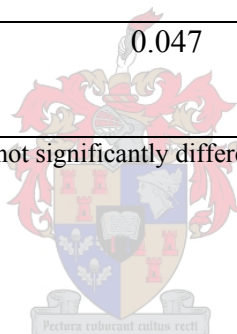




Table 4

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on the postharvest quality of Red Globe table grapes packed in non-, micro- and 2 mm perforated liners and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C during the 2004 season.

	<b>% SO<sub>2</sub></b>	<b>%</b>	<b>%</b>
	<b>Damage</b>	<b>Shatter</b>	<b>Split</b>
<b>Non perforated</b> <sup>1</sup>	26.5a <sup>3</sup>	0.1a	0.4a
<b>Micro perforated</b>	34.4a	0.0a	0.2a
<b>2 mm perforated</b>	31.8a	0.1a	0.3a
<b>LSD (0.05)</b>			
<b>P-Value (liner type)</b>	0.439	0.436	0.653
<b>No SO<sub>2</sub> fumigation</b> <sup>2</sup>	29.5a	0.0a	0.2a
<b>SO<sub>2</sub> fumigation</b>	31.8a	0.1a	0.4a
<b>LSD (0.05)</b>		0.055	
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.763	0.043	0.116
<b>P-Value (Liner type × SO<sub>2</sub> fumigation)</b>	0.269	0.971	0.915

<sup>1</sup> Data pooled across in-carton fumigation

<sup>2</sup> Data pooled across type of liner

<sup>3</sup> Means with the same letter in a column are not significantly different (P < 0.05)

Table 5

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on the development of decay on Red Globe table grapes packed in non-, micro- and 2 mm perforated liners and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C in the 2004 season.

% Decay		
	No SO <sub>2</sub> fumigation	SO <sub>2</sub> fumigation
<b>Non perforated</b>	0.1b	1.6a
<b>Micro perforated</b>	0.2b	0.2b
<b>2 mm perforated</b>	0.5b	0.1b
<b>LSD (0.05)</b>	1.111	
<b>P-value (liner type)</b>	0.334	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.157	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.040	

<sup>†</sup>Means with the same letter in a column are not significantly different (P < 0.05)

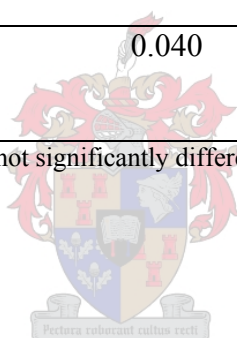


Table 6

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on the postharvest quality of Red Globe table grapes packed in non-, micro- and 2 mm perforated liners and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C in the 2003 season.

	%	%	%
	<b>Decay</b>	<b>Shatter</b>	<b>Split</b>
<b>Non perforated</b> <sup>1</sup>	0.8b <sup>3</sup>	0.8	2.6
<b>2 mm perforated</b>	1.5a	1.0	1.7
<b>Micro perforated</b>	0.9b	0.9	2.4
<b>LSD (0.05)</b>	0.513		
<b>P-Value (liner type)</b>	0.012	0.696	0.213
<b>No SO<sub>2</sub> fumigation</b> <sup>2</sup>	0.9	0.9	1.4b
<b>SO<sub>2</sub> fumigation</b>	1.1	1.0	3.1a
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.357	0.376	0.0001
<b>P-Value (Liner type × SO<sub>2</sub> fumigation)</b>	0.801	0.065	0.093

<sup>1</sup> Data pooled across in-carton fumigation

<sup>2</sup> Data pooled across type of liner

<sup>3</sup> Means with the same letter in a column are not significantly different (P < 0.05)

Table 7

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on the postharvest quality of Thompson Seedless table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 season.

	%	%	%	% SO <sub>2</sub>
	<b>Decay</b>	<b>Shatter</b>	<b>Split</b>	<b>Damage</b>
<b>Non perforated</b> <sup>1</sup>	46.8	3.1	15.0	5.2
<b>Micro perforated</b>	46.5	3.4	16.0	4.6
<b>2 mm perforated</b>	47.9	2.7	12.7	4.6
<b>LSD (0.05)</b>				
<b>P-Value (liner type)</b>	0.986	0.370	0.437	0.818
<b>No SO<sub>2</sub> fumigation</b> <sup>2</sup>	60.7a <sup>3</sup>	2.3b	11.2b	2.8b
<b>SO<sub>2</sub> fumigation</b>	33.5b	3.8a	17.9a	6.8a
<b>LSD (0.05)</b>				
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.0004	0.0002	0.003	0.0003
<b>P-Value</b> <b>(Liner type × SO<sub>2</sub> fumigation)</b>	0.965	0.192	0.055	0.988

<sup>1</sup> Data pooled across in-carton fumigation

<sup>2</sup> Data pooled across type of liner

<sup>3</sup> Means with the same letter in a column are not significantly different (P < 0.05)

Table 8

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on the postharvest quality of Thompson Seedless table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C in the 2004 season.

	%	%	%	% SO <sub>2</sub>
	<b>Decay</b>	<b>Shatter</b>	<b>Split</b>	<b>Damage</b>
<b>Non perforated</b> <sup>1</sup>	0.4	1.9	9.3a <sup>3</sup>	29.6
<b>Micro perforated</b>	1.5	1.4	5.8ab	34.5
<b>2 mm perforated</b>	3.7	1.5	3.6b	23.6
<b>LSD (0.05)</b>			4.085	
<b>P-Value (liner type)</b>	0.051	0.202	0.021	0.484
<b>No SO<sub>2</sub> fumigation</b> <sup>2</sup>	2.3	2.1a	6.6	27.2
<b>SO<sub>2</sub> fumigation</b>	1.2	1.2b	6.2	31.6
<b>LSD (0.05)</b>		0.495		
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.245	0.006	0.985	0.541
<b>P-Value (Liner type × SO<sub>2</sub> fumigation)</b>	0.236	0.245	0.631	0.771

<sup>1</sup> Data pooled across in-carton fumigation

<sup>2</sup> Data pooled across type of liner

<sup>3</sup> Means with the same letter in a column are not significantly different (P < 0.05)

Table 9

Effect of different in-carton SO<sub>2</sub> concentrations on the postharvest quality of Barlinka table grapes harvested after adverse weather conditions and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C in the 2003 and 2004 seasons.

	%	%	%	% SO <sub>2</sub>
	Decay	Shatter	Split	Damage
<b>2003</b>				
<b>No in-carton SO<sub>2</sub> fumigation<sup>2</sup></b>	6.6a <sup>1</sup>	0.1	1.0	0.5b
<b>4.5 mL/kg</b>	2.4b	0.3	1.8	1.3a
<b>6.0 mL/kg</b>	2.5b	0.4	1.6	1.3a
<b>7.5 mL/kg</b>	1.9b	0.3	1.5	1.2a
<b>LSD (0.05)</b>				0.599
<b>P-Value</b>	0.001	0.172	0.480	0.031
<b>2004</b>				
<b>No in-carton SO<sub>2</sub> fumigation<sup>2</sup></b>	0.6	1.7	2.3	10.0
<b>4.5 mL/kg</b>	0.8	1.2	2.8	17.5
<b>6.0 mL/kg</b>	0.7	1.4	3.8	17.2
<b>7.5 mL/kg</b>	2.1	1.4	2.9	16.0
<b>P-Value</b>	0.069	0.682	0.330	0.257

<sup>1</sup> Means with the same letter in a column are not significantly different (P < 0.05)

<sup>2</sup> No in-carton SO<sub>2</sub> fumigation treatments served as the control and were packed with a SO<sub>2</sub> generator pad

Table 10

Effect of different in-carton SO<sub>2</sub> concentrations on the postharvest quality of Dauphine table grapes harvested after adverse weather conditions and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 and 2004 seasons.

	%	%	%	% SO <sub>2</sub>
	Decay	Shatter	Split	Damage
<b>2003 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation</b>	3.6a <sup>1</sup>	0.4	0.3	0.8
<b>4.5 mL/kg</b>	1.0b	0.5	0.3	0.6
<b>6.0 mL/kg</b>	0.5b	0.7	0.4	0.6
<b>7.5 mL/kg</b>	0.4b	0.7	0.4	0.7
<b>LSD (0.05)</b>	1.449			
<b>P-Value</b>	0.0001	0.343	0.488	0.626
<b>2004 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation</b>	1.8	3.4	0.3	6.3
<b>4.5 mL/kg</b>	1.2	2.3	0.3	8.6
<b>6.0 mL/kg</b>	0.8	2.6	0.4	9.1
<b>7.5 mL/kg</b>	0.7	1.8	1.2	9.9
<b>P-Value</b>	0.440	0.761	0.182	0.613

<sup>1</sup> Means with the same letter in a column are not significantly different (P < 0.05)



## C. Article II

### **Control of *Botrytis cinerea* in table grapes using a prepack chlorine dioxide fumigation in conjunction with SO<sub>2</sub> generating pads.**

**Keywords:** ClO<sub>2</sub> fumigation, *Botrytis cinerea*, decay, split, shatter, SO<sub>2</sub> damage, pulp temperature, moisture, stem condition, table grapes

#### **Abstract**

Problems associated with the release of SO<sub>2</sub> from generator pads include phytotoxicity that is associated with bleaching of the berry and concern of residues on freshly consumed produce. SO<sub>2</sub> gas is highly corrosive to metal and extremely irritating to people. Food safety accompanied by regulations urge the transition away from SO<sub>2</sub> and alternatives are needed. ClO<sub>2</sub> is a strong oxidising agent with biocidal activity and it can be used in an integrated disease management system because of its disinfection potential. The objective of this study was to determine the optimum ClO<sub>2</sub> concentration and exposure time to sterilise the surface of table grapes before packing for grapes harvested in different weather conditions such as rain and high temperatures. Grapes were harvested in ideal and in adverse weather conditions and fumigated with 25, 50 and 75 µg/mL of ClO<sub>2</sub> gas with 15, 30 and 45 minute exposure periods prior to packing. The effect of pulp temperature was evaluated by fumigating grapes of 15°C, 20°C, 25°C, 30°C and 35°C. Exposure to ClO<sub>2</sub> gas may damage plant tissue which may predispose the tissue of the berry and stems to infections by *B. cinerea* or damage by SO<sub>2</sub>. Very low decay levels and the absence of infection spreading from one berry to another were observed on Red Globe and Dauphine. SO<sub>2</sub> reacts with the tissue of stems causing a lush green stem colour but prepack fumigation with ClO<sub>2</sub> caused no reaction of SO<sub>2</sub> with stems that were drier and had fungal growth on them. The formation of by-products at high concentrations decreases the stability of ClO<sub>2</sub>. The high concentrations and long exposure times were not effective to reduce decay in this study. Fumigation with ClO<sub>2</sub> diminishes negative effects of high pulp temperatures but excessive release of SO<sub>2</sub> was enhanced by

packing warm (> 30°C) grapes in non-perforated liners. Neither the prepack ClO<sub>2</sub> fumigation nor the SO<sub>2</sub> was capable to reduce decay levels on grapes harvested during these conditions.

## 1. INTRODUCTION

With new markets developing around the world, it becomes a challenge to store grapes for longer to reach the markets with superior quality. Consumer demands for residue-free produce are growing and this created a need for alternative forms of pest and disease control (Retamales *et al.*, 2003; Whiteman & Stewart, 1998). Consumers consider high quality fruit to be those with nice appearance, high nutritional value, good taste (Crisosto & Crisosto, 2002; Crisosto *et al.*, 2002 b) and free from pesticides. Food safety is an important attribute of superior quality, which is receiving a lot of attention around the world. This growing concern creates a need for alternative forms of postharvest preservation.

*Botrytis cinerea*, the common cause of bunch rot of grapes (Combrink & Ginsburg, 1972; Karabulut *et al.*, 2003; McClellan *et al.*, 1973; Palou *et al.*, 2002; Peiser & Yang, 1985; Thompson & Latorre, 1999; Witbooi *et al.*, 2000 a), is also commonly known as grey mould or slip skin (Combrink & Ginsburg, 1972; Ten Have, 2000). It is responsible for huge economic losses during storage and transport of the harvested crop (Nigro *et al.*, 1998; Leroux *et al.*, 1999; Ten Have, 2000). Rigorous handling at packing and at ports and insufficient temperature and humidity control are important factors that are conducive to development of latent infections after harvest (Nelson & Ahmedullah, 1976; Sommer, 1985). A wound is not necessary for infection when wet and/or high relative humidity conditions prevail (Comménil *et al.*, 1999; Holz, 1999; Keller *et al.*, 2003; Kupferman, 1999; Latorre *et al.*, 1997; Peacock & Smilanick, 1998; Thompson & Latorre, 1999; Ten Have, 2000).

Infection by the decay fungus occurs preharvest, during harvest and packing and even during storage. Control of preharvest infection is typically achieved through fungicide applications (Plotto *et al.*, 2003; Schena *et al.*, 1999). However, symptom expression is most prevalent on mature fruit and in table grapes occurs mostly during and after cold storage of harvested grapes. Latent *B. cinerea* in young berries invade the rest of the bunch during ripening and cause disease expression during postharvest stages (Keller *et al.*, 2003) with susceptibility that increases after exposure to low temperatures (Coertze & Holz, 1999). Surface sterilisation is therefore important to reduce inoculum levels and losses due to *B. cinerea* postharvest.

In America, sulphur dioxide (SO<sub>2</sub>) has been used for more than 70 years to reduce the occurrence of grey mould on table grapes (Chervin *et al.*, 2005; Crisosto *et al.*, 2002 a; László *et al.*, 1981; Palou *et al.*, 2002; Smilanick *et al.*, 1990). Two methods of applying the SO<sub>2</sub> gas have been adopted throughout the world: the use of regular fumigations, or the use of in-package SO<sub>2</sub> generator pads, or combinations of these (Karabulut, 2003; Lagunas-Solar *et al.*, 1992; Mustonen, 1992). Room fumigation is a common practice in California and is implemented by exposing the grapes to large doses before storage, which serves as a surface steriliser (Austin *et al.*, 1997; Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002 a; Droby & Lichter, 2004; Nelson & Ahmedullah, 1973; Palou *et al.*, 2002). This, however, does not kill inocula within the tissue and is followed by weekly fumigations during cold storage (Austin *et al.*, 1997; Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002 a; Droby & Lichter, 2004; Mustonen, 1992; Nelson & Ahmedullah, 1973). SO<sub>2</sub> generator pads, containing sodium metabisulphite, release SO<sub>2</sub> in a controlled manner upon reaction with water vapour, and sterilise the product during storage (Palou *et al.*, 2002).

Phytotoxicity due to SO<sub>2</sub> is associated with bleaching of the berry and hairline splits/cracks (Crisosto *et al.*, 2002 a; Gao *et al.*, 2003; Mustonen, 1992; Nelson, 1982; Palou *et al.*, 2002; Zhang *et al.*, 2003) and SO<sub>2</sub> toxicity increases with an increase in exposure time and SO<sub>2</sub> concentration. Another problem is the increasing concern of residues on freshly consumed produce. SO<sub>2</sub> gas is highly corrosive to metal and extremely irritating to people (Nelson & Ahmedullah, 1973; Smilanick & Henson, 1992; Zahavi *et al.*, 2000). The maximum residue limit for SO<sub>2</sub> has been established at 10 µL/L by the Environmental Protection Agency in America (Austin *et al.*, 1997; Crisosto *et al.*, 1994; Crisosto *et al.*, 2002 a; EPA, 1999; Palou *et al.*, 2002; Zoffoli *et al.*, 1999). Apart from the phytotoxicity and residues, it is still the aim to deliver high quality and decay-free grapes to the market. Grapes are subjected to different temperatures from harvest to storage and the release of SO<sub>2</sub> is influenced by these variations.

Chlorine dioxide (ClO<sub>2</sub>) is a selective bleaching agent widely used for water treatment and in the pulp and paper industries (Apel, 2002; Huang, 2001). It is a strong oxidising agent and has the ability to effectively kill microorganisms (Apel, 2002; Gagnon *et al.*, 2005; Han *et al.*, 1999; Lee *et al.*, 2004; Roberts & Reymond, 1994). The chemical is highly unstable and onsite production of the gas is essential to ensure efficacy (Apel, 2002). ClO<sub>2</sub> causes an interruption of cellular processes when the organic material acts with the ClO<sub>2</sub> (EPA, 1999).

It directly reacts with the cell wall of the microorganisms and can even kill the organism when it is inactive (Junli *et al.*, 1997; Anon, 2004; Steynberg *et al.*, 1993). Disinfection occurs in two steps with the formation of chlorate and chlorine in some instances. Both are, however, oxidizing agents, which dissociate to form sodium chloride (Dąbrowska *et al.*, 2003). The disinfection process entails a disruption of protein synthesis and permeability of the cell membrane. Several positive results have been achieved on controlling bacteria on peas, corn, tomatoes and potatoes (Spotts & Peters, 1980) and concentrations between 10 to 40 µL/L with high relative humidity control microbial activity (Lee *et al.*, 2004; Han *et al.*, 1999).

The objectives of this study were 1.) to determine the optimum ClO<sub>2</sub> concentration and exposure time to sterilise the surface of table grapes before packing for grapes harvested in ideal and adverse weather conditions and 2.) to determine the effect of the pre-packing ClO<sub>2</sub> when fumigating grapes with different pulp temperatures.

## 2. MATERIALS AND METHODS

### *Grapes*

Dauphine, Red Globe and Barlinka were harvested according to the export standards and requirements of the National Department of Agriculture (16.6°Brix, 15.7°Brix and 16.8°Brix, respectively) in the De Wet area and Bonheur (18.1°Brix) in the Hexriver area.

### *Inoculation*

*B. cinerea* was isolated from naturally infected table grape berries (Kulakiotu, 2004) and the culture was grown on potato dextrose agar at 22°C (Karabulut *et al.*, 2005; Roberts & Reymond, 1994; Zahavi *et al.*, 2000). A spore suspension was prepared by washing cultures with distilled water to remove the spores (Karabulut *et al.*, 2005; Lee *et al.*, 2004; Lydakis & Aked, 2003; Spotts & Peters, 1980; Zahavi *et al.*, 2000). Grapes were inoculated by spraying them with the spore suspension allowing an one hour incubation period.

