CESAR SIMAS TELES

STORAGE OF SEEDLESS TABLE GRAPES EXPOSED TO HIGH CO_2 CONCENTRATIONS FOR SHORT PERIOD FOLLOWED BY CONTROLLED ATMOSPHERE, ASSOCIATED OR NOT WITH PRE-HARVEST APPLICATION OF $CaCl_2$ OR ClO_2

CONSERVAÇÃO DE UVAS APIRÊNICAS SUBMETIDAS A CURTA EXPOSIÇÃO DE ALTAS CONCENTRAÇÕES DE CO_2, SEGUIDA DE ARMAZENAMENTO SOB ATMOSFERA CONTROLADA, ASSOCIADA OU NÃO À APLICAÇÃO DE $CaCl_2$ OU ClO_2 NA PRÉ-COLHEITA

CAMPINAS

2013

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA AGRÍCOLA

CESAR SIMAS TELES

STORAGE OF SEEDLESS TABLE GRAPES EXPOSED TO HIGH CO_2 CONCENTRATIONS FOR SHORT PERIOD FOLLOWED BY CONTROLLED ATMOSPHERE, ASSOCIATED OR NOT WITH PRE-HARVEST APPLICATION OF $CaCl_2$ OR ClO_2

Orientador: Prof. Dr. Benedito Carlos Benedetti

"CONSERVAÇÃO DE UVAS APIRÊNICAS SUBMETIDAS A CURTA EXPOSIÇÃO DE ALTAS CONCENTRAÇÕES DE CO_2, SEGUIDA DE ARMAZENAMENTO SOB ATMOSFERA CONTROLADA, ASSOCIADA OU NÃO À APLICAÇÃO DE $CaCl_2$ OU ClO_2 NA PRÉ-COLHEITA"

Tese de Doutorado apresentada ao Programa de Pós Graduação em Engenharia Agrícola da Universidade Estadual de Campinas para obtenção do título de Doutor em Engenharia Agrícola, na área de concentração Tecnologia de Pós-colheita.

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO CESAR SIMAS TELES ORIENTADOPELA PROF DR. BENEDITO CARLOS BENEDETTI

Assinatura do Orientador :

CAMPINAS
2013

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Área de Engenharia e Arquitetura
Rose Meire da Silva - CRB 8/5974

Teles, Cesar Simas, 1974-
T236s Storage of seedless table grapes exposed to high CO_2 concentrations for short period followed by controlled atmosphere, associated or not with pre-harvest application of $CaCl_2$ or ClO_2 / Cesar Simas Teles. – Campinas, SP : [s.n.], 2013.

Orientador: Benedito Carlos Benedetti.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia Agrícola.

1. Uva - Pós-colheita - Doenças e pragas. 2. Pós-colheita - Doenças e pragas. 3. Uva - Tecnologia pós-colheita. 4. Uva - Pós-colheita. 5. Uva - Cultivo. I. Benedetti, Benedito Carlos,1956-. II. Universidade Estadual de Campinas. Faculdade de Engenharia Agrícola. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Conservação de uvas apirências submetidas a curta exposição de altas concentrações de CO_2, seguida de armazenamento sob atmosfera controlada, associada ou não à aplicação de $CaCl_2$ ou ClO_2 na pré colheita
Palavras-chave em inglês:
Grape - Postharvest - Diseases and pests
Postharvest - Diseases and pests
Grape - Post-harvest technology
Grape - Post-harvest
Grape - Growing
Área de concentração: Tecnologia Pós-Colheita
Titulação: Doutor em Engenharia Agrícola
Banca examinadora:
Benedito Carlos Benedetti [Orientador]
Patrícia Cia
Eliane Aparecida Benato Rodrigues da Silva
Cláudio Luiz Messias
Paulo Ademar Martins Leal
Data de defesa: 17-07-2013
Programa de Pós-Graduação: Engenharia Agrícola

Este exemplar corresponde à redação final da **Tese de Doutorado** defendida por **Cesar Simas Teles,** aprovada pela Comissão Julgadora em 17 de julho de 2013, na Faculdade de Engenharia Agrícola da Universidade Estadual de Campinas.

Prof. Dr. Benedito Carlos Benedetti - Presidente e Orientador
Feagri/Unicamp

Dra. Patrícia Cia– Membro Titular
IAC

Dra. Eliane Aparecida Benato Rodrigues da Silva - Membro Titular
ITAL

Prof. Dr. Cláudio Luiz Messias - Membro Titular
Feagri/Unicamp

Prof. Dr. Paulo Ademar Martins Leal - Membro Titular
Feagri/Unicamp

"Quanto mais aumenta nosso conhecimento,
mais evidente fica nossa ignorância".

(John F. Kennedy)

Dedico esta tese minha avó, Thereza, por sua coragem e força (*in memorian*).

AGRADECIMENTOS:

Durante esses quatro anos, mais até do que meu objeto de estudo, aprendi que para atingir um objetivo é necessário a ajuda de várias pessoas. Nesse período muitos cruzaram meu caminho, alguns por um curto período outros participaram de boa parte da minha jornada e todos contribuíram de alguma forma fazendo diferença.

Agradeço: ao Ministério da Agricultura, instituição a qual tenho orgulho de pertencer, por conceder a licença que me permitiu realizar o doutorado;
à CAPES, que me concedeu a bolsa de pesquisa para que eu realizasse meu doutorado sanduiche no Estados Unidos;
à FEAGRI/UNICAMP, instituição que me acolheu e conduziu o meu aperfeiçoamento e
à Universidade da Califórnia – Davis, por ter me proporcionado uma maravilhosa experiência acadêmica e de vida;
à família, por todo o carinho e companherismo durante esses dias, em especial minha mãe, que sempre me apoiou, minha esposa, Eliane, e filha amada, Luíza, que superaram todos os desafios que a decisão de fazer doutorado acarretou;
à pesquisadora Claire Sarantóupolos, que me incentivou a começar o doutorado e diversas vezes foi conselheira;
ao Professor Benedetti por ter aceitado me orientar e pelo apoio durante todo o Doutorado;
aos professores da UNICAMP por terem compartilhado seus conhecimentos;
à banca de tese pelas inúmeras sugestões que ajudaram a aperfeiçoar este trabalho;
à Dra. Eliane Benato pelo apoio desde o primeiro esboço de projeto de tese;
à Rosa Helena, que foi uma ajuda decisiva no processo de mudança de Campinas para Davis;

aos meus colegas de trabalho Elizabeth Morgado, Edson Amorim, Ailena Salgado, Raquel Teixeira, Pedro Cabral, Fernando Penariol e em especial ao Fábio Florêncio, sem o qual eu não haveria cursado este doutorado;

ao Dr Crisosto que abriu as portas da UCDavis para mim, e desde o primeiro momento foi o amigo com quem eu e minha família contamos durante nossa estada em Davis;

à Gayle Crisosto pelo carinho e boa vontade;

a todos os amigos do Laboratório do Dr. Crisosto e do Laboratório de Pós-Colheita, cito alguns que foram companhia mais constante: Megan, pelas inúmeras aulas de inglês e sua amizade; Helen pela melhor risada da UCDavis; Michelle pelas inúmeras revisões e por ter me ensinado a usar a bendita Pivot Table; aos colegas brasileiros Francine, Sérgio, Paulo, Aquidauana e em especial Rita, pela amizade verde e amarela; Bill Biasi, pelo suporte técnico; Veronique, pelo suporte técnico e incondicional boa vontade; Ioannis por me guiar nos primeiros dias de UCDavis; aos inúmeros visitantes que passaram pelo laboratório durante meus experimentos.

aos "office mates" Omprya e Macarena por tolerarem minha bagunça;

ao Dr. Gubbler e sua equipe, em especial Robert e Cristina, por me ajudarem durante o trabalho no laboratório de fitopalogia;

ao Dr. Matthew Fidelibus e sua equipe por todo suporte durante o experimento de campo.