### ***ClO<sub>2</sub> Fumigation***

ClO<sub>2</sub> gas was generated from liquid Harvest Wash (ClO<sub>2</sub>, BTC Products and Services, SL (120g/L) by using a BTC Laboratory generator (BTC Products and Services, South Africa). The concentration of ClO<sub>2</sub> was determined by titration with sodium thiosulphate (Roberts & Reymond, 1994; Spotts & Peters, 1980). Freshly generated ClO<sub>2</sub> was first dissolved in 20 mL potassium iodide (KI) and then in 100 mL distilled water to which 5 mL hydrochloric acid (2.5N HCl) was added. Freshly generated ClO<sub>2</sub> was collected in an impinger with a 2% KI solution and titrated with 0.1N sodium thiosulphate (Anon, 2005). A polyethylene bag was used as fumigation chamber with an inlet for ClO<sub>2</sub> at the top and an outlet for excess gas at the bottom (Du *et al.*, 2002; Lee *et al.*, 2004). The fumigation chamber was flushed with the desired ClO<sub>2</sub> concentration for ±10 minutes. Once the fumigation chamber was filled with grapes, excess air was removed with a vacuum pump. ClO<sub>2</sub> decomposes easily in light and no provision was made to conduct fumigations in the dark (Du *et al.*, 2002).

#### ***Trial 1 – Optimum ClO<sub>2</sub> concentration and exposure time***

Grapes were fumigated with three different ClO<sub>2</sub> concentrations (25, 50 and 75 µg/mL) with three exposure times (15, 30 and 45 minutes) for each concentration. Each concentration and time combination had six replicates of 2 kg of grapes. All treatments were packed with a SO<sub>2</sub> generator with the control not receiving any ClO<sub>2</sub> fumigation.

#### ***Trial 2 – Different pulp temperatures***

Grapes with pulp temperatures of 15°C, 20°C, 25°C, 30°C and 35°C were fumigated with 50 µg/mL ClO<sub>2</sub> for 30 minutes (Sholberg *et al.*, 1996). Grapes were harvested in the morning and either cooled or heated in a room to the desired pulp temperature. Each pulp temperature treatment had six replications of 2 kg grapes. All treatments were packed with a SO<sub>2</sub> generator and the control did not receive any ClO<sub>2</sub> fumigations.

### ***Trial 3 – Optimum ClO<sub>2</sub> concentration and exposure time for adverse weather conditions***

This trial was conducted in 2003 following at least 5 mm rain and a wetting period longer than eight hours in the area. Grapes were fumigated with three different ClO<sub>2</sub> concentrations (25, 50 and 75 µg/mL) and each concentration had three exposure times (15, 30 and 45 minutes). Each concentration and time combination had six replicates of 2 kg of grapes and was packed with a SO<sub>2</sub> generator. The control did not have any ClO<sub>2</sub> fumigation.

### ***Packing***

After fumigation sub-standard berries were removed and grapes from all treatments were packed in 2 kg cartons with non-perforated liners and UVAS Quality SO<sub>2</sub> sheets (970 mg. kg<sup>-1</sup> Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>). Grapes were stored at -0.5°C and 85 to 90% relative humidity (RH) for six weeks and one week at 15°C (Crisosto *et al.*, 1994). Evaluations were conducted directly following cold storage (-0.5°C) and again after a shelf life simulation (15°C). Fruit were evaluated for dry stems on a scale of one to five where one is green and five is brown (Crisosto *et al.*, 1994; Perkins-Veazie, 1992; Sholberg *et al.*, 1996; Witbooi *et al.*, 2000 b), presence of moisture on a scale of one to three where one is dry, two condensation and three presence of free moisture (Sholberg *et al.*, 1996; Witbooi *et al.*, 2000 b). Decay, split and loose berries (shatter) were expressed as a percentage of the nett mass of the grape sample (Franck *et al.*, 2005; Witbooi *et al.*, 2000 b). A random sample of berries (100 berries) was picked, juiced, and filtered to get a clear sample. Total soluble solids (TSS) were determined by measuring the refractive index with an Atago DBX-55 digital refractometer (Atago Bussan Co. Ltd, Japan) and the acidity was measured by titration with a 719S automatic titrator (Metrohm, South Africa) using 0.1N NaOH to pH end point of 8.2.

### ***Statistical analyses***

Trials one and three had a completely randomised experimental design with a 3 × 4 factorial design with 3 ClO<sub>2</sub> concentrations and 4 exposure times with six single carton replicates. Trial two had a completely randomised experimental design with five pulp temperature treatments. Defect data were subjected to analyses of variance using the General Linear

means procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1999). Student's t-Test Least Significant Difference was calculated at the 5% confidence level to compare treatment means ( $P < 0.05$ ).

### 3. RESULTS

#### *Trial 1 – Optimum concentration and exposure time*

Analysis of variance of defect data of Red Globe showed a significant interaction between concentration and exposure time for decay and split berries (Table 1). Other quality parameters, SO<sub>2</sub> damage and shatter had a non significant interaction between ClO<sub>2</sub> concentration and exposure time and data were pooled across the two main effects (Table 2). The level of SO<sub>2</sub> damage was very high with all treatments, showing no significant differences (Table 2). Likewise, shatter was unaffected by ClO<sub>2</sub> concentration or exposure time (Table 2).

With the late season cultivar, Dauphine, significant interactions between ClO<sub>2</sub> concentration and exposure time were observed for quality parameters decay and SO<sub>2</sub> phytotoxicity (Table 3). Exposure time did not affect shatter or berry split (Table 4). The 50 µg/mL ClO<sub>2</sub> fumigation significantly reduced shatter in comparison to non fumigated Dauphine, but the application of 25 µg/mL and 75 µg/mL ClO<sub>2</sub> increased the level of berry split compared to the control. Visual observations of grape appearance indicated the presence of crystals (presumably NaCl) on all cultivars treated with the higher ClO<sub>2</sub> concentration (75 µg/mL).

Quality parameters of Bonheur showed no positive interaction between ClO<sub>2</sub> concentration and exposure time. When data were pooled across exposure times no significant difference was observed between the ClO<sub>2</sub> concentrations for decay and split berries ( $P = 0.198$  and  $P = 0.330$ , respectively; data not shown). SO<sub>2</sub> damage was significantly higher on control grapes whereas the treatment had significantly less shatter (data not shown).

No significant interactions between ClO<sub>2</sub> concentration and exposure time were observed for free moisture on Red Globe grapes and ClO<sub>2</sub> fumigated grapes were not significantly different from the control (data not shown). Data of stem condition of this cultivar showed a positive interaction ( $P = 0.006$ ) between ClO<sub>2</sub> concentration and exposure time (data not shown). This



phenomenon was also evident with moisture and stem condition of Dauphine ( $P = 0.003$  and  $P = 0.0004$ , respectively) and Bonheur ( $P = 0.023$  and  $P = 0.017$ , respectively) table grapes (data not shown).

A positive interaction was found between  $\text{ClO}_2$  concentration and exposure time for TSS and acidity of Red Globe and Bonheur (data not shown). This was also observed in acidity of Dauphine ( $P = 0.007$ ) but TSS had a non significant interaction ( $P = 0.552$ ) (data not shown). None of the treatments differed significantly from each other (data not shown).

### ***Trial 2 – Pulp temperature***

Pulp temperature of table grapes at harvest is an important factor that influences the postharvest quality of the grapes. For Dauphine table grapes, significant differences were observed for decay and  $\text{SO}_2$  phytotoxicity at the various pulp temperatures ( $P = 0.021$  and  $P < 0.0001$ , respectively; Fig.1). Lowest decay levels were observed on grapes of  $15^\circ\text{C}$  pulp temperature (Fig.1), but this did not differ significantly from grapes of  $30^\circ\text{C}$  and  $35^\circ\text{C}$ . Highest  $\text{SO}_2$  damage was recorded on grapes at  $30^\circ\text{C}$  pulp temperature (Fig. 1), which differed significantly from all other pulp temperatures.

With Red Globe, shatter and split berries were influenced significantly by the different pulp temperatures ( $P = 0.014$  and  $P = 0.002$ , respectively; Fig.2). Highest decay levels were recorded on fruit at  $30^\circ\text{C}$  pulp temperature ( $P = 0.012$ ; Fig.2), which differed significantly from the other pulp temperatures. Unacceptable and very high  $\text{SO}_2$  damage was observed on Red Globe and the lower pulp temperatures ( $15^\circ$ ) had significantly less damage (Fig. 2).

Highest decay levels were recorded on Bonheur grapes at  $25^\circ\text{C}$  pulp temperature, which was significantly higher than levels on grapes with  $35^\circ\text{C}$  pulp temperature ( $P = 0.032$ ; data not shown). No significant differences occurred between the decay levels of  $15^\circ\text{C}$  and  $35^\circ\text{C}$  pulp temperature grapes (0.8% and 0.7%, respectively; data not shown). This was also evident with quality variables such as  $\text{SO}_2$  damage and split berries (data not shown).

Significant differences occurred between the treatments for stem condition in Bonheur, Dauphine and Red Globe ( $P < 0.0001$ ,  $P = 0.037$  and  $P < 0.0001$ , respectively; data not

shown). With Bonheur and Dauphine stem condition of 15°C pulp temperature grapes did not differ significantly from 35°C pulp temperature grapes (data not shown). In Red Globe pulp temperatures above 30°C resulted in drier stems (data not shown).

Condensation occurred on Bonheur grapes at 15°C, 20°C and 35°C pulp temperatures, which differed significantly from other treatments. Significantly less moisture occurred on Dauphine grapes at 25°C and 30°C pulp temperatures (data not shown). The opposite was observed with the free moisture on Red Globe, with significantly more free moisture on grapes at 35°C than 20°C pulp temperatures (data not shown).

Fumigation with ClO<sub>2</sub> did not have any significant effect on the TSS of Dauphine and Red Globe table grapes (P = 0.595 and P = 0.313, respectively; data not shown). In Bonheur, lower pulp temperatures (15°C and 20°C) had significantly higher TSS than grapes at 35°C pulp temperatures (data not shown). The acidity of Bonheur grapes at 15°C pulp temperature was also significantly less than other treatments but no differences were observed amongst treatments of Dauphine and Red Globe (data not shown).

The crest of the University of Stellenbosch, featuring a shield with various symbols, topped with a crown and surrounded by a ribbon with the motto 'Pectora roburant cultus recti'.

***Trial 3 – Optimum concentration and exposure time for grapes harvested in adverse weather conditions***

Analysis of variance of defect data of Barlinka showed a significant interaction between concentration and exposure time for decay and SO<sub>2</sub> damage (Table 5). Very high decay levels (Table 5) were recorded with the 75 µg/mL ClO<sub>2</sub> and 15 minute exposure period, but these tended to decrease towards longer exposure times. Lowest decay levels were recorded on grapes that were not fumigated with ClO<sub>2</sub> (control). The positive interaction between concentration and exposure time was not evident with quality parameters such as shatter and split berries and data were pooled across the variable (Table 6). ClO<sub>2</sub> concentration did not significantly affect shatter or split although levels were slightly higher in all ClO<sub>2</sub> treated grapes. Likewise, exposure time did not influence split but there was a significant increase in shatter with exposure times of 15 minutes (Table 6).

In Dauphine, analysis of variance showed a significant interaction between concentration and exposure time for all quality parameters except SO<sub>2</sub> damage (P = 0.435 – SO<sub>2</sub> damage; Tables

7 and 8). Fungal growth, caused by latent *B. cinerea* infections, occurred on the rachises of Dauphine bunches.

Both stem condition and moisture of Barlinka and Dauphine showed no positive interaction between ClO<sub>2</sub> concentration and time. The stems of Barlinka that tended to be drier with increasing ClO<sub>2</sub> concentration (data not shown). Greenest stems were observed on 25 µg/mL ClO<sub>2</sub> treated grapes that differed significantly from other treatments (data not shown).

No interaction was evident for TSS and acidity in Barlinka grapes (P = 0.952 and P = 0.051; respectively) with significantly lower acid in control grapes (data not shown). Treatment did not have any effect on the TSS of this cultivar (data not shown). Acidity of Dauphine showed a significant interaction between ClO<sub>2</sub> concentration and time (P = 0.007), which was not evident in the TSS (P = 0.860; data not shown).

#### 4. DISCUSSION

ClO<sub>2</sub> treatments were applied as a prepack surface steriliser and the purpose was to eradicate decay fungi, specifically *B. cinerea* on the surface of the fruit before packing with standard packing material. It is, however, difficult to quantify the sterilising effect on grapes that are stored for long periods, as latent infections can occur during the storage period.

Significant interactions observed for decay of Red Globe and Dauphine were attributed to the significant differences in exposure times. ClO<sub>2</sub> concentrations used in this study were higher than the concentrations that reduced microorganisms on apples, lettuce and carrots (Singh *et al.*, 2002). Any exposure to ClO<sub>2</sub> gas might damage the plant tissue as it acts by oxidation. This predisposes the grapes to new infections of *B. cinerea* or damage by SO<sub>2</sub>. The SO<sub>2</sub> generator that continuously sterilises the environment surrounding the grapes has the purpose of preventing new infections in grapes. It is difficult to eradicate microorganisms that settled in plant tissue (Singh *et al.*, 2002) and one decaying berry infects neighbouring berries and infection spreads over an entire bunch (Droby & Lichter, 2004). The lowest decay levels were recorded on control fruit from Red Globe and Dauphine grapes indicating that prepack ClO<sub>2</sub> sterilisers cause some reaction with the berry tissue making it more susceptible to the decay fungus.

Optimal coverage of the fruit surface is an essential requirement for an effective steriliser. Bunches of Red Globe are stragglier than those of Dauphine, exposing more berry surface to ClO<sub>2</sub> gas. Differences in epidermal structure may also make a cultivar either sensitive to the gas or to infection by *B. cinerea* (Crisosto *et al.*, 1994; Zhang *et al.*, 2003). The sensitivity of Red Globe to SO<sub>2</sub> gas was evident with very high damage levels. Extremely high SO<sub>2</sub> damage was caused by the non-perforated liner, in which relative humidity increases quickly to cause condensation and the excessive release of SO<sub>2</sub> (Gao *et al.*, 2003; Palou *et al.*, 2002 a). The epidermal tissue of the berry differs from the stems and reaction of SO<sub>2</sub> on the berry causes damage whereas this enhances green colour of stems (Mustonen, 1992), which is conflicting with results from this study. All treatments were packed with a SO<sub>2</sub> generator and in the absence of a prepack fumigation treatment with ClO<sub>2</sub> stems are lush green after the storage period. In this study, the prepack fumigation with ClO<sub>2</sub> caused no further reaction of SO<sub>2</sub> gas with stems, resulting in drier stems on fumigated grapes and the presence of fungal growth.

The presence of supposedly sodium chloride (NaCl) crystals on high concentration treatments indicated that chlorate and chlorine were formed, which dissociated to form NaCl (Dąbrowska *et al.*, 2003; Anon, 2004). The formation of chlorine decreases the stability of ClO<sub>2</sub> (Prusky *et al.*, 2001), which may explain the insignificant differences at these high concentrations. The organoleptic properties could also be negatively influenced by these treatments, but the effect was not quantified in this study.

Many studies indicate the efficacy of ClO<sub>2</sub> to be enhanced at temperatures from 9°C to 28°C (Han *et al.*, 1999; Han *et al.*, 2000 a; Lee *et al.*, 2004), but in this research fumigation at pulp temperatures higher than 30°C was also highly effective in reducing decay. This contradicts results from Lee *et al.* (2004) who found reducing efficacy with increasing temperatures. The optimum temperature for infection by *B. cinerea* is between 20°C and 25°C (Blakeman, 1980) and these high pulp temperature conditions were not favourable for the development of the fungus. Ambient temperatures in the grape producing areas in South Africa can rise to ± 40°C during the harvesting season resulting in pulp temperatures substantially higher than those mentioned above. This is a factor that negatively affects postharvest grape quality and current control methods (SO<sub>2</sub> generators) are also influenced by it. Fumigation with ClO<sub>2</sub> before packing diminishes these negative effects but excessive release of SO<sub>2</sub> was enhanced by packing warm (> 30°C) grapes in non-perforated liners. It may therefore be of value to

evaluate further trials in a scenario where fumigation occurs during forced air cooling of grapes already packed in cartons.

Barlinka is a cultivar that is sensitive to decay and it also tends to split around the pedicel end of the berry. Harvesting after rain, with high disease pressure and relative humidity, aggravated damage due to split berries. The split around the pedicel end of the berry creates an entrance port for *B. cinerea* and fumigation with  $\text{ClO}_2$  might increase severity. Holz *et al.* (2004) found the attachment zone of the berry-pedicel to be an important site for development of the fungus and the  $\text{ClO}_2$  acts with the fungi (Junli *et al.*, 1997; Anon, 2004; Steynberg *et al.*, 1993). With long exposure periods, the oxidation process continues (in the absence of fungi) causing an interruption of the cellular processes of the berry (EPA, 1999). Underlying tissue with a high sugar content is exposed, which is an ideal environment for development of *B. cinerea*. Neither the prepack  $\text{ClO}_2$  fumigation nor the  $\text{SO}_2$  were capable of reducing decay levels on grapes harvested after rainfall.

Several researchers found the 30 minute exposure period sufficient to sterilise the surface of fruits (Beuchat *et al.*, 2004; Du *et al.*, 2002; Han *et al.*, 2000 a; Singh *et al.*, 2002), but with considerably lower concentrations than used in this study. It is important to establish which of the two variables, concentration or exposure time, is the critical factor that determines sterilisation as this will determine the economic viability to implement it as a control measure.  $\text{ClO}_2$  gas has a great penetration ability (Han *et al.*, 2000 a; b) to effectively kill fungi within tight bunches and the extremely high concentrations in this study, were therefore, unnecessary. Since no provision was made to protect freshly generated  $\text{ClO}_2$  against direct light (Han *et al.*, 2000 b), it is impossible to establish the exact concentrations that grapes were exposed to and whether this was  $\text{ClO}_2$  or  $\text{Cl}_2$ . The extremely high concentrations and the presence of NaCl crystals open the possibility of  $\text{Cl}_2$  as a steriliser. This, however, is highly corrosive and the application would be as in-carton fumigation (Prusky *et al.*, 2001; Roberts & Reymond, 1994; Spotts & Peters, 1980) or impregnated onto a sheet (Zoffoli *et al.*, 1999).

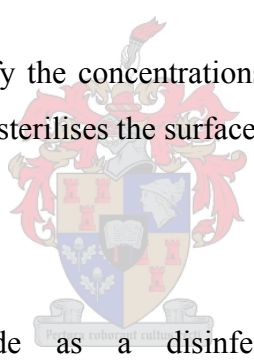
Postharvest and prepack fumigation treatments are contradictory to standard practices in South Africa. Such a treatment is an additional treatment to standard  $\text{SO}_2$  generating pads and concentrations above 10  $\mu\text{g/mL}$  are not economical (Spotts & Peters, 1980; Roberts & Reymond, 1994). Extremely high  $\text{ClO}_2$  concentrations applied in this study caused slight

discomfort to humans and good ventilation is necessary during fumigation (Roberts & Reymond, 1994). It is important to determine the by-products that are formed during ClO<sub>2</sub> fumigation and the effect these may have on grape quality and food safety. Market pressure and consumer acceptance highlight the need for postharvest disease control to move to an integrated control strategy.

These prepack treatments in combination with a SO<sub>2</sub> generator were effective in eradicating *B. cinerea* spores on table grapes harvested during optimum weather conditions but ineffective when high relative humidity, as in adverse weather conditions, prevails. Very high concentrations and the presence of by-products make it difficult to assign the efficacy to ClO<sub>2</sub> alone. Consumer focus is on food safety and the presence of by-products on the grapes makes these treatments unacceptable. The sterilising ability of the prepack ClO<sub>2</sub> fumigation treatment did not work with the various pulp temperatures but it does eliminate negative effects of high pulp temperatures on defects other than decay and SO<sub>2</sub> damage.

Further study is proposed to quantify the concentrations that grapes are exposed to and the exact chemical, whether ClO<sub>2</sub> or Cl<sub>2</sub> sterilises the surface.

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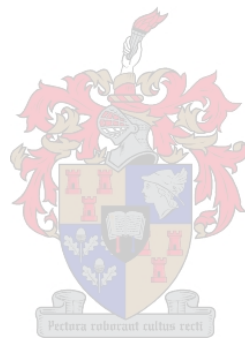


Table 1

Effect of ClO<sub>2</sub> concentration and exposure time on the development of postharvest decay caused by *Botrytis cinerea* and berry splits on Red Globe table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

% Decay				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	0.9a <sup>1</sup>	0.8a	1.4ade	0.5ac
50 µg/mL	0.9a	0.6ac	1.3ad	0.8a
75 µg/mL	0.9a	1.7bde	0.4acf	0.3acf
LSD (0.05)		0.753		
P-Value (concentration)		0.139		
P-Value (exposure time)		0.027		
P-Value (Concentration × Time)		0.002		
% Split				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	0.3a	0.9a	3.5b	0.4a
50 µg/mL	0.3a	1.1a	1.4a	0.8a
75 µg/mL	0.3a	0.9a	0.3a	0.8a
LSD (0.05)		1.392		
P-Value (concentration)		0.065		
P-Value (exposure time)		0.032		
P-Value (Concentration × Time)		0.003		

<sup>1</sup>Means with the same letter are not significantly different (P < 0.05)



Table 2

Effect of ClO<sub>2</sub> concentration and exposure time on the SO<sub>2</sub> phytotoxicity and berry shatter of Red Globe table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

	% SO <sub>2</sub> Damage	% Shatter
<sup>1</sup> No ClO <sub>2</sub> fumigation	63.9	0.0
25 µg/mL	60.6	0.2
50 µg/mL	61.5	0.3
75 µg/mL	62.9	0.3
<b>P-Value (concentration)</b>	0.341	0.416
<sup>2</sup> No ClO <sub>2</sub> fumigation	63.9	0.0
15 minute	62.7	0.3
30 minute	60.5	0.2
45 minute	61.8	0.3b
<b>P-Value (Time)</b>	0.367	0.578
<b>P-Value</b>	0.060	0.899
<b>(Concentration × Time)</b>		

<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

Table 3

Effect of ClO<sub>2</sub> concentration and exposure time on the development of postharvest decay caused by *Botrytis cinerea* and SO<sub>2</sub> phytotoxicity on Dauphine table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

	% Decay			
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	3.6a <sup>1</sup>	2.6a	4.6a	2.6a
50 µg/mL	3.6a	2.4a	4.4a	10.7b
75 µg/mL	3.6a	1.5a	4.9a	3.7a
LSD (0.05)		4.073		
P-Value (concentration)		0.123		
P-Value (exposure time)		0.012		
P-Value (Concentration × Time)		0.0173		
	% SO <sub>2</sub> damage			
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	3.0a <sup>1</sup>	7.0bcd	2.2ae	3.7ac
50 µg/mL	3.0a	2.8ae	1.7ae	1.0ae
75 µg/mL	3.0a	3.2a	4.6acd	2.2ae
LSD (0.05)		2.338		
P-Value (concentration)		0.005		
P-Value (exposure time)		0.011		
P-Value (Concentration × Time)		0.010		

<sup>1</sup>Means with the same letter in a column are not significantly different (P < 0.05)

Table 4

Effect of ClO<sub>2</sub> concentration and exposure time on the quality of Dauphine table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

	<b>% Shatter</b>	<b>% Split</b>
<sup>1</sup> <b>Control</b>	1.9a <sup>3</sup>	0.5b
<b>25 µg/mL</b>	1.9a	1.2a
<b>50 µg/mL</b>	1.2b	0.6b
<b>75 µg/mL</b>	1.7ab	1.1a
<b>LSD (0.05)</b>	0.596	0.532
<b>P-Value (concentration)</b>	0.033	0.005
<sup>2</sup> <b>Control</b>	1.9	0.5
<b>15 minute</b>	1.5	1.0
<b>30 minute</b>	1.9	0.8
<b>45 minute</b>	1.6	1.2
<b>P-Value (time)</b>	0.217	0.209
<b>P-Value</b>	0.219	0.107

**(Concentration × Time)**

<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>3</sup> Means with the same letter in a column do not differ significantly

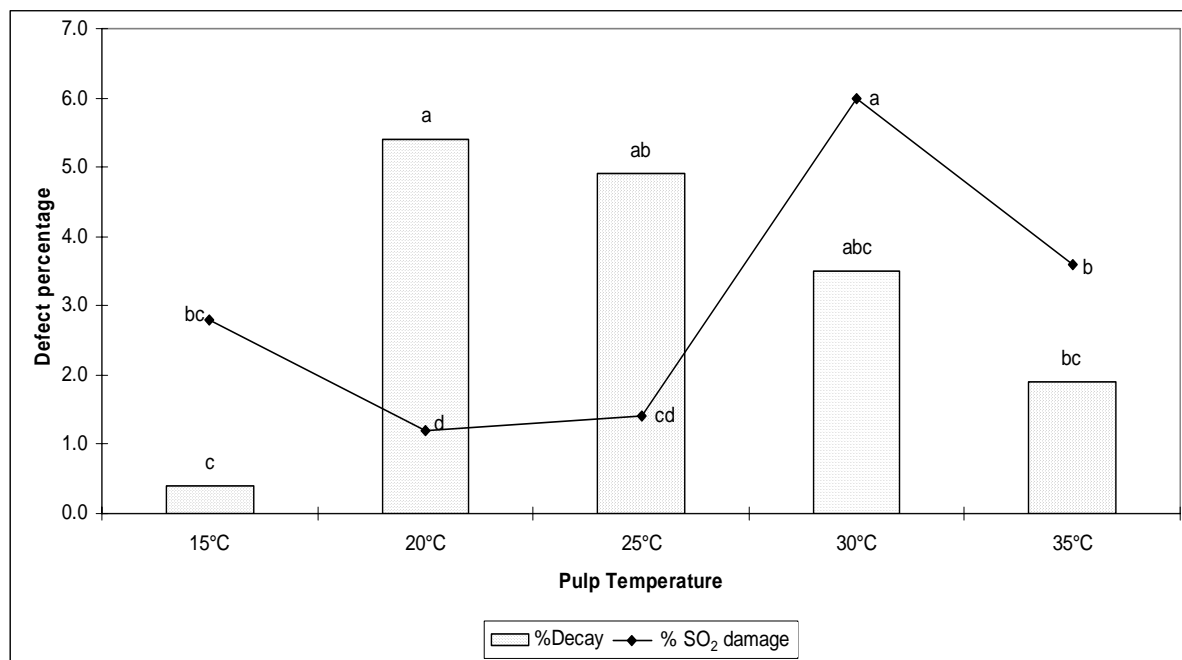
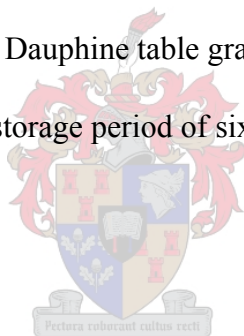


Figure 1 Effect of pulp temperature on the development of postharvest decay caused by *Botrytis cinerea*, and SO<sub>2</sub> damage on Dauphine table grapes fumigated with 50 µg/mL ClO<sub>2</sub> for 30 minutes and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C.



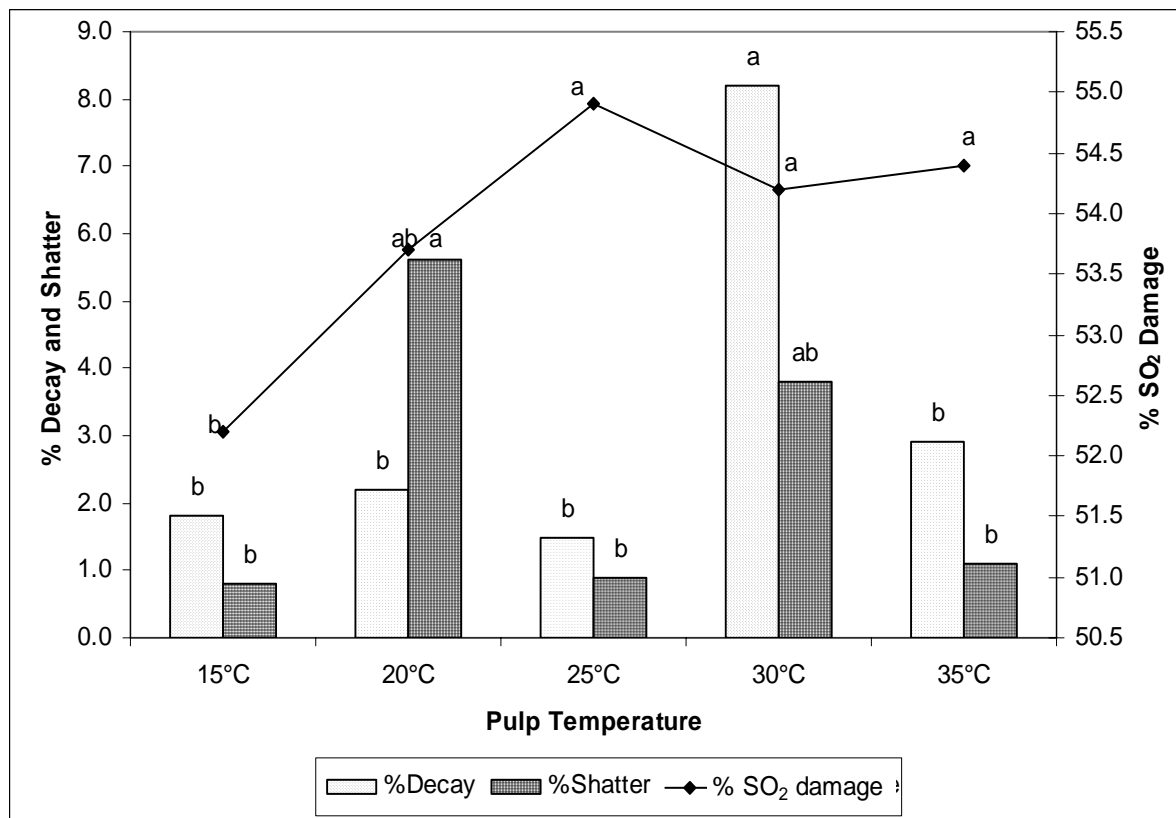


Figure 2 Effect of pulp temperature on the postharvest quality of Red Globe table grapes fumigated with 50  $\mu\text{g}/\text{mL}$   $\text{ClO}_2$  for 30 minutes and evaluated after a storage period of six weeks at  $-0.5^\circ\text{C}$  and shelf life of one week at  $15^\circ\text{C}$ .

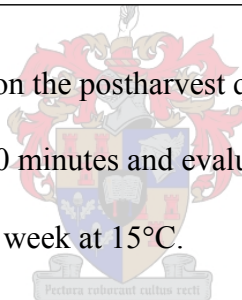


Table 5

Effect of ClO<sub>2</sub> concentration and exposure time on the development of postharvest decay caused by *Botrytis cinerea* and SO<sub>2</sub> damage on Barlinka table grapes, harvested after rain and stored for six weeks at -0.5°C and a shelf life of one week at 15°C.

	% Decay			
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	2.3a <sup>1</sup>	20.8b	7.0a	7.6a
50 µg/mL	2.3a	7.4a	11.2a	3.7a
75 µg/mL	2.3a	25.6bc	2.3a	2.4a
LSD (0.05)		10.743		
P-Value (concentration)		0.153		
P-Value (exposure time)		<0.0001		
P-Value (Concentration × Time)		0.0106		
	% SO <sub>2</sub> damage			
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	1.8a <sup>1</sup>	0.7a	1.5a	2.4ac
50 µg/mL	1.8a	2.1ac	0.7a	0.9a
75 µg/mL	1.8a	2.2ac	3.7bc	1.2a
LSD (0.05)		1.271		
P-Value (concentration)		0.023		
P-Value (exposure time)		0.480		
P-Value (Concentration × Time)		0.0001		

<sup>1</sup>Means with the same letter are not significantly different (P < 0.05)

Table 6

Effect of ClO<sub>2</sub> concentration and exposure time on the quality of Barlinka table grapes, harvested after rain and stored for six weeks at -0.5°C and a shelf life of one week at 15°C.

	<b>% Shatter</b>	<b>% Split</b>
<sup>1</sup> <b>Control</b>	0.5	2.9
<b>25 µg/mL</b>	0.6	4.7
<b>50 µg/mL</b>	0.9	4.3
<b>75 µg/mL</b>	0.9	4.4
<b>P-Value (concentration)</b>	0.123	0.249
<sup>2</sup> <b>Control</b>	0.5bc <sup>3</sup>	2.9
<b>15 minute</b>	1.1a	4.4
<b>30 minute</b>	0.9ab	4.5
<b>45 minute</b>	0.4c	4.4
<b>LSD (0.05)</b>	0.498	
<b>P-Value (time)</b>	0.003	0.949
<b>P-Value</b>	0.508	0.216

**(Concentration × Time)**

<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>3</sup> Means with the same letter in a column are not significantly different (P < 0.05)

Table 7

Effect of ClO<sub>2</sub> concentration and exposure time on the development decay and berry splits of Dauphine table grapes harvested after rain and stored for six weeks at -0.5°C and a shelf life of one week at 15°C.

% Decay				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	1.2ac <sup>1</sup>	2.2a	1.9a	2.4a
50 µg/mL	1.2ac	2.1a	3.5d	3.4d
75 µg/mL	1.2ac	3.5	6.2b	2.9a
LSD (0.05)		1.744		
P-Value (concentration)		<0.0001		
P-Value (exposure time)		0.040		
P-Value (Concentration × Time)		0.018		
% Split				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	0.0a <sup>1</sup>	0.1a	0.2a	0.4ac
50 µg/mL	0.0a	0.7bc	0.2a	0.1a
75 µg/mL	0.0a	0.3a	0.2a	0.1a
LSD (0.05)		0.437		
P-Value (concentration)		0.301		
P-Value (exposure time)		0.258		
P-Value (Concentration × Time)		0.034		

<sup>1</sup>Means with the same letter in a column are not significantly different (P < 0.05)



Table 8

Effect of ClO<sub>2</sub> concentration and exposure time on berry shatter of Dauphine table grapes harvested after rain and stored for six weeks at -0.5°C and a shelf life of one week at 15°C.

	% Shatter			
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	0.5a	0.8a	1.1a	0.6a
50 µg/mL	0.5a	0.6a	3.2bde	1.4b
75 µg/mL	0.5a	0.8a	1.1a	2.1bd
LSD (0.05)		0.863		
P-Value (concentration)		0.0004		
P-Value (exposure time)		0.0004		
P-Value (Concentration × Time)		<0.0001		

<sup>1</sup>Means with the same letter in a column are not significantly different (P < 0.05)



## D. Article III

### Potential prepack treatments for surface sterilisation of table grapes

**Keywords:** *Botrytis cinerea*, decay, ClO<sub>2</sub>, Citrofresh, Sporekill, dip, spray, table grapes, leaves

#### Abstract

Postharvest decay caused by *Botrytis cinerea*, result in huge economic losses for table grapes. Disease management relies heavily on postharvest SO<sub>2</sub> fumigation. There is increasing concern of pesticide residues on freshly consumed produce and governments consequently impose certain regulations and restrictions. Sulphite residues and phytotoxicity that include bleaching and hairline splitting on the berry surface are the main problems with SO<sub>2</sub>. The objective of this study was to compare the surface sterilising ability of selected “Generally Regarded as Safe” (GRAS) chemicals, a patented didecyldimethylammonium chloride formulation (Sporekill), a bioflavanoid complex (Citrofresh), and liquid ClO<sub>2</sub> with the contact fungicide fenhexamid (Teldor) applied as dip or spray treatments. The sterilising ability was screened on grapevine leaves and bunches. Fifth stage grapevine leaves and Red Globe table grapes were inoculated with dry *B. cinerea* conidia and incubated for 6 hours to allow germination and adhesion. Subsequently, leaves and grapes were then either dipped for 30 seconds or sprayed with a gravity feed mist spray gun to the point of run-off. Teldor, Sporekill and Citrofresh were effective in reducing *B. cinerea* on grapevine leaves with no significant difference between the application methods. On inoculated Red Globe grapes, Teldor, Sporekill and ClO<sub>2</sub> were equally effective and reduced decay levels to 8.9%, 11.0% and 12.7%, respectively, compared with the water sprayed control (27.1%). No difference was observed between spray and dip application of these compounds. Postharvest spray application of these GRAS chemicals, therefore, offers an alternative to chemical treatment for decay and should be investigated further.