Agradecimento especial aos "interns": Vivian, Selene, Brian, Antoinette e Robert; sem os quais eu não teria conseguido realizar meus experimentos; e

aos amigos que fiz em Davis, Carlos Reisser e Carla, Sebastian e Angélica e

a todos aqueles me ajudaram.

MUITO OBRIGADO.

ABSTRACT:

Gray mold, caused by *Botrytis cinerea* Pers, is the main postharvest decay of table grapes. The use of sulfur dioxide (SO_2) is the common post-harvest practice for its control. Several researchers are looking for alternative methods of control, because SO_2 can cause allergic reactions, damage fruits and also it cannot be applied in organic production system. In this thesis, it was evaluated the effects of applying an atmosphere of 40% CO_2 for 24 or 48 hours (pre-storage) combined with controlled atmosphere storage (CA = 12% O_2 + 12% CO_2) in the control of *B. cinerea*, and the effects in the quality and sensory attributes of 'Flame Seedless' and 'Crimson Seedless' table grapes. In addition, it was evaluated the efficacy of $CaCl_2$ or ClO_2 application in pre-harvest alone or in combination with pre-storage of 40% CO_2 for 24 h + CA, to control gray mold on 'Crimson Seedless' table grapes, and the determination of the impact of these treatments on fruit quality. The treatments were applied in certified organic table grapes naturally infected, surface inoculated and nesting inoculated (inoculated with an infected berry). After 4 weeks of storage, the pre-storage in 40% CO_2 for 48 hours + CA reduced postharvest rot from 22% to 0.6%, and after 7 weeks, the decay was reduced from 100% to 7.4% in 'Flame Seedless' naturally infected. The pre-storage in 40% CO_2 alone also reduced the incidence of gray mold in fruits naturally infected and in artificially inoculated, but it was less effective than combined treatment. The application of $CaCl_2$ or ClO_2 pre-harvest reduced the incidence of gray mold on grapes 'Crimson Seedless' inoculated with a spore solution, but there was no

control when fruits were nesting inoculated. After 6 weeks at 0°C, the application of $CaCl_2$, and the ClO_2 in fruits surface inoculated, reduced the gray mold from 45% to 23.2% and 15.6%, respectively. The pretreatment with 40% CO_2 + CA did not affect quality and nor sensory attributes for both varieties tested. In vitro experiments, the treatment with 40% CO_2 for 24 or 48 h limited mycelial growth for at least 72 hours after treatment. Conidial germination of *B. cinerea* was delayed for 12 hours. Our results showed the potential that pre-treatment with 40% CO_2 associated with CA has to be adopted in commercial practice for preservation of organic grapes.

Keywords: *Vitis vinifera*, fungal spoilage, decay, table grapes, postharvest, calcium chloride, chlorine dioxide.

RESUMO:

Botrytis cinerea Pers, causador da doença conhecida como mofo cinzento, é o principal problema para a conservação pós-colheita de uvas de mesa. A utilização do dióxido de enxofre (SO_2) é a prática pós-colheita mais comum para o controle desta doença. Pesquisas buscam alternativas a este produto devido às reações que causa em pessoas alérgicas, danos que pode causar nos frutos e às restrições ao seu uso em sistemas de produção orgânico. Foram avaliados os efeitos da aplicação de uma atmosfera de 40% de CO_2 por 24 ou 48 horas (pré-armazenagem) combinado com armazenagem em atmosfera controlada (AC) (12% O_2 + 12% CO_2) no controle de *B. cinerea,* e nos atributos de qualidade de uvas 'Flame Seedless' e 'Crimson Seedless'. Também foram avaliados, em uvas 'Crimson Seedless', e os efeitos da associação deste tratamentos com aplicações pré-colheita de cloreto de cálcio ($CaCl_2$) ou dióxido de cloro (ClO_2). Os tratamentos foram aplicados em uvas orgânicas infectadas de três formas: infectadas naturalmente, suerficialmente inoculadas com conídios e inoculadas com uma baga coberta de micélio. Uvas 'Flame Seedless', naturalmente infectadas, tratadas com 40% de CO_2 por 48 horas + AC apresentaram redução da podridão pós-colheita, de 22% para 0,6%, após 4 semanas, e de 100% para 7,4%, após 7 semanas. O pré-armazenamento em 40% de CO_2 sozinho também limitou a incidência de mofo cinzento em frutos infectados naturalmente e em uvas inoculadas artificialmente, porém foi menos eficaz do que quando seguido pelo armazenamento em AC. A aplicação de $CaCl_2$ ou ClO_2 em pré-colheita reduziu a incidência do mofo cinzento em uvas 'Crimson Seedless' inoculadas com uma solução de conídios,

porém não houve controle quando os cachos de uva foram inoculados com micélio. A aplicação de $CaCl_2$ e ClO_2 reduziram o mofo cinzento de 45% para 23,2% e 15.6%, respectivamente, em cachos inoculados com conídios e avaliados após 6 semanas armazenadas 0^oC. O pré-tratamento com 40% CO_2 + CA não afetou as características físico-químicas e sensoriais de uvas 'Crimson Seedless' ou 'Flame Seedless'. Em experimentos *in vitro* os tratamentos com 40% CO_2 por 24 ou 48 h limitaram o crescimento micelial até 72 horas após o tratamento. A germinação dos conídios de *B. cinerea* foi retardada por 12h. Os resultados mostram que o pré-tratamento com 40% CO_2 + CA possui grande potencial para ser adotado como prática comercial para conservação de uvas orgânicas.

Palavras chaves: *Vitis vinifera*, fungos deterioradores, uva de mesa, pós-colheita, cloreto de cálcio, dióxido de cloro.

LISTA DE FIGURAS

CAPÍTULO 2

CAPÍTULO 3

APÊNDICE

LISTA DE TABELAS:

CAPÍTULO 2

CAPÍTULO 3

SUMÁRIO

1. INTRODUÇÃO

A uva de mesa é uma das frutas mais consumidas no mundo, possuindo várias propriedades benéficas à saúde do consumidor, entre elas a presença de antioxidantes. O Brasil é um grande importador de uva, porém esta situação pode ser modificada, pois vem ocorrendo a expansão de sua área de cultivo, com incrementos consideráveis em regiões não tradicionais de produção, como por exemplo, o Vale do Rio São Francisco.

O Brasil, devido as suas condições climáticas, produz a uva de mesa em épocas do ano onde existe escassez do produto no mercado mundial. Apesar desta vantagem, para se consolidar como exportador de uva de mesa, os produtores brasileiros deverão atender as novas demandas do consumidor tais como: variedades adequadas (uvas sem sementes), sustentabilidade ambiental e social e ausência de resíduos químicos.

A uva de mesa é uma fruta que possui dois grandes problemas para a sua conservação pós-colheita, sendo o primeiro a sua susceptibilidade à desidratação e o segundo a elevada incidência de podridões ocasionadas por fungos.

O principal método de conservação utilizado é a aplicação de dióxido de enxofre (SO_2), seja na forma de fumigação durante o armazenamento ou liberação gradativa no interior das embalagens. O SO_2, quando aplicado em dose adequada, reduz as perdas por desidratação e controla o desenvolvimento dos fungos deterioradores durante o armazenamento.