## 1. INTRODUCTION

Consumers consider high quality fruit to be those with good appearance, high nutritional value, good taste (Crisosto & Crisosto, 2002; Crisosto *et al.*, 2002 b) and free from pesticides. With new markets developing around the world, it becomes a challenge to store grapes for longer to reach the markets with superior quality. Food safety is an important attribute of superior quality, which is receiving a lot of attention around the world. This growing concern creates a need for alternative forms of postharvest preservation.

*Botrytis cinerea*, the common cause of bunch rot of grapes (Combrink & Ginsburg, 1972; Karabulut *et al.*, 2003; McClellan *et al.*, 1973; Palou *et al.*, 2002; Peiser & Yang, 1985; Thompson & Latorre, 1999; Witbooi *et al.*, 2000 a), is also commonly known as grey mould or slip skin (Combrink & Ginsburg, 1972; Ten Have, 2000). It is responsible for huge economic losses during storage and transport of the harvested crop (Leroux *et al.*, 1999; Nigro *et al.*, 1998; Ten Have, 2000). Rigorous handling at packing and ports and insufficient temperature and humidity control are important factors that are conducive to development of latent infections after harvest (Nelson & Ahmedullah, 1976; Sommer, 1985). The fungus normally enters through wounds, but this is not necessary when wet and/or high relative humidity conditions prevail (Comménil *et al.*, 1999; Holz, 1999; Keller *et al.*, 2003; Kupferman, 1999; Latorre *et al.*, 1997; Peacock & Smilanick, 1998; Thompson & Latorre, 1999; Ten Have, 2000).

Infection occurs mostly during the preharvest stages and control is typically achieved through preharvest fungicide applications (Holz, 1999; Plotto *et al.*, 2003; Schena *et al.*, 1999). However, symptom expression is most prevalent on mature fruit and in table grapes occurs mostly during and after cold storage of harvested grapes. Latent *B. cinerea* in young berries invade the rest of the berry during ripening and cause disease expression during postharvest stages (Keller *et al.*, 2003) with susceptibility increasing with exposure to cold temperatures (Coertze & Holz, 1999). Surface sterilisation is therefore important to reduce inoculum levels and losses due to postharvest *B. cinerea*.

Sulphur dioxide (SO<sub>2</sub>) had been used for more than 70 years to reduce the occurrence of postharvest grey mould on table grapes (Chervin *et al.*, 2005; Crisosto *et al.*, 2002 a; László *et al.*, 1981; Palou *et al.*, 2002; Smilanick *et al.*, 1990) and is applied as a room fumigant, in-package SO<sub>2</sub> generator pads, or combinations of these (Karabulut, 2003; Lagunas-Solar *et al.*,

1992; Mustonen, 1992). Grapes are exposed to high doses of SO<sub>2</sub> just after packing and this serves as a surface steriliser but does not kill inoculum inside the fruit (Austin *et al.*, 1997; Combrink & Ginsburg, 1972; Couey & Uota, 1961; Crisosto *et al.*, 2002 a; Droby & Lichter, 2004; Nelson & Ahmedullah, 1973; Palou *et al.*, 2002). SO<sub>2</sub> generator pads, containing sodium metabisulphite, releases SO<sub>2</sub> in a controlled manner upon reaction with water vapour, and sterilise the product during shipment and storage (Palou *et al.*, 2002). Bleaching of grapes, which is the result of SO<sub>2</sub> phytotoxicity, increase with an increase in exposure time and SO<sub>2</sub> concentration. This is also true when temperature and relative humidity increase (Gao *et al.*, 2003; Palou *et al.*, 2002). Temperature fluctuation results in free moisture in the carton. This enhances the hydration of sodium metabisulfite and the excessive release of SO<sub>2</sub> (Palou *et al.*, 2002).

Fungicides form an integral part of the control strategy for decay caused by *B. cinerea*, but the fungus has the ability to develop resistance and producers acts by increasing sprays or doses to combat the problem (Al-Haq *et al.*, 2002). Fungicides have adverse effects on both the environment and humans and the concern for residues on freshly consumed produce is increasing with government imposing restrictions and regulations accordingly (Al-Haq *et al.*, 2002; Gullino *et al.*, 1992). Some importing countries prohibit the use of postharvest fungicides (Al-Haq *et al.*, 2002), which includes SO<sub>2</sub> fumigation. The gas is irritating to the mucous membrane, lungs and eyes (Nelson, 1982), which creates an allergic reaction in some people (Crisosto *et al.*, 1994). A 10 µL/L residue limit has been placed on SO<sub>2</sub> and the “Generally Regarded as Safe” (GRAS) status has been revoked (Crisosto *et al.*, 1994; Smilanick *et al.*, 1990). Apart from these phytotoxicity and residue issues, it is still the primary aim to deliver high quality and decay-free grapes to the market, and alternatives to SO<sub>2</sub> fumigation are increasingly being investigated. Alternatives to SO<sub>2</sub> include chlorine (Cl<sub>2</sub>), chlorine dioxide (ClO<sub>2</sub>), ethanol, hydrogen peroxide, biocontrol agents and plant extracts (Anon., 2005; Apel, 2002; Chervin *et al.*, 2003; Gabler *et al.*, 2005; Huang, 2001; Isman, 2000; Junli *et al.*, 1997; Kulakiotu *et al.*, 2004; Plotto *et al.*, 2003; Prusky *et al.*, 2001; Rij & Forney, 1995).

Biocontrol agents for the control of postharvest decay include many bacteria and yeasts of which the latter is applied as postharvest dips. This has a negative effect on quality, by removing the bloom on the berries (Zahavi *et al.*, 2000). Yeasts are also very sensitive to temperature changes (Kulakiotu *et al.*, 2004). Any biocontrol agent must be established on

the berry surface before the decay fungus, which means that it should be applied during the preharvest stage. During this stage, however, nutrients are low and temperatures vary in the field (Schena *et al.*, 1999). As a consequence of these factors, very little success has been obtained with biocontrol of postharvest decay.

Cl<sub>2</sub> show potential as a postharvest steriliser but it is highly reactive, and has a corrosive nature (Prusky *et al.*, 2001; Roberts & Reymond, 1994; Spotts & Peters, 1980). The deleterious effects of SO<sub>2</sub> and ClO<sub>2</sub> were emphasised in previous chapters with high levels of phytotoxicity and instability of ClO<sub>2</sub> gas. ClO<sub>2</sub> has the ability to effectively kill microorganisms and is widely used for water treatment (Anon., 2005; Apel, 2002; Gagnon *et al.*, 2005; Han *et al.*, 1999; Huang, 2001; Junli *et al.*, 1997; Lee *et al.*, 2004; Roberts & Reymond, 1994) but the stability of it is negatively affected by organic substances (Hofmann *et al.*, 2004). ClO<sub>2</sub> can be used in an integrated disease management system because of its disinfestation potential (Han *et al.*, 1999; Roberts & Reymond, 1994; Spotts & Peters, 1980). Liquid ClO<sub>2</sub> eradicated *Escherichia coli* on fresh cut lettuce and carrots (Singh *et al.*, 2002). These bacteria are capable of causing food-borne illnesses. Beuchat *et al.* (2004) also confirmed the sporicidal activity of ClO<sub>2</sub> against various bacteria on the surface of raw fruit and vegetables. Positive results and effective reductions were obtained with fungal pathogens on apples, pears and cherries (Roberts & Reymond, 1994; Spotts & Peters, 1980). For these fruits, ClO<sub>2</sub> is applied in dump tanks. ClO<sub>2</sub> also has GRAS status from the Food and Drug Administration in the United States of America (FDA-USDA, 1998; Singh *et al.*, 2002).

Citrofresh is an orange extract containing a bioflavanoid complex and is certified by the National Association for Sustainable Agriculture Australia Limited (NASAA) and also has GRAS status from the United States Food and Drug Administration (Anon, 2006 a; b; FDA-USDA, 1998). It has broad-spectrum anti-microbial activity and when applied as postharvest treatment, it extends the shelf life of fruits, vegetables and flowers (Anon, 2006 b; Van der Westhuizen, 2006).

Sporekill is a patented didecyldimethylammonium chloride formulation that has contact fungicidal activity, leaves no residue and therefore has no withholding period (Anon., 2006 c). Its postharvest use has been registered on apples, pears, avocados, mangoes and citrus as a drench application. Results from Fourie & Halleen (2006) proved Sporekill to be most effective to limit fungal infections in grapevine nurseries where propagation material is

handled. It was applied as repeated soak treatments and did not affect any of the growth parameters (Fourie & Halleen, 2006). An extinct gymnosperm species was also conserved by soaking seeds in Sporekill, which effectively killed *Penicillium crustosum* and ensured germination of the seeds (Whitaker *et al.*, 2004). The potential postharvest use of this compound against *B. cinerea* decay on table grapes has not been evaluated.

Other chemicals such as ethanol and hydrogen peroxide reduced postharvest decay and showed potential as sterilisers but caused quality defects such as dry stems and drying out of internal tissue (Chervin *et al.*, 2003; Gabler *et al.*, 2005; Rij & Forney, 1995; Sholberg *et al.*, 1996). These quality defects are a major constraint hampering implementation of these chemicals as pre-pack treatments against postharvest decay. Therefore, emphasis should also be placed on application methods that would maximise coverage and control, yet minimise detrimental effects on quality.

Auger *et al.* (2005) used an electrostatic typhoon sprayer that applied a contact fungicide (fenhexamid) to grapes on packlines prior to packing. The minute (<50 micron) electrostatically charged droplets ensured very effective coverage of grape bunches without exceeding maximum residue levels (MRLs). Together with an in-package SO<sub>2</sub> generator, this allowed for increased storage periods of Chilean table grapes. Increasing market pressure towards 'zero tolerance' on chemical residues might hamper full-scale implementation of this treatment, especially on fruit destined for sensitive markets. Further work, therefore, needs to be conducted on the prepack treatment of table grapes with chemicals that have the potential to surface sterilise bunches, without leaving any detectable residues and/or are GRAS chemicals.

The objective of this study was to evaluate the surface sterilising ability of selected GRAS chemicals applied as dip or spray treatments on grapevine leaves and bunches.

## 2. MATERIALS AND METHODS

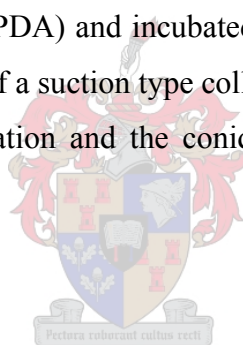
### *Chemical treatments*

The chemicals were initially screened for efficacy on grape leaves, followed by a postharvest trial on Red Globe grapes. Leaves and grapes were treated with Citrofresh (Citrofresh®

International Limited, EC), Sporekill (a patented didecyldimethylammonium chloride; ICA International (Pty) Ltd; SL (120g/L)) and Teldor (fenhexamid, Bayer; SC (500 g/L)) at the recommended dosages (4%, 0.1% and 0.75% (v/v) respectively) and Harvest Wash (ClO<sub>2</sub>; BTC Products and Services; SL (20 g/L)) at 0.001% (Spotts & Peters, 1980). These treatments were applied as dip and spray applications. For the spray treatment, a gravity feed mist spray gun (ITW DEVILBLISS Spray Equipment Products, Glendale Heights, USA) was used and the desired chemical was sprayed to the point of run-off. For the dip treatment, samples were dipped for 30 s in each chemical. Sterile deionised water served as the control. For grapes, an additional treatment included bunches that were dip or sprayed with water and packed with a UVASYS (Grapetek, South Africa) SO<sub>2</sub> generator (0.85g/kg Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>).

### ***Inoculation***

A virulent isolate of *B. cinerea* was grown on apricot halves and conidiophores were transferred to potato dextrose agar (PDA) and incubated at 22°C. Dry spores were collected from 14-day-old cultures by means of a suction type collector and stored at 5°C (1 – 8 weeks). Storage time did not affect germination and the conidia could be used in all experiments (Spotts & Holz, 1996).



### ***Leaves***

Fifth stage leaves were harvested from a greenhouse in Stellenbosch and surface sterilised with 70% ethanol (30 s), 10% NaOCl (2min) and 70% ethanol (30 s) and air-dried to eliminate microorganisms on the leave surface. One day later, leaves were inoculated with 3 mg dry spores in a settling tower (Plexiglass, 3×1×1 m; Salinas *et al.*, 1989) as described by Coertze & Holz (1999). Inoculated leaves were incubated in a moist chamber (Perspex; 0.6×0.3×0.6 m) for 6 hours at high humidity (≥92%) and 22°C. High humidity was created in the chamber by lining the back wall with moist chromatography paper suspended in sterile water. This incubation period would allow germination and adhesion of conidia, but would not be sufficient for penetration (Coertze *et al.*, 2001). After incubation, leaves were dipped- or sprayed with each chemical and allowed to dry for 12 hours. In order to assess infection on a specific leaf, 5 sections (5 mm diameter) were excised with a sterile cork-borer and isolated onto a Petri dish containing water agar supplemented with paraquat (Brink, 2005). For each leaf, this assessment was repeated 9 times. Plates were incubated at 22°C under diurnal light



and monitored for the development of *B. cinerea* after 1 week. Infection was rated as the percentage of leaf segments per Petri dish that were colonised by *B. cinerea*.

### ***Grapes***

Red Globe grapes were harvested at 15°Brix and packed in 4.5 kg cartons and transported to the cold store where it was kept for 5 days before the experiment was conducted. Before experimentation, grapes were surface sterilised with 70% ethanol (30 s), 10% NaOCl (2 min) and 70% ethanol (30 s) and air-dried. Grapes were subjected to lots of handling after harvest that included sterilisation and inoculation. This created wounds, which is an important site of entry for infection (Coertze *et al.*, 2001). For the inoculation of grapes, a spore suspension of  $1 \times 10^5$  spores/ml was prepared in sterile water. Grapes were spray-inoculated with the suspension to the point of run-off and incubated in grape cartons with a non-perforated polyethylene bag for 6 hours at 22°C. This allowed germination and adhesion of most *B. cinerea* conidia on the berry surface, but not penetration (Coertze *et al.*, 2001). After dip- and spray-treatment with the chemicals, grapes were allowed to dry for  $\pm 12$  hours. The spray and dip treatments of a specific chemical, including the treatment with water plus UVASYS SO<sub>2</sub> generator, were subsequently re-packed in a 4.5 kg carton in 2 mm perforated polyethylene liners. Each treatment had five replications. Cartons were stored at  $-0.5^\circ\text{C}$  for 4 weeks and evaluated for decay development caused by *B. cinerea*, which was expressed as a percentage of the weight of treated bunches.

### ***Statistical analyses***

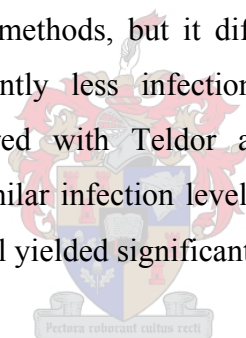
For leaves, the experimental design was a completely randomised design with a  $5 \times 2$  factorial treatment design including 5 chemical treatments and 2 sub-treatments (dip and spray) replicated nine times, and four repeated measurements per leaf. For grapes, the experimental design was a completely randomised split plot design with 6 main plot chemical treatments and 2 sub-plot method treatments (spray and dip), with 5 replications of each combination. Infection data were subjected to analyses of variance using the General Linear Means procedure of the Statistical Analysis System (SAS Institute Inc., 1999). Student's t-Test Significant Difference was calculated at the 5% confidence level to compare treatment means ( $P < 0.05$ ).



### 3. RESULTS

#### *Leaves*

Analysis of variance of infection data on grape leaves indicated a significant 3-factor interaction between experiment, application method and chemical treatment ( $P < 0.0015$ ; Table 1). This interaction was attributed to the infection values of the ClO<sub>2</sub> dip-treatment in experiment 1, which did not differ from the water-treated control (83.9% vs 83.3%), whereas this treatment differed significantly from the control in experiment 2 (43.3% vs 91.1%; results not shown). A significant interaction between application method and chemical treatment ( $P < 0.0001$ ; Table 1) was also observed. This was attributed to significant differences between infection data for dip and spray treatments in the water-treated control and ClO<sub>2</sub>-treatments, but not for the other treatments (Table 2). Teldor and Sporekill were two of the most effective chemicals to eliminate *B. cinerea* spores on the surface of leaves by reducing infection percentages to levels below 1%. There was no significant difference between these two chemicals or their application methods, but it differed significantly from the control. ClO<sub>2</sub> treated leaves had significantly less infection than the control treatments, but significantly higher levels compared with Teldor and Sporekill treated leaves. Dip application of Citrofresh yielded similar infection levels than those of Teldor and Sporekill, but spray-application of this chemical yielded significantly higher levels.



#### *Grapes*

No significant interaction between application method and chemical was observed for the grape infection data (Table 3), nor was a significant effect observed for application method. Means of decay levels obtained following dip- and spray-application of chemicals therefore did not differ significantly (15.6% and 16.4%, respectively; data not shown). Significant differences were, however, observed between chemical treatments. Means of decay levels for chemical treatments are summarised in Table 4. Teldor-, Sporekill and ClO<sub>2</sub>-treated grapes yielded the lowest decay levels, which were significantly lower than those in the water-treated control. Citrofresh and UVASYS were less effective, although these decay levels were significantly lower than the control.