Este produto é utilizado nas principais regiões produtoras de uva do mundo, porém os mercados consumidores vêm colocando restrições ao seu uso devido aos problemas de saúde ocasionados em pessoas alérgicas. Vários mercados consumidores (Ex.: União Européia) proibiram a presença de resíduos de SO_2 nos cachos de uva e a agência americana de alimentos (FDA) retirou o nome deste produto da lista dos aditivos geralmente reconhecidos como seguros. A busca por alternativas de conservação comercialmente viáveis é um grande desafio.

Neste trabalho foram estudadas alternativas ao uso do SO_2 para a conservação pós-colheita de variedades apirênicas de uva de mesa.

Como premissa dessa pesquisa foi considerada a hipótese de que é possível controlar o *B. cinerea* durante conservação pós-colheita da uva de mesa sem semente, pela utilização de

altas concentrações de CO_2 por um curto período de aplicação associadas a atmosfera controlada, sem comprometer a qualidade dos frutos; e que a associação do alto CO_2 com aplicações em pré-colheita de cloreto de cálcio ($CaCl_2$) ou dióxido de cloro (ClO_2) proporciona uma melhor proteção dos cachos de uva do que a sua utilização isoladamente.

Desta forma, destacamos o objetivo geral e os específicos propostos para os experimentos desenvolvidos durante o curso de doutorado em Engenharia Agrícola.

2. OBJETIVO GERAL

Conservar uva de mesa de variedades apirênicas (simulando as condições de comercialização e exportação) utilizando, individualmente ou em associação, a aplicação de altas concentrações de CO_2, atmosfera controlada e aplicações de $CaCl_2$ ou ClO_2 em pré-colheita .

2.1 Objetivos específicos

1- Avaliar o efeito de altas concentrações de CO_2, aplicadas durante curto período, em cachos de uvas 'Flame Seedless' e 'Crimson Seedless', no controle de doenças e sobre a qualidade físico-química e sensorial.

2- Avaliar *in vitro* os efeitos de altas concentrações de CO_2 durante curto período, sobre o crescimento e a germinação de conídios de *Botrytis cinerea*.

3- Avaliar o efeito de aplicações de $CaCl_2$ ou ClO_2, em pré-colheita, combinadas com o armazenamento em 40% CO_2 por 24 h + atmosfera controlada (12% O_2 + 12% CO_2), no controle de doenças e sobre a qualidade físico-química e sensorial de cachos de uvas 'Crimson Seedless'.

3. CAPÍTULO 1 - REVISÃO BIBLIOGRÁFICA

3.1 Importância da cultura da videira

A videira é uma das principais fruteiras cultivadas no mundo e ocupava no ano de 2003 uma área de 75 milhões de hectares, com produção anual de 62 milhões de toneladas (t) (FAO, 2004), das quais 8,5 milhões t eram de uva para mesa.

Após uma série consecutiva de aumentos na produção de uvas no Brasil, no ano de 2009 houve uma redução de 4,08% devido à crise econômica mundial, que afetou fortemente o consumo de uvas de mesa, e às condições climáticas adversas. Neste ano, o Brasil possuía a área plantada de 81,9 mil hectares, com produção de 1,34 milhões de toneladas. Na safra de 2012 a produção brasileira aumentou para 1,47 milhões de toneladas, tendo aproximadamente 50% da produção destinada ao consumo em natura. A região Sul é a maior produtora nacional de uvas, com cerca de 907.000 t no ano de 2009, correspondente a 67% da produção nacional, seguida pelas regiões Nordeste (18,5%) e Sudeste (14%). Em São Paulo, principal produtor da região Sudeste, há predominância no cultivo de uvas de mesa (MELLO, 2010, 2012a).

A cultura da videira tem grande importância econômica e social no Brasil, uma vez que envolve grande número de negócios voltados tanto para o comércio interno quanto para o externo, gerando grande número de empregos diretos e indiretos. No cenário internacional, a vitivinicultura brasileira ocupou, em 2010, a vigésima posição em área cultivada e a décima quarta em produção. Em relação às transações comerciais do ano de 2010, o Brasil ocupou a décima sétima posição em quantidade de uvas exportadas e a décima em valor de exportações (MELLO, 2011, 2012b). Em 2010, o volume de uvas exportadas foi de aproximadamente 60.805 t e o volume importado de 24.794 t (IBRAF, 2013). A mais importante de mesa do mundo é a 'Itália' (Muscat) com uma produção de 700 000 t/ano no começo da década de 1990. A variedade Thompson Seedless é mundialmente comercializada durante quase o ano inteiro. No ano de 2000 o estado da Califórnia, responsável por 90% da produção de uvas dos Estados Unidos, produziu aproximadamente 224.000 t de uvas 'Thompson Seedless', enquanto que o Chile produziu 176.000 t (CHERVIN, AKED e CRISOSTO, 2012).

3.2 Aspectos botânicos e desenvolvimento dos frutos

A videira é uma planta vigorosa da família Vitidaceae Juss. (syn. Ampelidaceae; Vitacae). A uva européia *Vitis vinifera* (L.) é hermafrodita, enquanto as espécies nativas da América do Norte pertencentes ao gênero *Vitis* são monóicas. Vários botões são produzidos lateralmente na safra anterior e as flores são polinizadas pelo vento (CHERVIN, AKED e CRISOSTO, 2012).

O desenvolvimento da baga da uva segue um padrão duplo sigmóide, sendo dividido em três fases: fase I: desenvolvimento inicial; fase II: latência; e fase III: amadurecimento dos frutos. A primeira fase corresponde a formação do embrião e ao crescimento exponencial do fruto, ocorrendo o acúmulo de diversos solutos, predominantemente ácido málico e tartárico. Na segunda fase, o tamanho do fruto permanece estável. Na terceira, as bagas voltam a crescer, coincidindo com o início da maturação. A palavra francesa "véraison" usada para descrever a mudança na cor da pele da baga, indicador do início de maturação, tem sido adotada para descrever o início do amadurecimento. As mudanças mais drásticas na composição química das bagas de uva ocorrem durante a fase de amadurecimento. As bagas passam de um estado onde elas são pequenas, duras, ácidas e com pouco açúcar, para um estado onde eles são maiores, mais macias, mais doces, com menos ácido, fortemente aromatizadas e coloridas. O sabor formado na uva é principalmente o resultado do balanço acidez/açúcar e da síntese de compostos aromáticos e de sabor, ou precursores destes. O desenvolvimento destas características determinará em grande parte a qualidade do produto final. O ácido málico é metabolizado e utilizado como fonte de energia durante a fase de amadurecimento, resultando em uma significativa diminuição dos seus níveis em relação ao ácido tartárico, cuja concentração geralmente permanece quase constante depois do "véraison" (CONDE *et al.*, 2007).

3.3 Colheita e Pós-colheita

A uva é uma fruta não climatérica, com baixa taxa respiratória (5 a 10 mg CO_2 kg^{-1} h^{-1}, à 5 °C) com valores similares a nozes e muito baixa produção de etileno (<0,1 μL kg^{-1} h^{-1}, à 20 °C) (KADER, 2002b; GARDEA *et al.*, 2004). Por apresentar baixa sensibilidade ao dano pelo frio, pode ser armazenada por longos períodos a 0 °C e 95 % de umidade relativa (KADER, 2002b). Os cachos de uva embalados devem ser resfriados rapidamente e levados

para a estocagem em câmaras frias até a sua comercialização. Recomenda-se que a uva seja armazenada com temperatura de polpa entre -0,5 °C e 0 °C, com 95% de umidade relativa, e que seja mantida na câmara fria em temperaturas entre -1 e 0 °C, pois nesta faixa de temperatura evita-se o congelamento da polpa e cachos, prolongando ao máximo a vida de prateleira dos cachos de uva (ZOFFOLI, LATORRE e NARANJO, 2008).

Assim como outras frutas não climatéricas, a uva não amadurece após ser colhida, e por isso recomenda-se a colheita ao atingir um estádio ótimo de consumo quanto à aparência, aroma, sabor e textura. A exposição de uvas de mesa ao gás etileno não afeta a aparência das bagas e nem da ráquis. Atributos físico químicos, tais como firmeza da polpa, concentração de sólidos solúveis, também não são afetados (PALOU *et al.*, 2003). Segundo este autores o controle de etileno em câmaras de armazenagem não traz benefícios comerciais.

Os açúcares e os ácidos são os mais importantes constituintes do sabor da fruta e a relação sólidos solúveis/acidez titulável é o parâmetro que melhor define o grau de maturação das uvas; por isso em diversos países as uvas são colhidas baseadas na medição do teor de sólidos solúveis e na acidez titulável (CRISOSTO e MITCHELL, 2002). Durante o processo de maturação, o teor de sólidos solúveis aumenta e o de ácidos orgânicos diminui. A norma brasileira não leva em consideração esta relação para a classificação das uvas finas de mesa (MAPA, 2002), esta norma considera a concentração de 14 ^{o}Brix, aferida por refratômetro, como valor mínimo para a comercialização de uvas (MAPA, 2002).

O padrão de qualidade da Califórnia estabelece que, com exceção das uvas ‘Thompson Seedless’ e ‘Perlette’, a uva deve ser considerada madura, quando o suco apresentar relação de sólidos solúveis/acidez titulável igual ou superior a 20:1 (NELSON, 1985). Esta relação se torna mais importante em variedades com elevada acidez. A aceitabilidade quanto ao sabor é maior com o aumento da relação sólido solúveis/acidez titulável. Como aspecto negativo, frutos muito maduros são mais susceptíveis a descoloração interna, escurecimento superficial e infecção fúngicas (FOURIE, 2008; ZOFFOLI, LATORRE e NARANJO, 2008).

Preferências étnicas podem determinar a aceitação do consumidor. Estudo com consumidores americanos e chineses mostrou que a acidez titulável influenciou na aceitação de uvas 'Red Globe' (CRISOSTO e CRISOSTO, 2002).

Variedades de uva vermelhas e pretas possuem requerimentos mínimos de cor, baseado na percentagem de bagas do cacho que exibem um mínimo de intensidade de cor e mínima cobertura. A boa aparência, espessura da casca, textura, tamanho e sabor são outros critérios que podem ser utilizados para classificar a qualidade de uvas de mesa (CONDE *et al.*, 2007).

A colheita deve ocorrer nas horas mais frescas do dia e deve ser realizada rapidamente para prevenir a desidratação, que pode comprometer a aparência e prejudicar a sua comercialização (FOURIE, 2008). Considerando a baixa taxa respiratória da uva, a desidratação dos cachos é o mais importante processo fisiológico que pode comprometer a sua aparência, provocando o escurecimento da ráquis e pedicelo, a degrana das bagas, murcha e enrugamento, influindo diretamente no período de armazenamento das uvas de mesa (CRISOSTO, SMILANICK e DOKOOZLIAN, 2001; CRISOSTO e MITCHELL, 2002).

Durante a colheita, costuma-se realizar a primeira limpeza dos cachos que consiste na retirada de restos foliares, ramos secos, gavinhas e bagas defeituosas e danificadas (CHOUDHURY e COSTA, 2004; FOURIE, 2008). Dois tipos principais de embalagens primárias são usadas para empacotar as uvas: sacos perfurados e caixas plásticas perfuradas (CRISOSTO e MITCHELL, 2002; CHERVIN, AKED e CRISOSTO, 2012). O uso de bolsas plásticas reduz muito os danos durante a comercialização (LUVISI *et al.*, 1995), e as perdas por transpiração também são reduzidas pelo uso destas bolsas (GARDEA *et al.*, 2004). Os cachos de uva após embalados nas bolsas plásticas são colocadas em caixas que podem ser feitas de poliestireno (Styrofoam), papelão corrugado ou madeira, e a escolha do material depende da duração de estocagem prevista e do preço final do produto (GARDEA *et al.*, 2004).

A uva deve ser resfriada rapidamente logo após a colheita, pois um retardamento de 8 horas neste processo pode ocasionar perdas de massa de 0,5 a 2,1% em várias cultivares (CRISOSTO, SMILANICK e DOKOOZLIAN, 2001). Ainda neste estudo, perdas de água

iguais ou superiores a 2,0% ocasionaram escurecimento do pedúnculo, após sete dias de armazenamento a 0 ^{o}C, em uvas 'Perlette', 'Flame Seedless', 'Thompson Seedless', 'Ruby Seedless' e 'Fantasy Seedless'. A ráquis possui taxa respiratória 25 vezes maior do que os frutos, o que explica a deterioração mais rápida da ráquis, fator que é um dos limitantes para maior vida de prateleira da uva de mesa (GARDEA *et al.*, 2004).

A cera natural presente na superfície das bagas de uva, denominada pruína, também é um importante fator de qualidade (CONDE *et al.*, 2007). A manipulação inadequada durante a colheita, embalagem, armazenamento e comercialização eliminam a pruína, comprometendo significativamente a aparência dos frutos e também a sua qualidade (CRISOSTO e MITCHELL, 2002).

O escurecimento das bagas e o ressecamento do ráquis são desordens de natureza fisiológica, ocasionadas, quase sempre, durante o armazenamento refrigerado. O emprego de embalagens adequadas pode reduzir a incidência desses processos fisiológicos prejudiciais à qualidade dos cachos de uva, minimizando essas perdas, que podem comprometer o valor comercial das uvas (NEVES *et al.*, 2008).

A utilização de filme de polietileno linear de baixa densidade (PELBD) 25 μm, com ou sem injeção de mistura gasosa, e de PVC 17 μm, aliadas ao armazenamento refrigerado (1°C e 90% de umidade relativa), reduziram a perda de massa de matéria fresca dos cachos, mas não foi eficiente nas reduções da incidência de podridões em uvas 'Niagara rosada', e a embalagem de PELBD com 50 μm de espessura acarretou a fermentação da uva (CIA *et al.*, 2010).

Segundo CENCI e CHITARRA (1994) as perdas pós-colheita da uva no Brasil estão em torno de 35%, sendo as principais causas: perda de massa, escurecimento do engaço, amolecimento das bagas, degrana, problemas com embalagens, manuseio e transporte do produto.

Filmes plásticos reduzem a perda de umidade e também auxiliam a retenção de SO_2, quando os frutos são fumigados com sachês de metabissulfito. Sacolas plásticas de polietileno de baixa densidade (PEBD) perfuradas conservaram a qualidade de uvas 'Itália' e 'Red Globe' da região de Jales-SP até, respectivamente, seis e sete semanas em refrigeração (CASTRO,

1999).

A espessura e o tipo de filme plástico utilizado na embalagem também influenciam na penetração do ozônio e de outros gases. Apesar das diferenças não serem significativas, a difusão de ozônio foi maior em PEAD do que em PEBD. A difusão foi aumentada com o aumento da área de ventilação das embalagens (KARACA e SMILANICK, 2011).