#### 4. DISCUSSION

Chemicals that were evaluated in this study as potential prepack treatments against postharvest decay ranged from fungicides to organic products. Treatments were applied as surface sterilisers and the purpose was to eradicate decay fungi, specifically *B. cinerea*, on the surface of the grapevine leaves or bunches. The fungicide fenhexamid, Teldor, was highly effective but has restricted postharvest use due to residue issues and market acceptance. Moreover, fenhexamid is classified as a medium-risk fungicide in terms of fungicide resistance build-up, and its frequency of use would therefore be restricted (NRA, 2001). This fungicide cannot be used for more than two consecutive sprays and preharvest sprays should be monitored to justify a postharvest application (NRA, 2001). Sporekill, and to a lesser extent ClO<sub>2</sub> and Citrofresh, were as effective as Teldor with the advantage that they are GRAS chemicals (Anon, 2006 a; b; FDA-USDA, 1998; Singh *et al.*, 2002). Results obtained with Sporekill were consistent on both leaves and grapes but this was not experienced with ClO<sub>2</sub> and Citrofresh. The efficacy of ClO<sub>2</sub> was higher on grapes than on leaves, while the opposite was observed with Citrofresh. This might be ascribed to the difference in epidermal tissue of leaves and grapes. ClO<sub>2</sub> is a chemical that is commonly used to control fungal spores in dump tanks in packhouses and is therefore formulated to adhere better to this type of tissue. Citrofresh, however, can be used during different stages, but protection last only for short-term storage (Anon, 2006 a). Manual monitoring of the chemical concentration throughout the dipping/spraying process is essential, as it tend to decrease (Prusky *et al.*, 2001; Roberts & Reymond, 1994). A highly reactive chemical such as ClO<sub>2</sub> quickly evaporates from the surface and in both these cases plant tissue were expose to infection.

The sterilising effect of chemicals is logistically difficult to quantify on grapes. Therefore, preliminary screening of compounds on leaves can be considered as an ideal screening method for potential postharvest treatments, despite of the inconsistency observed with ClO<sub>2</sub> and Citrofresh. Naturally, promising compounds should also be evaluated in semi-commercial packing trials and compared with standard practices. In this study, the standard practice of in-carton SO<sub>2</sub> fumigation proved to be less effective than the pre-pack sterilising treatments with Teldor, Sporekill and ClO<sub>2</sub>. This finding was not expected, and was attributed to the extended incubation period as a result of the drying-off period (up to 18 hours) following dip and spray treatment, in this case with sterile water. This extended incubation period provided sufficient time for the fungus to germinate and infect the berry tissue. This observation confirms that SO<sub>2</sub> fumigation generally provides a surface sterilising

effect and is not effective in eradicating established infections (Mustonen, 1992). Singh *et al.* (2002) also experienced difficulty in eradicating microorganisms that settled in tissue with sterilising treatments.

For surface-sterilising treatments, optimal coverage of fruit surfaces is an essential requirement for efficacy (Crisosto *et al.*, 2002 a). Postharvest *B. cinerea* is associated with rachises, pedicels and berry bases (Holz *et al.*, 2003) and insufficient coverage during application would leave these tissues unprotected against infection. Sequential dipping ensured better coverage with highly reactive chemicals (Singh *et al.*, 2002). However, liquid dip-treatments have negative effects on quality and storage potential (Chervin *et al.*, 2003; Zoffoli *et al.*, 1999). These detrimental effects might not be experienced with spray application, although sufficient spray coverage should still be ensured. Better coverage was obtained with charged droplets and electrostatic sprays (Law & Cooper, 2001; Auger *et al.*, 2005).

In the present study, spray- and dip-application methods did not differ on grapes. Charged droplets with electrostatic sprays might reduce the drying time of fruit, which will be an important logistical requirement in commercial packhouses. A spraying method is conflicting with current commercial packing regimes in South Africa, but postharvest sprays (Typhoon) are successfully implemented on bananas and grapes in other countries (USA and Chile, respectively) (Law & Cooper, 2001; Auger *et al.*, 2005). Installation of spraying machines in pre-cooling rooms might eliminate the problems with long drying periods and logistical problems in the packhouse. The combination of natural/organic antimicrobial agents might serve as an option to satisfy consumers and ensure prolonged protection against decay caused by *B. cinerea*. A detailed study is anticipated to quantify the effects on the fungus, grape quality and the organoleptic properties of the grape.

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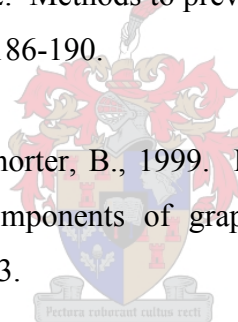
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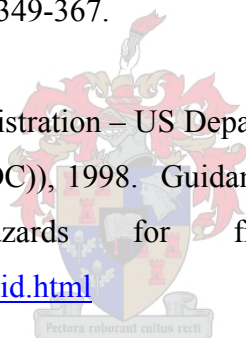
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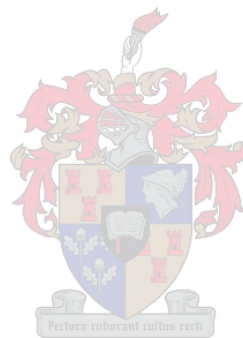


Table 1.

ANOVA of the infection data of table grape leaves treated with fungicides as spray and dip application methods to act as steriliser to control *Botrytis cinerea*.

	<b>Df</b>	<b>SS</b>	<b>MS</b>	<b>F Value</b>	<b>P Value</b>
<b>Experiment (Exp)<sup>1</sup></b>	1	5335.556	5335.556	5.28	0.035
<b>Error a</b>	16	15158.889	1010.556		
<b>Chemical<sup>2</sup></b>	4	497018.889	124254.722	159.24	< 0.0001
<b>Method<sup>3</sup></b>	1	41102.222	41102.222	52.67	< 0.0001
<b>Chemical × Method</b>	4	81947.778	20486.944	26.25	< 0.0001
<b>Exp × Chemical</b>	4	12358.889	3089.722	3.96	0.0004
<b>Exp × Method</b>	1	802.222	802.222	1.03	0.3123
<b>Exp × Chemical × Method</b>	4	14425.556	3606.389	4.62	0.0015
<b>Error b</b>	144	112364.444	780.309		
<b>Sample Error</b>	540	143400.000	265.556		
<b>Corrected Total</b>	719	924924.444			

<sup>1</sup>Experiment was repeated once

<sup>2</sup>Chemical refers to the different chemicals namely Citrofresh, ClO<sub>2</sub>, Sporekill, Teldor and water

<sup>3</sup>Method refers to the different methods used for sterilisation, which were spraying or dipping

Table 2.

Means of *Botrytis cinerea* infection percentages on table grape leaves that were inoculated with dry conidia, incubated for 6 hours and subsequently treated with spray and dip applications of different chemicals

Chemical	Application method <sup>1</sup>	
	Dip <sup>2</sup>	Spray <sup>2</sup>
Citrofresh	4.4ef	10.3e
ClO <sub>2</sub>	63.6b	30.8d
Sporekill	0.8f	0.8f
Teldor	0.6f	0.0f
H <sub>2</sub> O	87.2a	39.2c
<b>LSD (0.05)</b>	7.731	

<sup>1</sup>Data were pooled across experiments

<sup>2</sup>Means with the same letter in a column do not differ significantly ( $P < 0.05$ )

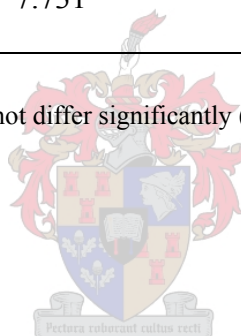


Table 3

ANOVA of the infection data of table grapes treated with fungicides as spray and dip application methods to act as steriliser to control *Botrytis cinerea*.

<b>Chemical<sup>1</sup></b>	5	2163.082	432.616	10.62	< 0.0001
<b>Error a</b>	24	977.672	40.736		
<b>Method<sup>2</sup></b>	1	10.736	10.736	0.190	0.6690
<b>Chemical × Method</b>	5	285.314	57.063	1.000	0.4414
<b>Error b</b>	24	1375.596	57.3164		
<b>Corrected Total</b>	59	4812.399			

<sup>1</sup>Chemical refers to the different chemicals namely Citrofresh, ClO<sub>2</sub>, Sporekill, Teldor and water

<sup>2</sup>Method refers to the different methods used for sterilisation, which were spraying or dipping

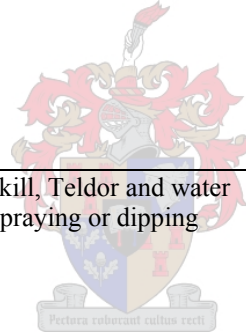


Table 4.

Means of *Botrytis cinerea* infection percentages on Red Globe grapes that were inoculated with conidia suspension, incubated for 6 hours and subsequently treated with spray and dip applications of different chemicals.

<b>Chemical</b>	<b>% Decay<sup>1</sup></b>
<b>Citrofresh</b>	18.2b
<b>ClO<sub>2</sub></b>	12.7bc
<b>Sporekill</b>	11.0c
<b>Teldor</b>	9.0c
<b>H<sub>2</sub>O + UVASYS</b>	18.1b
<b>H<sub>2</sub>O</b>	27.1a
<b>LSD (0.05)</b>	6.243

<sup>1</sup>Means with the same letter do not differ significantly ( $P < 0.05$ )





## GENERAL DISCUSSION AND CONCLUSION

Delivery of fresh produce to an export market currently relies on extensive use of chemicals but food safety and consumer preferences necessitates a move away from postharvest chemical control. Disease pressure and financial implications make it impossible to quickly find and implement alternative solutions. SO<sub>2</sub> provides positive control for decay caused by *Botrytis cinerea* in table grapes, but the margin between control and damage is very narrow and the allergic reactions of some people to this gas make its use undesirable. However, until safer and more acceptable postharvest disease control is available, SO<sub>2</sub> application needs to be optimised.

The epidermal structure of different table grape cultivars is the factor that determines either sensitivity to SO<sub>2</sub> gas or to the *Botrytis cinerea* fungus. A high SO<sub>2</sub> concentration during initial fumigation is an important surface steriliser for table grapes and packing with SO<sub>2</sub> generators provides the continuous sterilising effect during storage. High SO<sub>2</sub> concentrations in water form sulphurous acid that injures the waxy cuticle and expose underlying tissue. In this study, Red Globe is identified as one of the most sensitive cultivars to SO<sub>2</sub>. It absorbs most of the gas through the berry, leaving the stems drier after the storage period. With red cultivars the berry colour is bleached while hairline splits appear on the berry of white cultivars. A split exposes underlying tissue that is high in sugar content and provides optimum growing conditions for this fungus. In this study, higher SO<sub>2</sub> concentrations obtained with the use of Dosigas, resulted in lower disease incidence but also aggravated SO<sub>2</sub> damage. The use of SO<sub>2</sub> fumigation on Red Globe and Thompson Seedless reduced decay levels but this reduction was not of commercial value as markets have a 0.5% disease tolerance level on *B. cinerea*.

In this study it was experienced that the Dosigas applicator damaged the berries in a packed carton where space is limited. Cartons used for South African packaging of table grapes are not equipped with a centre space to insert the Dosigas applicator. This causes an uneven distribution of SO<sub>2</sub> gas in the carton. Modified cartons for easy insertion of the Dosigas applicator and bunches that are not packed tightly will reduce the problems of damage during application. Polyethylene liners stabilise emission of SO<sub>2</sub> gas from the generators in the carton and create a high relative humidity environment. The Dosigas applicator causes a large perforation in the liner and with hasty removal after fumigation, gas escapes and causes

discomfort to the person operating the device. SO<sub>2</sub> gas that was lost through the natural perforations of the polyethylene liner was insignificant. The advantages of high humidity in non perforated liners were evident in stem condition but it did not aggravate decay as was expected. Perforated liners combined with SO<sub>2</sub> fumigation and forced air cooling contributed to the loss of SO<sub>2</sub> in the immediate environment of the grapes. It is important to quantify the amount of gas that is absorbed by packaging and the berries and also lost through packaging. As such, the minimum SO<sub>2</sub> concentration must be quantified for the different packaging combinations in South Africa.

The formation of sulphurous acid in the presence of water and high disease pressure makes packing grapes after rain a high-risk activity with huge financial implications. High initial fumigation concentrations sterilise the berry surface but with high disease pressure latent infections need to be controlled with continuous sterilisation through a SO<sub>2</sub> generator. The initial fumigation concentration and type of SO<sub>2</sub> generator should be adjusted according to disease pressure prior to harvest and the type of liner that will be used. Further study is proposed to determine the minimum SO<sub>2</sub> concentration that kills *B. cinerea* spores to disinfect (rather than sterilise) the berry surface. Consumer focus is on food safety and SO<sub>2</sub> fumigation is therefore only a solution for the interim and alternatives with natural/organic products are required.

ClO<sub>2</sub> treatments were applied as prepack surface sterilisers and the purpose was to eradicate decay fungi, specifically *B. cinerea* on the surface of the fruit before packing with standard packing material. It is, however, difficult to quantify the sterilising effect on grapes that is stored for long periods, as latent spores can cause infections during the storage period. ClO<sub>2</sub> concentrations used in this study were much higher than those that reduced microorganisms on other commodities, which make the implementation of treatment with these concentrations economically unviable. Optimal coverage of the fruit surface is an essential requirement for an effective steriliser and ClO<sub>2</sub> has great penetration ability.

ClO<sub>2</sub> acts by oxidation and exposure to gas damages the plant tissue and predisposes the tissue of the berry and stems to latent infections of *B. cinerea* or damage by SO<sub>2</sub>. Very low decay levels on Red Globe and Dauphine and the absence of infection that spreads from one berry to another indicates that prepack ClO<sub>2</sub> sterilisers lowered the viability of fungal spores. Neither the prepack ClO<sub>2</sub> fumigation nor the SO<sub>2</sub> were capable of reducing decay levels on

grapes harvested during these conditions. Prepack fumigation with  $\text{ClO}_2$  cause no further reaction of  $\text{SO}_2$  gas with stems, with drier stems on fumigated grapes and the presence of fungal growth. The organoleptic properties could also be negatively influenced by the presents of crystals on the berry surface and this is an unacceptable attribute of food quality.

Fumigation with  $\text{ClO}_2$  at pulp temperatures higher than  $30^\circ\text{C}$ , were also highly effective in reducing decay and these temperatures are not favourable for development of *B. cinerea*. Harvesting at high pulp temperatures affects postharvest grape quality negatively but fumigation with  $\text{ClO}_2$  before packing diminishes these negative effects. However, excessive release of  $\text{SO}_2$  was enhanced by packing warm ( $> 30^\circ\text{C}$ ) grapes in non-perforated liners. Postharvest and prepack fumigation treatments are contradictory to standard practices in South Africa and the concentrations used in this study cause slight discomfort to humans. It is important to establish which of the two variables, concentration or exposure time, is the critical factor that determines sterilisation as this will determine the economic viability to implement it as a control measure.

The fungicide fenhexamid, Teldor, was highly effective in reducing decay but has restricted postharvest use due to residue issues and market acceptance. Sporekill, and to a lesser extent  $\text{ClO}_2$  (liquid) and Citrofresh, applied as spray and dip treatments were as effective as Teldor with the advantage that they are Generally Regarded as Safe (GRAS) chemicals. Sterilising treatments with Citrofresh do not last for long storage periods and an additional or continuous steriliser is necessary for protection during the storage period. The standard practice of a  $\text{SO}_2$  generator proved to be less effective than the pre-pack sterilising treatments with Teldor, Sporekill and  $\text{ClO}_2$ , which was attributed to an extended incubation period as a result of the drying-off period.

$\text{ClO}_2$  is a highly reactive chemical and monitoring of the chemical concentration throughout the dipping/spraying process is essential. The sterilising effect of chemicals is logistically difficult to quantify on grapes. Spray- and dip-application methods did not differ on grapes but dip-treatments have negative effects on quality and storage potential that can be eliminated by spray application if sufficient spray coverage is ensured. A spraying method is conflicting with commercial packing practices in South Africa but installation of spraying machines in pre-cooling rooms might eliminate the problems with long drying periods and logistical problems in the packhouse.