O uso de sacolas plásticas de polietileno promove grande redução de danos devido a manipulação dos consumidores (LUVISI *et al.*, 1995). Sacolas e caixas plásticas são perfuradas para permitir o fluxo de ar através das embalagens. As embalagens individuais são colocadas em caixas, que podem ser feitas de madeira laminada, papel ondulado, plástico ondulado, plástico retornável ou poliestireno. As embalagens de madeira laminada e poliestireno são utilizadas principalmente para o armazenamento a frio durante longos períodos, pois estas embalagens mantém sua resistência física mesmo em ambientes de alta umidade ventilação (CHERVIN, AKED e CRISOSTO, 2012).

3.4 Principais doenças pós-colheita

Nas condições de armazenamento requeridas pelas uvas de mesa, baixas temperaturas e umidade relativa alta e constante, as podridões podem gerar enormes prejuízos.

3.4.1 *Botrytis cinerea* ou mofo cinzento

O desenvolvimento de fungos durante o armazenamento e transporte é uma das principais causas de perdas pós-colheita, sendo *Botrytis cinerea* Pers.:Fr., agente causal do mofo cinzento, a praga chave desta cultura nas principais regiões produtoras do mundo (FOURIE, 2008). As lesões causadas pelo mofo cinzento possuem coloração claro até café escuro. Em condições de umidade adequada, as lesões são cobertas por micélio que ao esporular possuem cor cinza (CASTRO, PARK e HONÓRIO, 1998).

Botrytis cinerea é um fitopatógeno que possui uma ampla gama de hospedeiros, causando perdas em frutas, hortaliças e plantas ornamentais. Para o cultivo de uvas de mesa *B. cinerea* é considerada praga chave, devido as grandes perdas econômicas que pode causar

(KASSEMEYER e BERKELMANN-LÖHNERTZ, 2009).

Este fungo pode sobreviver saprofitamente em restos de culturas e produzir esclerócios para a sobrevivência por longos períodos. Os conídios são propágulos que sobrevivem por curto período e disseminam o fungo através do vento, água da chuva e insetos. O fungo penetra na superfície do hospedeiro secretando enzimas como lipases, cutinases e pectinases, porém a via preferencial de penetração ocorre através de aberturas naturais ou ferimentos presentes na superfície das bagas (KRETSCHMER, KASSEMEYER e HAHN, 2007). A germinação dos conídios, o crescimento do tubo germinativo, a penetração e colonização do tecido do hospedeiro são etapas críticas do ciclo de infecção. A germinação de conídios e infecções ocorrem sob alta umidade relativa (> 94%), e longos períodos de elevada umidade favorecem o desenvolvimento de *B. cinerea* e aumentam a incidência da doença. Nestas condições, os conídios germinam 1-3 h após a inoculação, e podem penetrar a epiderme do fruto em 15 h quando armazenados a temperaturas entre 13 e 24 °C (KASSEMEYER e BERKELMANN-LÖHNERTZ, 2009).

Chuvas durante a época de colheita aumentam a incidência de mofo cinzento. Recomenda-se aguardar três dias após chuvas para que as uvas sejam colhidas. Este intervalo é necessário para que bagas contaminadas pelo mofo cinzento exibam os sintomas da doença, e sejam removidas durante a limpeza dos cachos que precede ao empacotamento (LUVISI *et al.*, 1992; CHERVIN, AKED e CRISOSTO, 2012). Os cachos colhidos após chuvas devem ser estocados separadamente dos cachos colhidos no período seco e a sua venda deve ocorrer rapidamente (LUVISI *et al.*, 1992).

Cachos de uva imaturos são mais susceptíveis a danos pela aplicação de SO_2, enquanto que a colheita de cachos muito maduros está associada a altos níveis de infecção fúngicas e degrana, devido ao desprendimento das bagas do pedicelo (FOURIE, 2008). Durante o amadurecimento, as uvas ficam mais susceptíveis a infecção do *B. cinerea* devido a vários fatores tais como: (1) enfraquecimento das defesas do hospedeiro; (2) redução de substâncias fungistáticas, ex.: protoantocianidinas; (3) mudanças na cutícula e epiderme, devido ao amadurecimento das sementes que ocasionam microfissuras (KRETSCHMER, KASSEMEYER e HAHN, 2007).

Em infecções quiescentes, a doença se desenvolve a partir do inóculo presente internamente nas bagas infectadas durante o florescimento, causando perdas significativas, em todas as regiões produtoras do mundo. A doença pode ocorrer no campo e causar danos, mas em geral, é na fase de armazenamento que o patógeno é mais agressivo, devido a sua capacidade de desenvolver-se em temperaturas de refrigeração muito baixas, como por exemplo, a 0,5 ^{o}C, e disseminar-se por crescimento micelial baga a baga (CRISOSTO e MITCHELL, 2002; LICHTER, MLIKOTA GABLER e SMILANICK, 2006).

Os conídios do *B. cinerea* podem infectar as bagas de uva de diversas formas. Infecções no início do desenvolvimento dos frutos ocorrem durante a abertura do estigma; os conídios do fungo ficam na forma quiescente durante o desenvolvimento do fruto, manifestando sintomas apenas quando o fruto estiver maduro (LUVISI *et al.*, 1992; ELMER e MICHAILIDES, 2004). As infecções quiescentes não são controladas pelas aplicações de fungicidas no campo nem pela aplicação de dióxido de enxofre em pós colheita, por que o fungo fica protegido no interior dos frutos (LUVISI *et al.*, 1992).

A germinação de conídios na superfície dos frutos é outro mecanismo de infecção, nesta forma de infecção o conídio germina e penetra a epiderme do fruto, porém o desenvolvimento da infecção só se completa quando os frutos estão maduros. As bagas podem ser infectadas através do micélio de frutos infectados, mecanismo quando frutos com sintomas de infecção visíveis não são removidos durante o empacotamento, a partir deles o micélio do botrytis se dissemina de um fruto para outro (LUVISI *et al.*, 1992). A penetração do patógeno é favorecida pela presença de microfissuras ou ferimentos.

No escuro e sob condições de alta umidade relativa, ocorre rápido crescimento de hifas, e o fungo dissemina-se rapidamente de uma baga para outra, nestas condições uma única baga contaminada pode infectar um cacho inteiro de uvas. Além da formação de “ninhos” de bagas e micélio, outros sintomas que identificam o mofo cinzento facilmente são: a formação de áreas marrons nos frutos e o fácil desprendimento da epiderme dos frutos quando friccionado com os dedos, deixando a polpa exposta (LUVISI *et al.*, 1992; CHERVIN, AKED e CRISOSTO, 2012).

3.4.2 Outras doenças

Outros fungos, como por exemplo *Rhizopus stolonifer* e *Aspergillus* spp., podem causar perdas em casos de falhas na refrigeração ou durante a exposição da fruta para a comercialização (LICHTER, MLIKOTA GABLER e SMILANICK, 2006). Os principais fitopatógenos deterioradores encontrados em uvas produzidas no agropolo irrigado da região do semiárido brasileiro pertencem aos gêneros *Cladosporium, Alternaria, Aspergillus, Penicillium* e *Rhizopus* (CHOUDHURY, RESENDE e COSTA, 2001). Em cultivos de uvas 'Crimson Seedless' e 'Itália', em jovens parreirais implantados no Estado de Roraima, foram encontrados fungos deterioradores dos gêneros *Botrytis, Penicillium, Plasmopara, Alternaria* e *Colletotrichum*, com maior incidência dos dois primeiros (NEVES *et al.*, 2008).

Pesquisas realizadas com 4 variedades apirênicas cultivadas na região do sub-médio São Francisco identificaram a grande incidência dos fungos *Aspergillus niger, Alternaria alternata, Cladosporium herbarum, Lasiodiploidia theobroma*e, e em menor incidência *Rhizopus stolonifer* e *Penicillium expansum* (CAMARGO *et al.*, 2011).

BENATO et al. (1998) não constaram a presença de *B. cinerea* em uva 'Itália', cultivada na região de Jales/SP e colhidas no mês de outubro, quando as temperaturas variaram de 18 a 32 °C. Neste trabalho identificou-se os fungos *Colletotrichum gloeosporioides, Alternaria alternata, Rhizopus sp*., *Lasiodiplodia theobromae* (=Botryodiplodia), *Penicillium sp., Aspergillus sp*. e leveduras como causadores das podridões pós-colheita.

Penicillium expansum é um fungo que causa grande perdas econômicas em uvas estocadas sob refrigeração durante períodos superiores a 60 d; este fungo é um problema para exportações de uvas chilenas da variedade Red Globe (FRANCK *et al.*, 2005).

3.5 Métodos de controle de doenças pós-colheita

A incidência de podridões fúngicas constitui-se como um dos principais problemas à qualidade das uvas produzidas em regiões quentes e úmidas. Por melhor que seja o tratamento fitossanitário efetuado no campo, é necessária a realização de fumigações com SO_2 durante a

pós-colheita (SMILANICK *et al.*, 2010). Por essa razão, o SO_2 geralmente é empregado no controle das podridões de pós-colheita de uvas de mesa combinado ao armazenamento sob baixas temperaturas (LUVISI *et al.*, 1992; ZAHAVI *et al.*, 2000; ZOFFOLI, LATORRE e NARANJO, 2008).

Nesta revisão foram abordados o uso de métodos culturais para a redução de podridões pós-colheita, uso de SO_2 e de métodos alternativos ao SO_2 utilizados durante esta pesquisa.

3.5.1 Manejo Pré-Colheita

Várias práticas culturais podem reduzir a incidência de podridões pós-colheita causadas pelo *B. cinerea*. A remoção de restos vegetais infectados de parreirais durante a poda de inverno, pode reduzir o inóculo produzido na temporada seguinte. A aplicação de fungicidas sistêmicos e de contato em pré-colheita reduzem a infecção em flores e infecções nos cachos (LUVISI *et al.*, 1992).

A abertura da copa das parreiras de uva é umas das práticas recomendadas, a remoção das folhas adjacentes aos cachos melhora a cobertura de fungicidas e aumenta a velocidade do ar no interior da copa das plantas, diminuindo a umidade. Essas medidas associadas a rigorosa retirada de bagas infectadas no momento da colheita e antes do empacotamento são fundamentais para redução das perdas pós-colheita (LUVISI *et al.*, 1992).

Para retardar o desenvolvimento da doença deve-se evitar vegetação excessiva através do manejo de porta-enxerto e do uso criterioso da adubação nitrogenada. O aumento de aeração e da exposição dos cachos ao sol, o controle de doenças e pragas capazes que danificar as bagas da uva, promovem grande redução na incidência do mofo cinzento (PEARSON e GOHEEN, 1988).

O controle químico é normalmente necessário, mas pode ser conduzido apenas com tratamentos preventivos. Um programa de quatro aplicações, conhecido como o método "padrão" na Europa, tem apresentado resultados satisfatórios. A primeira aplicação ocorre no fim da floração e/ou início da formação dos frutos; a segunda, imediatamente antes do fechamento do cachos; a terceira, no início do “véraison” (mudança de cor); e uma quarta, três

semanas antes da colheita. O uso de fungicidas exige um manejo adequado, para evitar que cepas *de B. cinerea* desenvolvam resistência aos fungicidas, como já aconteceu com os benzimidazóis e dicarboximidas. O ajuste adequado do equipamento de pulverização é fundamental para promover boa penetração e cobertura do fungicida nos cachos (PEARSON e GOHEEN, 1988).

3.5.2 Dióxido de enxofre (SO_2)

O uso do SO_2, seja aplicado por fumigação na câmara fria de estocagem ou através de liberadores no interior das embalagens, permanece como a mais efetiva forma de controle pós-colheita da deterioração por *Botrytis* (CRISOSTO e MITCHELL, 2002; LURIE *et al.*, 2006; FOURIE, 2008).

A fumigação com SO_2 apresenta a grande vantagem de não necessitar a manipulação das uvas, o que ajuda a explicar a grande adesão ao uso deste produto (MLIKOTA GABLER *et al.*, 2010). O tratamento com SO_2 limita o crescimento fúngico e permite que a uva de mesa seja armazenada em ambientes com alta umidade, reduzindo o ressecamento do cacho, que é um fator importante de depreciação do valor do fruto (CHERVIN, WESTERCAMP e MONTEILS, 2005).

Devido ao crescente interesse no mercado exportador, há crescente demanda pela utilização de saches geradores de SO_2, especialmente para o transporte a grandes distâncias. O metabissulfito de sódio é incorporado dentro de sachês ou papéis geradores, que ao reagirem com a umidade proveniente da uva, liberam o SO_2 durante o transporte e a comercialização (CRISOSTO e MITCHELL, 2002; LURIE *et al.*, 2006; FOURIE, 2008). Diferentes tipos de liberadores de SO_2 estão disponíveis no mercado mundial, com o Chile e África do Sul liderando a sua produção (FOURIE, 2008).

Recomenda-se concentrações de 3,6 a 5,5 μmol/kg h para a inibição do crescimento de micélio a partir de frutos com infecção quiescente, essa liberação pode ser ajustada através da dosagem do sal de metabissulfito e do tamanho dos grânulos de sais (PALOU *et al.*, 2002). No interior das caixas de uvas são utilizados filmes plásticos perfurados para reduzir a perda de massa dos cachos de uva e para aumentar a eficiência dos liberadores de SO_2 (PALOU *et al.*, 2002).

O dióxido de enxofre podem reagir com materiais na superfície da epiderme das bagas, incluindo as estruturas dos fungos (conídios e/ou micélio), ou pode penetrar através das aberturas naturais ou feridas a reagir com constituintes de células epidérmicas, ou ser diluído nos líquidos de tecidos. Todos estes processos são normalmente designados como adsorção do gás pela uva. O controle dos fungos dependerá da concentração de gás e de outros fatores (ex.: temperatura e umidade relativa). Doses excessivas poderão causar fitotoxicidade nos cachos de uva, e/ou deixar resíduos de sulfito acima dos limites tolerados (PALOU *et al.*, 2002).

O emprego do gerador de liberação rápida de SO_2 reduziu significativamente a taxa de deterioração e perda de massa dos cachos, permitindo a boa conservação de uvas 'Red Globe' por até seis semanas em refrigeração a 4 ^{o}C (CASTRO, PARK e HONÓRIO, 1998). O uso de gerador de fase rápida permitiu a conservação refrigerada de uvas "Itália" por quatro semanas (CHERVIN, AKED e CRISOSTO, 2012).

O maior problema em se utilizar o SO_2 como erradicante de *B. cinerea* é a proximidade entre as concentrações capazes de provocar toxicidade às uvas e as requeridas para o controle do patógeno. Altas concentrações de SO_2 podem causar danos aos cachos, como branqueamento, sabor desagradável, acelerada desidratação e lesões (LUVISI *et al.*, 1992; CRISOSTO e MITCHELL, 2002; LYDAKIS e AKED, 2003).