## APPENDICES

Appendix I – Complete data of Article I.....	124
Table 1 Effect of different in-carton SO <sub>2</sub> concentrations on the postharvest quality of Bonheur table grapes.....	124
Table 2 Effect of different in-carton SO <sub>2</sub> concentrations on the postharvest quality of Sunred Seedless table grapes.....	125
Table 3 Effect of different in-carton SO <sub>2</sub> concentrations on the TSS and acidity of Bonheur, Sunred Seedless, Red Globe and Thompson Seedless table grapes.....	126
Table 4 Effect of different in-carton SO <sub>2</sub> concentrations on the TSS and acidity of Red Globe and Thompson Seedless table grapes.....	127
Table 5 Effect of different in-carton SO <sub>2</sub> concentrations on the stem condition and moisture of Bonheur, Sunred Seedless, Red Globe and Thompson Seedless table grapes.....	128
Table 6 Effect of different in-carton SO <sub>2</sub> concentrations on the stem condition and moisture of Red Globe and Thompson Seedless table grapes.....	129
Table 7 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on SO <sub>2</sub> damage and decay development on Bonheur table grapes packed in non-, micro and 2 mm perforated liners.....	130
Table 8 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on shatter and split on Bonheur table grapes packed in non-, micro and 2 mm perforated liners.....	131
Table 9 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on decay development of Sunred Seedless table grapes packed in non-, micro and 2 mm perforated liners.....	132
Table 10 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on the postharvest quality of Sunred Seedless table grapes packed in non-, micro and 2 mm perforated liners.....	133
Table 11 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on TSS and acidity of Red Globe, Thompson- and Sunred Seedless table grapes packed in non-, micro- and 2 mm perforated liners.....	134
Table 12 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on TSS and acidity of Bonheur table grapes packed in non-, micro and 2 mm perforated liners.....	136
Table 13 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on TSS and acidity of Red Globe and Thompson Seedless table grapes packed in non-, micro- and 2 mm perforated liners.....	137
Table 14 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on stem condition of Bonheur, Red Globe, Thompson Seedless and Sunred Seedless table grapes packed in non-, micro and 2 mm perforated liners.....	138
Table 15 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on moisture of Bonheur and Red Globe in 2003 and Red Globe in 2004 packed in non-, micro and 2mm perforated liners.....	139
Table 16 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on moisture of Sunred- and Thompson Seedless table grapes packed in non-, micro and 2 mm perforated liners.....	140
Table 17 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on stem condition of Red Globe table grapes packed in non-, micro and 2 mm perforated liners.....	141

Table 18 The effect of a 6.0 mL/kg in-carton SO <sub>2</sub> fumigation on stem condition and moisture of Thompson Seedless table grapes packed in non-, micro and 2 mm perforated liners .....	142
Table 19 Effect of different in-carton SO <sub>2</sub> concentrations on the TSS and acidity of Barlinka and Dauphine table grapes harvested during adverse weather conditions .....	143
Table 20 Effect of different in-carton SO <sub>2</sub> concentrations on the TSS and acidity of Barlinka and Dauphine table grapes harvested during adverse weather conditions .....	144
Table 21 Effect of different in-carton SO <sub>2</sub> concentrations on the stem condition and moisture of Barlinka and Dauphine table grapes harvested during adverse weather conditions .....	145
Table 22 Effect of different in-carton SO <sub>2</sub> concentrations on the stem condition and moisture of Barlinka and Dauphine table grapes harvested during adverse weather conditions .....	146

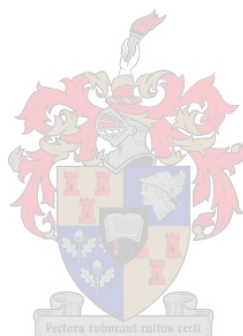
Fig.1 Weather conditions prior and after packing during the 2003 and 2004 season

1469

Appendix II – Complete data for Article II.....	15050
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Table 1 Effect of ClO <sub>2</sub> concentration and exposure time on the postharvest quality of Bonheur table grapes .....	150
Table 2 Effect of ClO <sub>2</sub> concentration and exposure time on moisture of Red Globe table grapes .....	151
Table 3 Effect of ClO <sub>2</sub> concentration and exposure time on the stem condition of Red Globe table grapes .....	152
Table 4 Effect of ClO <sub>2</sub> concentration and exposure time on the stem condition and moisture of Dauphine table grapes.....	153
Table 5 Effect of ClO <sub>2</sub> concentration and exposure time on the stem condition and moisture of Bonheur table grapes.....	154
Table 6 Effect of ClO <sub>2</sub> concentration and exposure time on the TSS and acidity of Red Globe table grapes .....	155
Table 7 Effect of ClO <sub>2</sub> concentration and exposure time on the TSS and acidity of Bonheur table grapes.....	156
Table 8 Effect of ClO <sub>2</sub> concentration and exposure time on TSS of Dauphine table grapes .....	157
Table 9 Effect of ClO <sub>2</sub> concentration and exposure time on acidity of Dauphine table grapes .....	158
Table 10 Effect of pulp temperature on the postharvest quality of Dauphine table grapes fumigated with 50 µg/mL ClO <sub>2</sub> for 30 minutes .....	159
Table 11 Effect of pulp temperature on the postharvest quality of Red Globe table grapes fumigated with 50 µg/mL ClO <sub>2</sub> for 30 minutes .....	160
Table 12 Effect of pulp temperature on the postharvest quality of Bonheur table grapes fumigated with 50 µg/mL ClO <sub>2</sub> for 30 minutes .....	161

Table 13 Effect of pulp temperature on the stem condition of Bonheur, Dauphine and Red Globe table grapes fumigated with 50 µg/mL ClO <sub>2</sub> for 30 minutes .....	16262
Table 14 Effect of pulp temperature on the moisture of Bonheur table grapes fumigated with 50 µg/mL ClO <sub>2</sub> for 30 minutes .....	163
Table 15 Effect of pulp temperature on the TSS and acidity of Bonheur, Dauphine and Red Globe table grapes fumigated with 50 µg/mL ClO <sub>2</sub> for 30 minutes .....	164
Table 16 Effect of ClO <sub>2</sub> concentration and exposure time on stem condition and moisture of Barlinka table grapes .....	165
Table 17 Effect of ClO <sub>2</sub> concentration and exposure time on stem condition and moisture of Dauphine table grapes .....	166
Table 18 Effect of ClO <sub>2</sub> concentration and exposure time on TSS and acidity of Barlinka table grapes.....	167
Table 19 Effect of ClO <sub>2</sub> concentration and exposure time on acidity of Dauphine table grapes .....	168
Table 20 Effect of ClO <sub>2</sub> concentration and exposure time on TSS of Dauphine table grapes .....	16969



## Appendix I – Complete data of Article I

Table 1

Effect of different in-carton SO<sub>2</sub> concentrations on the postharvest quality of Bonheur table grapes evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C during 2003.

	%	%	%	% SO <sub>2</sub>
	Decay	Shatter	Split	Damage
<b>No SO<sub>2</sub></b>	19.7a <sup>1</sup>	3.6	3.6b	1.5b
<b>4.5 mL/kg</b>	2.9b	1.7	6.0a	3.2a
<b>6.0 mL/kg</b>	2.9b	2.1	7.8a	2.7a
<b>7.5 mL/kg</b>	1.5b	2.8	6.2a	2.4ab
<b>LSD (0.05)</b>	9.690		2.267	1.067
<b>P-Value</b>	0.001	0.079	0.007	0.015

<sup>1</sup> Means with the same in a column letter do not differ significantly (P < 0.05)

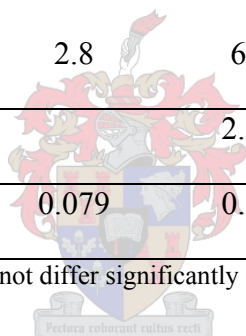


Table 2

Effect of different in-carton SO<sub>2</sub> concentrations on the postharvest quality of Sunred Seedless table grapes evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 season.

	%	%	%	% SO <sub>2</sub>
	Decay	Shatter	Split	Damage
<b>No SO<sub>2</sub></b>	2.2	4.8	1.2	8.8
<b>4.5 mL/kg</b>	0.8	3.3	1.1	10.2
<b>6.0 mL/kg</b>	0.7	3.7	0.7	8.1
<b>7.5 mL/kg</b>	1.0	3.1	0.6	7.9
<b>P-Value</b>	0.218	0.403	0.249	0.611

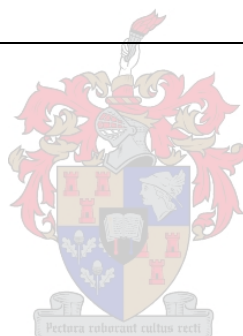




Table 3

Effect of different in-carton SO<sub>2</sub> concentrations on the TSS and acidity of Bonheur, Sunred Seedless, Red Globe and Thompson Seedless table grapes evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C during 2003.

	<b>Bonheur</b>	<b>Sunred Seedless</b>	<b>Red Globe</b>	<b>Thompson Seedless</b>
<b>% TSS</b>				
<b>No SO<sub>2</sub></b>	17.2	20.8	17.8	15.3
<b>4.5 mL/kg</b>	16.7	21.1	18.0	15.7
<b>6.0 mL/kg</b>	17.4	21.0	18.4	15.7
<b>7.5 mL/kg</b>	17.4	21.3	18.1	16.2
<b>P-Value</b>	0.407	0.509	0.589	0.455
<b>%Acid</b>				
<b>No SO<sub>2</sub></b>	0.56	0.65	0.48a <sup>1</sup>	0.63a
<b>4.5 mL/kg</b>	0.55	0.66	0.48a	0.62a
<b>6.0 mL/kg</b>	0.54	0.65	0.48a	0.60b
<b>7.5 mL/kg</b>	0.57	0.65	0.45b	0.59b
<b>LSD (0.05)</b>			0.018	0.022
<b>P-Value</b>	0.225	0.744	0.001	0.001

<sup>1</sup> Means with the same letter in a column are not significantly different (P < 0.05)

Table 4

Effect of different concentrations of in-carton SO<sub>2</sub> fumigation on the TSS and acidity of Red Globe and Thompson Seedless table grapes evaluated after a storage period of six weeks at – 0.5°C and shelf life of one week at 15°C in 2003 and 2004.

	Red Globe		Thompson Seedless	
	% TSS	%Acid	% TSS	%Acid
<b>2003 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation<sup>2</sup></b>	15.3	0.38b <sup>1</sup>	18.6a	0.74a
<b>4.5 mL/kg</b>	14.7	0.41ab	17.4b	0.68ab
<b>6.0 mL/kg</b>	14.8	0.42a	18.2ab	0.67b
<b>7.5 mL/kg</b>	14.8	0.42a	17.7b	0.63b
		0.038	0.900	0.057
<b>P-Value</b>	0.201	0.039	0.029	0.003
<b>2004 season</b>				
<b>No in-carton SO<sub>2</sub> fumigation<sup>2</sup></b>	17.8	0.48a	15.3	0.63a
<b>4.5 mL/kg</b>	18.0	0.48a	15.7	0.62a
<b>6.0 mL/kg</b>	18.4	0.48a	15.7	0.60b
<b>7.5 mL/kg</b>	18.1	0.45b	16.2	0.59b
		0.018		0.022
<b>P-Value</b>	0.589	0.001	0.455	0.001

<sup>1</sup> Means with the same letter in a column do not differ significantly (P < 0.05)

<sup>2</sup>No in-carton SO<sub>2</sub> fumigation treatments served as the control and were packed with a SO<sub>2</sub> generator pad

Table 5

Effect of different in-carton SO<sub>2</sub> concentrations on the stem condition and moisture of Bonheur, Sunred Seedless, Red Globe and Thompson Seedless table grapes evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C during 2003.

<b>Stem Condition<sup>1</sup></b>				
	<b>Bonheur</b>	<b>Sunred Seedless</b>	<b>Red Globe</b>	<b>Thompson Seedless</b>
<b>No SO<sub>2</sub></b>	3.1a <sup>2</sup>	2.4	4.7	1.8b
<b>4.5 mL/kg</b>	2.4b	2.3	4.3	2.2
<b>6.0 mL/kg</b>	1.8c	2.4a	4.6	2.3a
<b>7.5 mL/kg</b>	1.7c	2.9	4.4	2.2a
<b>LSD (0.05)</b>	0.981			0.938
<b>P-Value</b>	< 0.0001	0.070	0.147	0.009
<b>Moisture<sup>3</sup></b>				
<b>No SO<sub>2</sub></b>	1.1b	1.1	1.4	1.3
<b>4.5 mL/kg</b>	1.3b	1.0	1.5	1.3
<b>6.0 mL/kg</b>	2.0a	1.0	1.4	1.4
<b>7.5 mL/kg</b>	1.8a	1.0	1.3	1.7
<b>LSD (0.05)</b>	0.758			
<b>P-Value</b>	< 0.0001	0.344	0.601	0.104

<sup>1</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

<sup>2</sup> Means with the same letter in a column do not differ significantly (P < 0.05)

<sup>3</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

Table 6

Effect of different in-carton SO<sub>2</sub> concentrations on the stem condition and moisture of Red Globe and Thompson Seedless table grapes evaluated after a storage period of six weeks at – 0.5°C and shelf life of one week at 15°C during 2004.

	Red Globe		Thompson Seedless	
	Stem condition <sup>1</sup>	Moisture <sup>2</sup>	Stem condition <sup>1</sup>	Moisture <sup>2</sup>
<b>No SO<sub>2</sub></b>	2.3	2.0	1.6b <sup>3</sup>	1.8c
<b>4.5 mL/kg</b>	2.6	2.7	2.5a	3.2a
<b>6.0 mL/kg</b>	2.5	2.7	2.6a	2.3b
<b>7.5 mL/kg</b>	2.3	2.3	2.9a	2.3b
<b>LSD (0.05)</b>			0.559	0.473
<b>P-Value</b>	0.845	0.157	< 0.0001	< 0.0001

<sup>1</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

<sup>2</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

<sup>3</sup> Means with the same letter in a column do not differ significantly (P < 0.05)

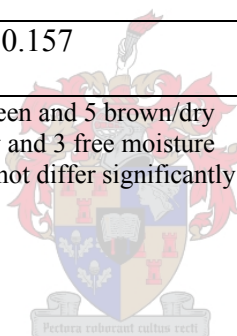


Table 7

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on SO<sub>2</sub> damage and decay development on Bonheur table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during 2003.

	% Decay		% SO <sub>2</sub> Damage	
	No SO <sub>2</sub> fumigation	SO <sub>2</sub> fumigation	No SO <sub>2</sub> Fumigation	SO <sub>2</sub> fumigation
<b>Non perforated</b>	20.6b	5.6c	3.1bc	4.9ab
<b>Micro perforated</b>	46.6a	7.5c	3.3bc	6.8a
<b>2 mm perforated</b>	49.8a	7.2c	4.5bc	2.4c
<b>LSD (0.05)</b>	12.243		2.196	
<b>P-value (liner type)</b>	0.0006		0.125	
<b>P-value (SO<sub>2</sub> fumigation)</b>	<0.0001		0.113	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.005		0.002	

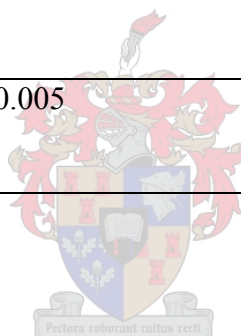


Table 8

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on shatter and split on Bonheur table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 season.

	<b>%Shatter</b>	<b>%Split</b>
<b>Non perforated</b>	1.7	6.2
<b>Micro perforated</b>	2.1	5.7
<b>2 mm perforated</b>	2.1	6.5
<b>LSD (0.05)</b>	1.533	7.390
<b>P-Value (liner type)</b>	0.893	0.975
<b>No SO<sub>2</sub> fumigation</b>	1.5	3.4
<b>SO<sub>2</sub> fumigation</b>	2.4	8.7
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.120	0.082
<b>P-Value</b>	0.428	0.443
<b>(Liner type × SO<sub>2</sub> fumigation)</b>		

Table 9

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on decay development of Sunred Seedless table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during 2003.

	% Decay	
	No SO <sub>2</sub> fumigation	SO <sub>2</sub> fumigation
<b>Non perforated</b>	0.4b	0.3b
<b>Micro perforated</b>	0.5b	0.2b
<b>2 mm perforated</b>	1.5a	0.5b
<b>LSD (0.05)</b>	0.401	
<b>P-value (liner type)</b>	<0.0001	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.0002	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.004	



Table 10

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on the postharvest quality of Sunred Seedless table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during 2003.

	%	%	% SO <sub>2</sub>
	<b>Shatter</b>	<b>Split</b>	<b>Damage</b>
<b>Non perforated</b> <sup>1</sup>	2.8a <sup>3</sup>	0.8	23.0a
<b>2 mm perforated</b>	2.4ab	0.9	12.2b
<b>Micro perforated</b>	2.0b	1.1	15.5b
<b>LSD (0.05)</b>	0.576		3.982
<b>P-Value (liner type)</b>	0.030	0.200	<0.0001
<b>No SO<sub>2</sub> fumigation</b> <sup>2</sup>	2.3	0.9	15.2
<b>SO<sub>2</sub> fumigation</b>	2.5	1.0	18.5
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.373	0.352	0.048
<b>P-Value</b>	0.118	0.723	0.056
<b>(Liner type × SO<sub>2</sub> fumigation)</b>			

<sup>1</sup> Data pooled across in-carton fumigation

<sup>2</sup> Data pooled across type of liner

<sup>3</sup> Means with the same letter in a column do not differ significantly (P < 0.05)



Table 11

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on TSS and acidity of Red Globe, Thompson- and Sunred Seedless table grapes packed in non-, micro- and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 season.

	Red Globe		Thompson		Sunred Seedless	
	% TSS	% Acid	% TSS	% Acid	% TSS	% Acid
<b>Non perforated<sup>1</sup></b>	17.5	0.45b <sup>3</sup>	20.4b	0.65a	15.8	0.60
<b>Micro perforated</b>	17.4	0.46ab	20.7ab	0.64ab	15.0	0.62
<b>2 mm perforated</b>	17.5	0.47a	21.1a	0.63b	15.2	3.01
<b>LSD (0.05)</b>		0.014	0.518	0.013		
<b>P-Value (liner type)</b>	0.948	0.036	0.030	0.041	0.185	0.369
<b>No SO<sub>2</sub> fumigation<sup>2</sup></b>	18.4a	0.46	20.7	0.64	15.7	0.61
<b>SO<sub>2</sub> fumigation</b>	16.5b	0.46	20.7	0.64	15.0	2.20
	0.495					

<b>P-Value (SO<sub>2</sub> fumigation)</b>	< 0.0001	0.738	0.990	0.210	0.094	0.322
<b>P-Value (Liner type × SO<sub>2</sub> fumigation)</b>	0.675	0.126	0.743	0.156	0.208	0.368

<sup>1</sup> Data pooled across in-carton fumigation

<sup>2</sup> Data pooled across type of liner

<sup>3</sup> Means with the same letter in a column do not differ significantly ( $P < 0.05$ )

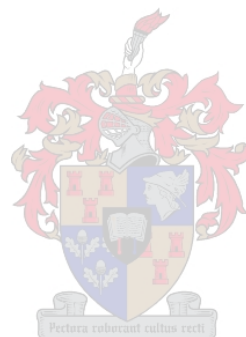


Table 12

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on TSS and acidity of Bonheur table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 season.

% TSS		
	No SO <sub>2</sub> fumigation	SO <sub>2</sub> fumigation
<b>Non perforated</b>	16.5ab	16.4ab
<b>Micro perforated</b>	16.4ab	15.9b
<b>2 mm perforated</b>	15.9b	16.8a
<b>LSD (0.05)</b>	0.814	
<b>P-value (liner type)</b>	0.397	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.617	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.037	
%Acid		
<b>Non perforated</b>	0.60a	0.59ab
<b>Micro perforated</b>	0.58ab	0.56b
<b>2 mm perforated</b>	0.58ab	0.60a
<b>LSD (0.05)</b>	0.023	
<b>P-value (liner type)</b>	0.038	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.375	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.046	

Table 13

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on TSS and acidity of Red Globe and Thompson Seedless table grapes packed in non-, micro- and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2004 season.