O branqueamento das bagas ocorre quando concentrações excessivas de SO_2 penetram na inserção do cacho com a baga, nas lenticelas ou danos na epiderme, causam branqueamento ou manchas aquosas. Microfissuras, seguida de exsudação de líquidos é outro sintoma da aplicação excessiva de SO_2 (ZOFFOLI, LATORRE e NARANJO, 2008; ZOFFOLI *et al.*, 2009). Altas concentrações de metabissulfito (SO_2) provocaram elevada desidratação das bagas e da ráquis, e ocasionaram grande porcentagem de degrana para uvas 'Itália' e 'Crimson Seedless' (NEVES *et al.*, 2008).

Apesar da excelente resposta ao controle desta infecção fúngica e prevenção da desidratação do cacho, a aplicação do SO_2 tem sido bastante restringida em vários países. O uso deste produto para frutas e hortaliças *in natura* não é autorizado na União Européia (Directiva 95/2/CE).

O SO_2 foi retirado da lista da substâncias geralmente reconhecidas como seguras (GRAS), e um limite máximo de resíduo em frutas de 10 μL L^{-1} de SO_2 foi estabelecido pelo

Departamento Americano de Administração de Alimentos e Drogas (FDA). Este órgão também regula o número máximo de aplicações permitidas para cada cultivar (CRISOSTO e MITCHELL, 2002). Desde que o SO_2 foi retirado pelo FDA da lista GRAS, grande esforço tem sido realizado pela cadeia de produção de uvas para diminuir a quantidade de SO_2. Exemplo deste esforço é o uso do sistema de utilização total, onde a fumigação com SO_2 é associada com a refrigeração forçada com ar. Este sistema reduz em 75% a quantidade de SO_2 aplicado (LUVISI *et al.*, 1992; CRISOSTO e MITCHELL, 2002).

Nos Estados Unidos, o uso de dióxido de enxofre é proibido para uvas orgânicas certificadas. Outra preocupação em relação ao uso SO_2 é a saúde dos trabalhadores, algumas agências regulatórias não permitem o descarte do SO_2 no ar após a fumigação e limitam a exposição dos trabalhadores ao valor máximo de 2 $\mu L\ L^{-1}$ (MLIKOTA GABLER *et al.*, 2010).

Resíduos de SO_2 são perigosos para pessoas alérgicas a sulfitos, também é levemente corrosivo para estruturas metálicas e, quando aplicado excessivamente, pode ocasionar toxidez em cachos de uva (CHERVIN, WESTERCAMP e MONTEILS, 2005). Por estas razões tem-se buscado métodos de preservação alternativos ao uso do SO_2.

3.5.3 Dióxido de cloro

O dióxido de cloro (ClO_2) é um poderoso oxidante, tal como o ozônio. Pode ser produzido através de duas reações diferentes: a reação de um ácido forte com clorato de sódio ou pela reação do clorito de sódio com gás cloro (MARI, BERTOLINI e PRATELLA, 2003). O ClO_2 é mais estável, e possui poder de oxidação 2,5 vezes maior do que o cloro, possuindo maior poder bactericida (BENARDE *et al.*, 1965). Ao contrário do cloro, a eficácia do ClO_2 permanece constante numa vasta gama de pH (BENARDE *et al.*, 1965). Ele produz menos compostos potencialmente cancerígenos e é menos corrosivo que o cloro e o ozônio. No entanto, uma vez que é explosivo, deve ser gerado no local e não pode ser estocado em cilindros sobre pressão (OLMEZ e KRETZSCHMAR, 2009). O dióxido de cloro é de difícil manuseio em ambientes fechados, porque quando um fluxo de água tratada é agitado ou gaseificado, parte do ClO_2 é liberado da solução para atmosfera e pode causar desconforto em trabalhadores (MARI, BERTOLINI e PRATELLA, 2003).

O dióxido de cloro pode ser usado para a lavagem e desinfecção de frutas e hortaliças, porém a concentração máxima permitida nos Estados Unidos é de 3 ppm, e os produtos devem ser enxaguados com água após o uso da solução de ClO_2 (OLMEZ e KRETZSCHMAR, 2009). Estudos com controle de patógenos em frutas e hortaliças tem demonstrados que maiores concentrações são necessárias para o controle destes micro-organismos (GLATZ *et al.*, 2000; PAO *et al.*, 2007).

O dióxido de cloro é um desinfetante que atua por contato e não possui efeito sistêmico, ele é eficaz apenas em propágulos expostos do fungo, tais como aqueles em suspensão na água ou na superfície do fruto. Ele não elimina os patógenos sob a pele de frutas ou infecções ativas (MARI, BERTOLINI e PRATELLA, 2003).

Concentrações de 75 ppm ou superiores são recomendadas para inibir a germinação de conídios de *Botrytis cinerea, Penicillium expansum* e *Rhizopus stolonifer* (ZOFFOLI *et al.*, 2005). A aplicação de 2.000 ppm de ClO_2 na forma de névoa em morangos reduziu a população de fungos na sua superfície (VARDAR, ILHAN e KARABULUT, 2012). Soluções de 120 d ppm de ClO_2 foram efetivas no controle de doenças pós-colheita (antracnose) e reduziu o escurecimento enzimático dos frutos de lichia (WU *et al.*, 2011). A aplicação de 1.000 ppm ClO_2 reduziu a incidência natural de podridões em figos e também reduziu a população de fungos na superfície da câmara de refrigeração (KARABULUT *et al.*, 2009).

O ClO_2 atua sobre os microrganismos de diversas formas, afetando o funcionamento de membranas e interferindo na atividade de algumas proteínas e ácido ribonucleico (ZOFFOLI *et al.*, 2005).

3.5.4 Atmosfera controlada e atmosfera modificada

O armazenamento em atmosfera controlada (AC) ou atmosfera modificada (AM) consiste no prolongamento da vida pós-colheita de produtos, por meio da modificação e/ou controle de gases no meio do armazenamento (CHITARRA e CHITARRA, 2005). Na AM ou AC, a composição inicial dos gases, presente no ambiente, é removida, adicionada ou alterada para criar uma composição atmosférica diferente da composição do ar. Para a conservação de frutas e hortaliças frescas, normalmente as concentrações de O_2 são reduzidas e as concentrações de CO_2 aumentadas, dentro de uma faixa de tolerância para cada produto. Neste

ambiente ocorre a redução da taxa respiratória e da taxa de produção de etileno (KADER, 2002a).

Na AM a alteração da composição dos gases é obtida pela combinação da taxa respiratória do vegetal e a permeabilidade do filme plástico que embala o produto. A combinação dos dois processos leva a um aumento na concentração de CO_2 e a redução da concentração do O_2 no interior da embalagem. Durante o estado de equilíbrio dos gases é mantido uma concentração de gases ideal para a conservação do produto até a sua exposição para venda (KADER, 2003). Na AC a modificação de atmosfera é feita no interior de câmaras frias onde a concentração de gases é alterada de acordo com o produto armazenado, neste sistema o nível de controle é maior do que na AM.

A AC e AM devem ser associadas a baixas temperaturas de refrigeração para evitar concentrações de CO_2 e O_2 indesejadas, pois concentrações muito baixas de O_2 podem induzir a fermentação, acelerar a deterioração do vegetal, gerar a produção de odores desagradáveis (KADER, 2003) e proporcionar condições favoráveis para o desenvolvimento de microrganismos patogênicos anaeróbicos (LIU, 2013).