	Red Globe		Thompson Seedless	
	% TSS	%Acid	% TSS	%Acid
<b>Non perforated</b> <sup>1</sup>	17.5	0.45a <sup>3</sup>	17.4	0.73
<b>Micro perforated</b>	17.5a	0.47a	17.2	0.74
<b>2 mm perforated</b>	17.2	0.47a	17.1	0.74
<b>LSD (0.05)</b>		0.020		
<b>P-Value (liner type)</b>	0.076	0.202	0.831	0.877
<b>No SO<sub>2</sub> fumigation</b> <sup>2</sup>	17.4	0.46	17.3	0.73
<b>SO<sub>2</sub> fumigation</b>	17.4	0.46	17.1	0.74
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.890	0.589	0.583	0.856
<b>P-Value (Liner type × SO<sub>2</sub> fumigation)</b>	0.914	0.199	0.599	0.592

<sup>1</sup> Data pooled across in-carton fumigation

<sup>2</sup> Data pooled across type of liner

<sup>3</sup> Means with the same letter do not differ significantly (P < 0.05)

Table 14

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on stem condition of Bonheur, Red Globe, Thompson Seedless and Sunred Seedless table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 season.

<b>Stem condition<sup>1</sup></b>				
	<b>Bonheur</b>	<b>Red Globe</b>	<b>Sunred</b>	<b>Thompson</b>
			<b>Seedless</b>	<b>Seedless</b>
<b>Non perforated<sup>3</sup></b>	2.3c <sup>2</sup>	3.2c	1.2c	2.0
<b>Micro perforated</b>	2.8b	3.5b	1.8b	2.1
<b>2 mm perforated</b>	3.2a	3.9a	2.4a	2.0
<b>LSD (0.05)</b>	1.020	0.793	0.993	
<b>P-Value (liner type)</b>	< 0.0001	< 0.0001	< 0.0001	0.985
<b>No SO<sub>2</sub> fumigation<sup>4</sup></b>	2.8	3.5	1.7	1.8b
<b>SO<sub>2</sub> fumigation</b>	2.7	3.5	1.9	2.2a
<b>LSD (0.05)</b>				1.042
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.324	0.798	0.092	0.015
<b>P-Value (Liner type × SO<sub>2</sub> fumigation)</b>	0.152	0.175	0.265	0.622

<sup>1</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

<sup>2</sup> Means with the same letter do not differ significantly (P < 0.05)

<sup>3</sup> Data pooled across in-carton fumigation

<sup>4</sup> Data pooled across type of liner

Table 15

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on moisture of Bonheur and Red Globe in 2003 and Red Globe in 2004 packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°.

<b>Moisture in carton<sup>1</sup></b>			
	<b>2003</b>		<b>2004</b>
	<b>Bonheur</b>	<b>Red Globe</b>	<b>Red Globe</b>
<b>Non perforated<sup>2</sup></b>	1.0a	1.6a	1.6
<b>Micro perforated</b>	1.0a	1.3b	1.4
<b>2 mm perforated</b>	1.0a	1.2b	1.4
<b>LSD (0.05)</b>		1.119	
<b>P-Value (liner type)</b>	0.880	< 0.0001	0.759
<b>No SO<sub>2</sub> fumigation<sup>3</sup></b>	1.0	1.3	1.4
<b>SO<sub>2</sub> fumigation</b>	1.0	1.4	1.5
<b>P-Value (SO<sub>2</sub> fumigation)</b>	0.702	0.278	0.370
<b>P-Value (Liner type × SO<sub>2</sub> fumigation)</b>	0.8418	0.069	0.098

<sup>1</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

<sup>2</sup> Data pooled across in-carton fumigation

<sup>3</sup> Data pooled across type of liner

<sup>4</sup> Means with the same letter do not differ significantly ( $P < 0.05$ )

Table 16

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on moisture of Sunred- and Thompson Seedless table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during the 2003 season.

<b>Moisture in carton<sup>1</sup></b>		
<b>Sunred Seedless</b>		
	<b>No SO<sub>2</sub> fumigation</b>	<b>SO<sub>2</sub> fumigation</b>
<b>Non perforated</b>	1.3b	1.5a
<b>Micro perforated</b>	1.0c	1.0c
<b>2 mm perforated</b>	1.0c	1.0c
<b>LSD (0.05)</b>	0.742	
<b>P-value (liner type)</b>	< 0.0001	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.023	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.007	
<b>Thompson Seedless</b>		
<b>Non perforated</b>	1.9ab	1.6bc
<b>Micro perforated</b>	2.0a	1.2d
<b>2 mm perforated</b>	1.4cd	1.5c
<b>LSD (0.05)</b>	1.888	
<b>P-value (liner type)</b>	0.059	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.0003	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.0008	

<sup>1</sup>Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

Table 17

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on stem condition of Red Globe table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15° during 2004.

<b>Stem condition<sup>1</sup></b>		
	<b>No SO<sub>2</sub> fumigation</b>	<b>SO<sub>2</sub> fumigation</b>
<b>Non perforated</b>	2.2d	2.9cd
<b>Micro perforated</b>	4.1cb	2.5cb
<b>2 mm perforated</b>	3.4a	3.7ab
<b>LSD (0.05)</b>	1.121	
<b>P-value (liner type)</b>	0.001	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.013	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	< 0.0001	

<sup>1</sup>Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

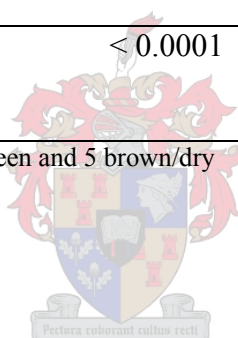




Table 18

The effect of a 6.0 mL/kg in-carton SO<sub>2</sub> fumigation on stem condition and moisture of Thompson Seedless table grapes packed in non-, micro and 2 mm perforated liners and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C during the 2004 season.

<b>Stem condition<sup>1</sup></b>		
	<b>No SO<sub>2</sub> fumigation</b>	<b>SO<sub>2</sub> fumigation</b>
<b>Non perforated</b>	2.3c	2.8bc
<b>Micro perforated</b>	3.7a	3.3b
<b>2 mm perforated</b>	3.3b	4.1a
<b>LSD (0.05)</b>	0.993	
<b>P-value (liner type)</b>	< 0.0001	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.143	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.0008	
<b>Moisture<sup>2</sup></b>		
<b>Non perforated</b>	1.5b	2.0a
<b>Micro perforated</b>	2.0a	2.0a
<b>2 mm perforated</b>	2.0a	2.0a
<b>LSD (0.05)</b>	0.466	
<b>P-value (liner type)</b>	0.002	
<b>P-value (SO<sub>2</sub> fumigation)</b>	0.023	
<b>P-value (Liner type × SO<sub>2</sub> fumigation)</b>	0.014	

<sup>1</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

<sup>2</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

Table 19

Effect of different in-carton SO<sub>2</sub> concentrations on the TSS and acidity of Barlinka and Dauphine table grapes harvested during adverse weather conditions and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C during 2003.

	<b>Barlinka</b>		<b>Dauphine</b>	
	<b>% TSS</b>	<b>%Acid</b>	<b>% TSS</b>	<b>%Acid</b>
<b>No SO<sub>2</sub></b>	16.7	0.60	15.9ab <sup>1</sup>	5.87
<b>4.5 mL/kg</b>	16.8	0.58	16.7a	0.59
<b>6.0 mL/kg</b>	16.6	0.58	16.2ab	0.58
<b>7.5 mL/kg</b>	16.6	0.57	15.4b	0.62
<b>LSD (0.05)</b>			0.959	
<b>P-Value</b>	0.744	0.196	0.046	0.404

<sup>1</sup> Means with the same letter in a column do not differ significantly (P < 0.05)



Table 20

Effect of different in-carton SO<sub>2</sub> concentrations on the TSS and acidity of Barlinka and Dauphine table grapes harvested during adverse weather conditions and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C during 2004.

	<b>Barlinka</b>		<b>Dauphine</b>	
	<b>% TSS</b>	<b>%Acid</b>	<b>% TSS</b>	<b>%Acid</b>
<b>No SO<sub>2</sub></b>	16.1	0.43bc <sup>1</sup>	16.9	0.59
<b>4.5 mL/kg</b>	14.7	0.45a	17.1	0.58
<b>6.0 mL/kg</b>	15.6	0.45ab	16.6	0.57
<b>7.5 mL/kg</b>	15.9b	0.43a	16.5	0.56
<b>LSD (0.05)</b>	1.350	0.021		
<b>P-Value</b>	0.187	0.032	0.584	0.211

<sup>1</sup> Means with the same letter in a column do not differ significantly (P < 0.05)

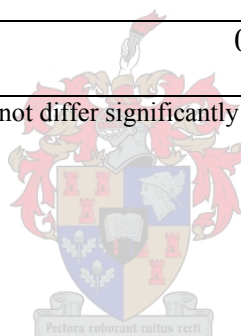


Table 21

Effect of different in-carton SO<sub>2</sub> concentrations on the stem condition and moisture of Barlinka and Dauphine table grapes harvested during adverse weather conditions and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during 2003.

	Barlinka		Dauphine	
	Moisture <sup>1</sup>	Stem Condition <sup>2</sup>	Moisture <sup>1</sup>	Stem Condition <sup>2</sup>
No SO <sub>2</sub>	1.5	2.5b <sup>3</sup>	1.5	1.8
4.5 mL/kg	1.5	2.2b	1.5	2.1
6.0 mL/kg	1.5	2.6b	1.5	2.1
7.5 mL/kg	1.5	3.3a	1.5	1.7
<b>LSD (0.05)</b>		0.939		1.003
<b>P-Value</b>	-	< 0.0001	< 0.0001	0.054

<sup>1</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

<sup>2</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

<sup>3</sup> Means with the same letter in a column do not differ significantly (P < 0.05)

Table 22

Effect of different in-carton SO<sub>2</sub> concentrations on the stem condition and moisture of Barlinka and Dauphine table grapes harvested during adverse weather conditions and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C during 2004.

	Barlinka		Dauphine	
	Moisture <sup>1</sup>	Stem Condition <sup>2</sup>	Moisture <sup>1</sup>	Stem Condition <sup>2</sup>
No SO <sub>2</sub>	1.2b <sup>3</sup>	2.3b	1.3c	2.1
4.5 mL/kg	1.9a	2.9ab	1.8ab	2.5
6.0 mL/kg	1.7ab	3.3a	1.9a	2.5
7.5 mL/kg	1.6b	3.0ab	1.5bc	2.5
<b>LSD (0.05)</b>	0.555	0.737	0.616	
<b>P-Value</b>	0.006	0.026	0.002	0.950

<sup>1</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

<sup>2</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

<sup>3</sup> Means with the same letter in a column do not differ significantly (P < 0.05)

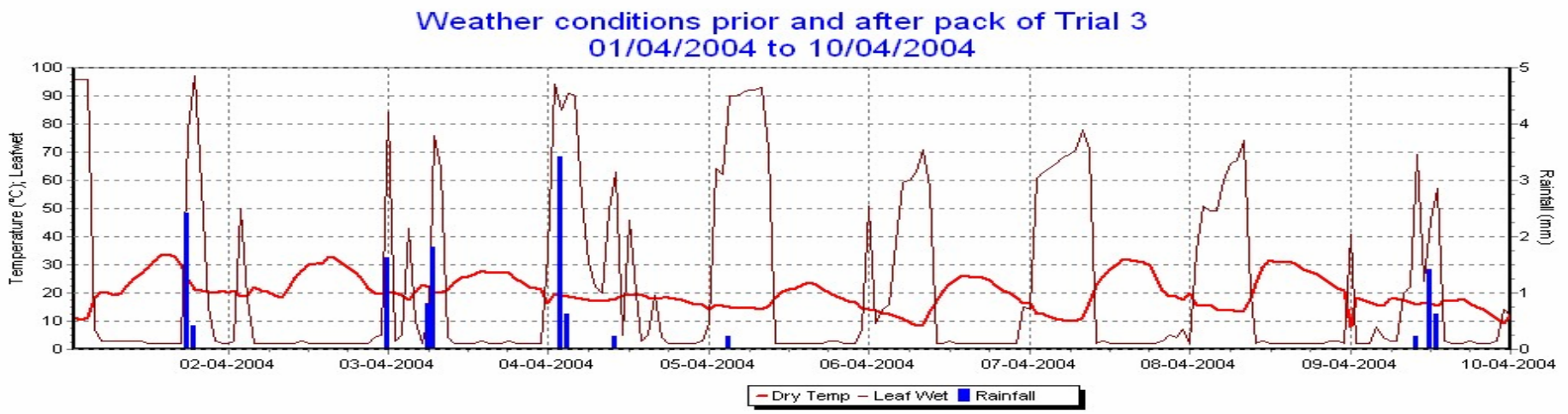
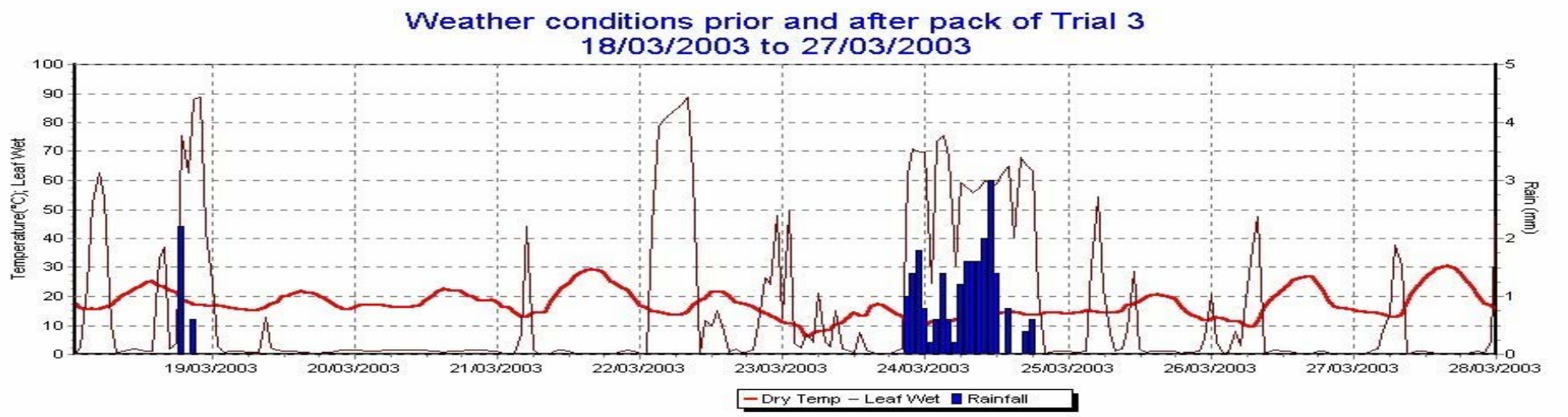


Figure 1. Weather conditions prior and after packing during the 2003 and 2004 season

## Appendix II – Complete data of Article II

Table 1

Effect of ClO<sub>2</sub> concentration and exposure time on the postharvest quality of Bonheur table grapes after six weeks of storage at –0.5°C and a shelf life of one week at 15°C.

	%	% SO <sub>2</sub>	%	%
	Decay	damage	Shatter	Split
<sup>1</sup> Control	1.8	14.4a <sup>3</sup>	0.7b	2.7
25 µg/mL	3.2	7.5bc	3.8a	1.6
50 µg/mL	1.2	10.2b	5.3a	2.0
75 µg/mL	3.2	5.7c	4.7a	1.7
LSD (0.05)		3.731	1.967	
P-Value (concentration)	0.198	0.0004	0.001	0.330
<sup>2</sup> Control	1.8	14.4	0.7	2.7
15 minute	1.9	6.6	4.1	1.4
30 minute	3.1	9.0	5.5	2.0
45 minute	2.6	7.8	4.3	1.8
P-Value (time)	0.550	0.316	0.187	0.453
P-Value	0.752	0.363	0.439	0.176
<b>(Concentration × Time)</b>				

<sup>1</sup> Data pooled across exposure times

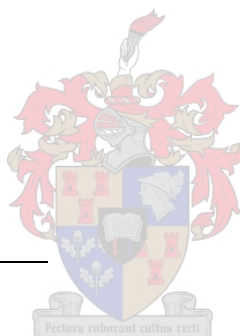
<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>3</sup> Means with the same letter in a column do not differ significantly

Table 2

Effect of ClO<sub>2</sub> concentration and exposure time on moisture of Red Globe table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

<b>Moisture<sup>1</sup></b>	
<sup>2</sup> <b>Control</b>	1.4ab <sup>4</sup>
<b>25 µg/mL</b>	1.5a
<b>50 µg/mL</b>	1.6a
<b>75 µg/mL</b>	1.2b
<b>LSD (0.05)</b>	2.476
<b>P-Value (concentration)</b>	0.002
<sup>3</sup> <b>Control</b>	1.4a
<b>15 minute</b>	1.4a
<b>30 minute</b>	1.4a
<b>45 minute</b>	1.5a
<b>P-Value (time)</b>	0.357
<b>P-Value</b>	0.320



**(Concentration × Time)**

<sup>1</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

<sup>2</sup> Data pooled across exposure times

<sup>3</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>4</sup> Means with the same letter in column do not differ significantly



Table 3

Effect of ClO<sub>2</sub> concentration and exposure time on the stem condition of Red Globe table grapes after storage period of six weeks at -0.5°C and a shelf life of one week at 15°C.

<b>Stem condition<sup>1</sup></b>				
	<b>Control</b>	<b>15 minutes</b>	<b>30 minutes</b>	<b>45 minutes</b>
<b>25 µg/mL</b>	1.8a	2.4b	2.8b	2.2a
<b>50 µg/mL</b>	1.8a	2.2a	2.1a	2.3b
<b>75 µg/mL</b>	1.8a	2.5b	1.9a	1.8a
<b>LSD (0.05)</b>		2.517		
<b>P-Value (concentration)</b>		0.007		
<b>P-Value (exposure time)</b>		0.132		
<b>P-Value (Concentration × Time)</b>		0.006		

<sup>1</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

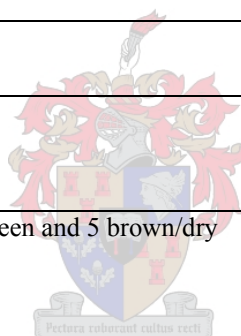


Table 4

Effect of ClO<sub>2</sub> concentration and exposure time on the stem condition and moisture of Dauphine table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

<b>Moisture<sup>1</sup></b>				
	<b>Control</b>	<b>15 minutes</b>	<b>30 minutes</b>	<b>45 minutes</b>
<b>25 µg/mL</b>	2.0a	1.9ab	1.9ab	1.5b
<b>50 µg/mL</b>	2.0a	1.5b	1.5b	1.4b
<b>75 µg/mL</b>	2.0a	1.5b	1.4b	1.4b
<b>LSD (0.05)</b>	1.625			
<b>P-Value (concentration)</b>	< 0.0001			
<b>P-Value (exposure time)</b>	0.0001			
<b>P-Value (Concentration × Time)</b>	0.003			
<b>Stem condition<sup>2</sup></b>				
	<b>Control</b>	<b>15 minutes</b>	<b>30 minutes</b>	<b>45 minutes</b>
<b>25 µg/mL</b>	2.8a	3.0ac	3.0ac	2.8ac
<b>50 µg/mL</b>	2.8a	2.6bc	2.7ac	3.0ac
<b>75 µg/mL</b>	2.8a	2.9a	3.0ac	3.0ac
<b>LSD (0.05)</b>	2.157			
<b>P-Value (concentration)</b>	0.005			
<b>P-Value (exposure time)</b>	0.404			
<b>P-Value (Concentration × Time)</b>	0.0004			

<sup>1</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

<sup>2</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

Table 5

Effect of ClO<sub>2</sub> concentration and exposure time on the stem condition and moisture of Bonheur table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

<b>Moisture<sup>1</sup></b>				
	<b>Control</b>	<b>15 minutes</b>	<b>30 minutes</b>	<b>45 minutes</b>
<b>25 µg/mL</b>	2.0a	1.8b	1.8b	1.6bc
<b>50 µg/mL</b>	2.0a	1.8b	2.0a	1.8b
<b>75 µg/mL</b>	2.0a	1.8b	1.7b	1.9a
<b>LSD (0.05)</b>	2.474			
<b>P-Value (concentration)</b>	0.050			
<b>P-Value (exposure time)</b>	0.413			
<b>P-Value (Concentration × Time)</b>	0.023			
<b>Stem condition<sup>2</sup></b>				
	<b>Control</b>	<b>15 minutes</b>	<b>30 minutes</b>	<b>45 minutes</b>
<b>25 µg/mL</b>	2.5a	3.9bc	3.7bdf	3.3bc
<b>50 µg/mL</b>	2.5a	3.6bf	3.5bf	3.5bf
<b>75 µg/mL</b>	2.5a	3.9be	3.5bf	4.2bd
<b>LSD (0.05)</b>	2.437			
<b>P-Value (concentration)</b>	< 0.0001			
<b>P-Value (exposure time)</b>	0.158			
<b>P-Value (Concentration × Time)</b>	0.017			

<sup>1</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

<sup>2</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

Table 6

Effect of ClO<sub>2</sub> concentration and exposure time on the TSS and acidity of Red Globe table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

% TSS				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	15.2a	14.9a	15.2a	15.3ac
50 µg/mL	15.2a	16.0b	15.2a	15.9bc
75 µg/mL	15.2a	15.9bc	16.2b	15.1a
LSD (0.05)	0.696			
P-Value (concentration)	0.009			
P-Value (exposure time)	0.637			
P-Value (Concentration × Time)	0.003			
%Acid				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	0.52ac	0.50a	0.46acd	0.50a
50 µg/mL	0.52ac	0.49bd	0.46bd	0.50a
75 µg/mL	0.52ac	0.48bd	0.50a	0.50a
LSD (0.05)	0.021			
P-Value (concentration)	0.005			
P-Value (exposure time)	< 0.0001			
P-Value (Concentration × Time)	0.0002			

Table 7

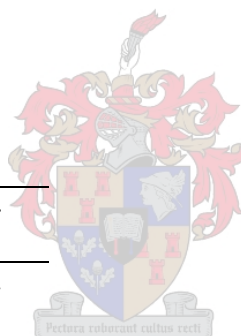
Effect of ClO<sub>2</sub> concentration and exposure time on the TSS and acidity of Bonheur table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

% TSS				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	16.3ad	15.9ad	16.6a	17.2ac
50 µg/mL	16.3ad	16.3ad	17.3bc	17.2bc
75 µg/mL	16.3ad	17.0ac	16.5a	17.0ac
LSD (0.05)	0.809			
P-Value (concentration)	0.145			
P-Value (exposure time)	0.006			
P-Value (Concentration × Time)	0.044			
% Acid				
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	0.54a	0.56a	0.56a	0.58acd
50 µg/mL	0.54a	0.54a	0.54a	0.57bd
75 µg/mL	0.54a	0.56a	0.62bcd	0.57bd
LSD (0.05)	0.026			
P-Value (concentration)	0.001			
P-Value (exposure time)	0.051			
P-Value (Concentration × Time)	0.004			

Table 8

Effect of ClO<sub>2</sub> concentration and exposure time on TSS of Dauphine table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

% TSS	
<sup>1</sup> Control	16.5
25 µg/mL	16.8
50 µg/mL	16.5
75 µg/mL	16.4
<b>P-Value (concentration)</b>	0.528
<sup>2</sup> Control	16.5
15 minute	16.5
30 minute	16.8
45 minute	16.4
<b>P-Value (time)</b>	0.404
<b>P-Value</b>	0.552
<b>(Concentration × Time)</b>	



<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>3</sup> Means with the same letter in a column do not differ significantly

Table 9

Effect of ClO<sub>2</sub> concentration and exposure time on acidity of Dauphine table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

	% Acid			
	Control	15 minutes	30 minutes	45 minutes
<b>25 µg/mL</b>	0.70ade	0.65ae	0.64ae	0.63be
<b>50 µg/mL</b>	0.70ade	0.73acd	0.63be	0.62be
<b>75 µg/mL</b>	0.70ade	0.67be	0.70bde	0.68be
<b>LSD (0.05)</b>				0.057
<b>P-Value (concentration)</b>				0.038
<b>P-Value (exposure time)</b>				0.042
<b>P-Value (Concentration × Time)</b>				0.007

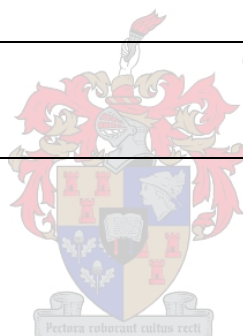


Table 10

Effect of pulp temperature on the postharvest quality of Dauphine table grapes fumigated with 50 µg/mL ClO<sub>2</sub> for 30 minutes and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C.

	<b>%</b>	<b>% SO<sub>2</sub></b>	<b>%</b>	<b>%</b>
	<b>Decay</b>	<b>damage</b>	<b>Shatter</b>	<b>Split</b>
<b>15°C</b>	0.4c	2.8bc	1.3	1.2
<b>20°C</b>	5.4a	1.2d	1.5	1.1
<b>25°C</b>	4.9ab	1.4cd	1.0	1.1
<b>30°C</b>	3.5abc	6.0a	1.3	0.9
<b>35°C</b>	1.9bc	3.6b	2.1	0.6
<b>LSD (0.05)</b>	3.293	1.484		
<b>P-Value</b>	0.021	< 0.0001	0.054	0.288

<sup>1</sup>Means with the same letter in a column do not differ significantly



Table 11

Effect of pulp temperature on the postharvest quality of Red Globe table grapes fumigated with 50  $\mu\text{g/mL}$   $\text{ClO}_2$  for 30 minutes and evaluated after a storage period of six weeks at  $-0.5^\circ\text{C}$  and shelf life of one week at  $15^\circ\text{C}$ .

	%	% $\text{SO}_2$	%	%
	Decay	damage	Shatter	Split
<b>15°C</b>	1.8	52.2	0.8b <sup>1</sup>	0.5b
<b>20°C</b>	2.2	53.7	5.6a	0.0c
<b>25°C</b>	1.5	54.9	0.9b	0.3bc
<b>30°C</b>	8.2	54.2	3.8ab	0.2bc
<b>35°C</b>	2.9	54.4	1.1b	1.0a
<b>LSD (0.05)</b>			3.283	0.490
<b>P-Value</b>	0.062	0.071	0.014	0.002

<sup>1</sup>Means with the same letter in a column do not differ significantly

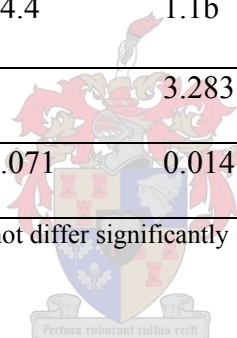


Table 12

Effect of pulp temperature on the postharvest quality of Bonheur table grapes fumigated with 50  $\mu\text{g/mL}$   $\text{ClO}_2$  for 30 minutes and evaluated after a storage period of six weeks at  $-0.5^\circ\text{C}$  and shelf life of one week at  $15^\circ\text{C}$ .

	<b>%</b>	<b>% SO<sub>2</sub></b>	<b>%</b>	<b>%</b>
	<b>Decay</b>	<b>damage</b>	<b>Shatter</b>	<b>Split</b>
<b>15°C</b>	0.9b	8.6	1.8ab	1.5
<b>20°C</b>	0.8b	11.8	2.4a	1.9
<b>25°C</b>	2.8a	11.7	1.7ab	2.4
<b>30°C</b>	1.8ab	8.1	1.0bc	1.2
<b>35°C</b>	0.7b	5.7	0.7c	2.7
<b>LSD (0.05)</b>	1.536		1.036	
<b>P-Value</b>	0.032	0.172	0.010	0.092

<sup>1</sup>Means with the same letter in a column do not differ significantly ( $P < 0.05$ )

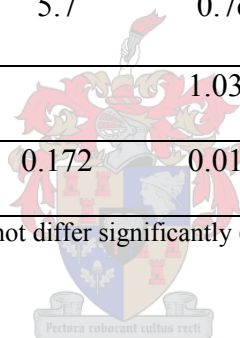


Table 13

Effect of pulp temperature on the stem condition of Bonheur, Dauphine and Red Globe table grapes fumigated with 50 µg/mL ClO<sub>2</sub> for 30 minutes and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C.

<b>Stem condition<sup>1</sup></b>			
	<b>Bonheur</b>	<b>Dauphine</b>	<b>Red Globe</b>
<b>15°C</b>	2.8cd <sup>2</sup>	3.0a	3.0d
<b>20°C</b>	3.3ab	2.8b	3.8c
<b>25°C</b>	3.0bc	3.0a	4.3c
<b>30°C</b>	3.5a	3.0a	4.9a
<b>35°C</b>	2.5d	3.0a	4.4b
<b>LSD (0.05)</b>	1.249	0.635	1.019
<b>P-Value</b>	< 0.0001	0.037	< 0.0001

<sup>1</sup> Data were ranked from 1 to 5 where 1 is green and 5 brown/dry

<sup>2</sup> Means with the same letter in a column do not differ significantly

Table 14

Effect of pulp temperature on the moisture of Bonheur table grapes fumigated with 50 µg/mL ClO<sub>2</sub> for 30 minutes and evaluated after a storage period of six weeks at –0.5°C and shelf life of one week at 15°C.

<b>Moisture<sup>1</sup></b>			
	<b>Bonheur</b>	<b>Dauphine</b>	<b>Red Globe</b>
<b>15°C</b>	5.2b <sup>2</sup>	6.4a	5.3
<b>20°C</b>	6.4a	6.8a	4.5
<b>25°C</b>	4.3b	3.9b	5.8
<b>30°C</b>	4.8b	3.9b	5.8
<b>35°C</b>	6.8a	6.4a	6.2
<b>LSD (0.05)</b>	0.911	1.004	
<b>P-Value</b>	< 0.0001	< 0.0001	0.154

<sup>1</sup> Data were ranked from 1 to 3 where 1 dry and 3 free moisture

<sup>2</sup> Means with the same letter in a column do not differ significantly

Table 15

Effect of pulp temperature on the TSS and acidity of Bonheur, Dauphine and Red Globe table grapes fumigated with 50 µg/mL ClO<sub>2</sub> for 30 minutes and evaluated after a storage period of six weeks at -0.5°C and shelf life of one week at 15°C.

% TSS			
	Bonheur	Dauphine	Red Globe
15°C	18.1a	15.2	15.6
20°C	18.1a	15.9	15.3
25°C	16.0c	15.8	15.3
30°C	17.4ab	15.8	16.2
35°C	16.3bc	16.2	15.6
<b>LSD (0.05)</b>	1.106		
<b>P-Value</b>	0.0003	0.595	0.313
% Acid			
15°C	0.52c	0.71	0.49
20°C	0.55b	0.71	0.49
25°C	0.57ab	0.69	0.50
30°C	0.56ab	0.74	0.50
35°C	0.58a	0.73	0.47
<b>LSD (0.05)</b>	0.024		
<b>P-Value</b>	0.0002	0.424	0.337

<sup>1</sup>Means with the same letter in a column do not differ significantly

Table 16

Effect of ClO<sub>2</sub> concentration and exposure time on stem condition and moisture of Barlinka table grapes, harvested during adverse weather conditions and evaluated after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

	Stem Condition <sup>4</sup>	Moisture <sup>5</sup>
<sup>1</sup> Control	2.2c <sup>3</sup>	1.5
25 µg/mL	3.6bc	1.5
50 µg/mL	2.7a	1.5
75 µg/mL	2.7ab	1.5
LSD (0.05)	2.367	
P-Value (concentration)	0.002	-
<sup>2</sup> Control	2.2c	1.5
15 minute	2.6ab	1.5
30 minute	2.7a	1.5
45 minute	2.4bc	1.5
LSD (0.05)	2.367	
P-Value (time)	0.021	-
P-Value	0.055	-
<b>(Concentration × Time)</b>		

<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>3</sup> Means with the same letter in a column do not differ significantly

<sup>4</sup> Data were ranked from 1 to 5 here 1 is green and 5 brown/dry

<sup>5</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

Table 17

Effect of ClO<sub>2</sub> concentration and exposure time on stem condition and moisture of Dauphine table grapes, harvested during adverse weather conditions and evaluated after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

	Stem Condition <sup>4</sup>	Moisture <sup>5</sup>
<sup>1</sup> Control	2.4a	1.5
25 µg/mL	2.1b	1.5
50 µg/mL	2.6a	1.5
75 µg/mL	2.6a	1.5
LSD (0.05)	1.939	
P-Value (concentration)	0.0003	-
<sup>2</sup> Control	2.4a	1.5
15 minute	2.1b	1.5
30 minute	2.5a	1.5
45 minute	2.6a	1.5
LSD (0.05)	1.939	
P-Value (time)	0.0002	-
P-Value	0.058	-
<b>(Concentration × Time)</b>		

<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>3</sup> Means with the same in a column letter do not differ significantly

<sup>4</sup> Data were ranked from 1 to 5 here 1 is green and 5 brown/dry

<sup>5</sup> Data were ranked from 1 to 3 where 1 is dry and 3 free moisture

Table 18

Effect of ClO<sub>2</sub> concentration and exposure time on TSS and acidity of Barlinka table grapes, harvested during adverse weather conditions and evaluated after six weeks of storage at – 0.5°C and a shelf life of one week at 15°C.

	<b>% TSS</b>	<b>% Acid</b>
<sup>1</sup> <b>Control</b>	17.9	0.58a
<b>25 µg/mL</b>	17.6	0.54bc
<b>50 µg/mL</b>	18.0	0.54c
<b>75 µg/mL</b>	17.4	0.56b
<b>LSD (0.05)</b>		0.015
<b>P-Value (concentration)</b>	0.093	< 0.0001
<sup>2</sup> <b>Control</b>	17.9	0.58a
<b>15 minute</b>	17.8	0.55bc
<b>30 minute</b>	17.8	0.54c
<b>45 minute</b>	17.4	0.56b
<b>LSD (0.05)</b>		0.0151
<b>P-Value (time)</b>	0.241	0.029
<b>P-Value</b>	0.952	0.051
<b>(Concentration × Time)</b>		

<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration

<sup>3</sup> Means with the same letter in a column do not differ significantly



Table 19

Effect of ClO<sub>2</sub> concentration and exposure time on acidity of Dauphine table grapes, harvested during adverse weather conditions and evaluated after six weeks of storage at – 0.5°C and a shelf life of one week at 15°C.

	% Acid			
	Control	15 minutes	30 minutes	45 minutes
25 µg/mL	0.67a	0.74bd	0.75bd	0.71ad
50 µg/mL	0.67a	0.61ac	0.74bd	0.69ad
75 µg/mL	0.67a	0.72ad	0.68a	0.73ad
LSD (0.05)		0.069		
P-Value (concentration)		0.028		
P-Value (exposure time)		0.292		
P-Value (Concentration × Time)		0.007		

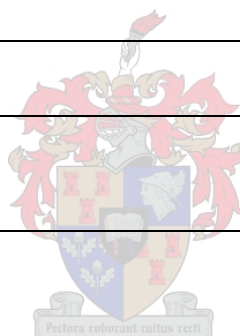


Table 20

Effect of ClO<sub>2</sub> concentration and exposure time on TSS of Dauphine table grapes after six weeks of storage at -0.5°C and a shelf life of one week at 15°C.

	<b>% TSS</b>
<sup>1</sup> <b>Control</b>	17.0
<b>25 µg/mL</b>	16.4
<b>50 µg/mL</b>	16.4
<b>75 µg/mL</b>	16.5
<b>P-Value (concentration)</b>	0.291
<sup>2</sup> <b>Control</b>	17.0
<b>15 minute</b>	16.3
<b>30 minute</b>	16.7
<b>45 minute</b>	16.3
<b>LSD (0.05)</b>	0.595
<b>P-Value (time)</b>	0.125
<b>P-Value</b>	0.860

**(Concentration × Time)**

<sup>1</sup> Data pooled across exposure times

<sup>2</sup> Data pooled across ClO<sub>2</sub> concentration