Entre os benefícios proporcionados pela AM destaca-se a redução de incidência de podridões (ARTÉS-HERNÁNDEZ, TOMÁS-BARBERÁN e ARTÉS, 2006) do escurecimento da ráquis e da perda de massa por desidratação, que também ocasiona o aumento da taxa de degrana (NEVES *et al.*, 2008).

Com a crescente restrição ao uso do SO_2, a AM tem sido testada para várias uvas de mesa como a 'Niágara Rosada' (CIA *et al.*, 2010), 'Napoleon' (ARTÉS-HERNÁNDEZ, ARTÉS e TOMAS-BARBERÁN, 2003), 'Autumn Seedless' (ARTÉS-HERNÁNDEZ, AGUAYO e ARTÉS, 2004), 'Flame Seedless' (MARTÍNEZ-ROMERO *et al.*, 2003), 'Crimson Seedless' e 'Itália' (NEVES *et al.*, 2008).

A AM foi eficiente na conservação de uvas 'Autumn Seedless' durante 60 dias de armazenamento, controlando a perda de peso, a incidência do *B. cinerea* e mantendo a aceitabilidade do produto. Neste trabalho, relata-se que a atmosfera de equilíbrio de 10% CO_2 e 15% O_2 ocorreu no interior de contêineres selados com um filme de polipropileno de 35 µm, após o trigésimo dia (ARTÉS-HERNÁNDEZ, AGUAYO e ARTÉS, 2004). O uso de embalagens de polipropileno de 30 µm gerou uma modificação de atmosfera que manteve

quase inalteradas as características sensoriais e químicas de uvas de mesas 'Superior Seedless' durante o armazenamento por 7 dias a 0 ^{o}C, seguido de 4 dias a 8 ^{o}C e 2 dias a 20 ^{o}C; a associação de AM com o tratamento de SO_2 trouxe poucos benefícios em relação ao uso isolado da AM. De qualquer forma, o curto período de armazenamento não permitiu uma boa avaliação do controle da infecção do *B. cinerea* pelo uso da AM (ARTÉS-HERNÁNDEZ, TOMÁS-BARBERÁN e ARTÉS, 2006).

A acumulação de água, proveniente da condensação e da respiração, na superfície interna das embalagens pode ser um empecilho para o uso da AM para a conservação de uvas. Este problema pode ser minimizado pelo uso de filmes anti-condensação, absorvedores de umidade e uso de filmes com alta permeabilidade ao vapor de água (LICHTER, MLIKOTA GABLER e SMILANICK, 2006).

LICHTER, MLIKOTA GABLER e SMILANICK (2006) concluem, em sua revisão, que o uso da AM pode ser uma alternativa viável ao uso do SO_2, desde que seja combinado com outro método alternativo de controle das podridões fúngicas a ser aplicado antes ou durante a estocagem.

A estocagem em AC reduziu a infecção por *B. cinerea* em uvas 'Red Globe' armazenadas a 0 ^{o}C durante 12 semanas, na qual foram usadas concentrações iguais ou superiores a 10% CO_2 combinadas com 3%, 6% e 12% O_2 (CRISOSTO, GARNER e CRISOSTO, 2002a). Combinações de atmosferas de 15% CO_2 com 3-12% O_2 controlaram a infecção de *B. cinerea* em uvas 'Thompson Seedless' de colheita tardia (sólidos solúveis de 19%) armazenadas a 0 ^{o}C durante 3 meses; porém estas atmosferas não foram adequadas para uvas colhidas precocemente (sólidos solúveis totais de 16,5%) (CRISOSTO, GARNER e CRISOSTO, 2002b).

A AC e AM com altas concentrações de CO_2 (> 10% CO_2) tem sido aplicadas para o controle *B. cinerea* em uvas de mesa (CRISOSTO, GARNER e CRISOSTO, 2002a, 2002b; RETAMALES *et al.*, 2003; ROMERO *et al.*, 2006). Altas concentrações de CO_2 retardem o desenvolvimento do mofo cinzento em uvas de mesa, apresentando resultados similares aos obtidos com liberadores de SO_2 (RETAMALES *et al.*, 2003). Por outro lado concentrações iguais ou superiores a 15% CO_2 causam escurecimento da ráquis e das bagas e também

ocasionam o desenvolvimento de odores estranhos (CRISOSTO, C. H. et al., 2002a; 2002b; RETAMALES et al., 2003).

A aplicação de 20% O_2 + 20% CO_2 durante 3 dias reduziu a incidência de podridão e preservou a qualidade de uvas 'Cardinal', os cachos de uva tratados possuíam menores concentrações de ARNm estibene sintase e trans-resveratrol do que uvas não tratadas, indicando que o tratamento não induziu estresse nos cachos de uva (SANCHEZ-BALLESTA *et al.*, 2006). Nestas condições, a expressão de genes para síntese de fenilpropanóide, a acumulação de antocianinas e a atividade antioxidante foram reduzidas pela aplicação do alto CO_2 (SANCHEZ-BALLESTA *et al.*, 2007), a efetividade deste pré-tratamento no controle de podridões fúngicas e na manutenção da qualidade dos cachos de uvas, após um mês de armazenamento em baixa temperatura, advém da capacidade de prevenir a formação de espécies reativas à oxigênio ao invés da inativação destas substâncias quando formadas (ROMERO *et al.*, 2008).

A aplicação de 20% O_2 + 20% CO_2 durante três dias reduziu o escurecimento da ráquis em uvas 'Cardinal' de severo, em uvas não tratadas, para moderado, em cachos tratados. Os autores concluem que o pré-tratamento ativou respostas específicas que levaram a redução do escurecimento durante o armazenamento em baixa temperatura, prevenindo a ação de genes da biossíntese de etileno, promovendo o ajustamento osmótico e induzindo a ativação do sistema enzimático antioxidante (ROSALES *et al.*, 2013). A aplicação de 20% O_2 + 20% CO_2 durante 3 d, em morangos 'Camarosa' armazenados a 0 ^oC, preveniu a acumulação de fenóis e antocianinas e provocou a redução da concentração de sacarose, o que pode indicar uma resposta adaptativa para mitigar os efeitos da armazenagem em baixa temperatura (BODELÓN *et al.*, 2010).

Altas concentrações de O_2 é outro enfoque utilizado para o armazenamento de uvas em atmosfera controlada. A aplicação de altas concentrações de O_2 em uvas 'Kyoho' mantiveram a textura e a integridade das membranas das bagas, e reduziram a incidência de mofo cinzento (DENG, WU e LI, 2005). O armazenamento de uvas 'Kyoho' em 4% O_2 + 30% CO_2 reduziu a atividade enzimática da polifenoloxidase (PPO) e a incidência de mofo cinzento, e manteve a força de destacamento dos frutos, porém ocorreu inaceitável sabor alcoólico e escurecimento dos frutos, após 45 dias de armazenamento. Quando os cachos de

uvas foram armazenados em 4% O_2 + 9% CO_2 ou 80% O_2 elas mantiveram boa qualidade até 60 d de armazenamento (DENG, WU e LI, 2006).

A AC é utilizada comercialmente no Brasil principalmente para o armazenamento de maçãs, não sendo normalmente utilizada para a conservação de uvas. Nos EUA, a AC também não é usada para a conservação de uvas, porém tem sido empregado para o tratamento quarentenário de insetos de uvas comercializadas para a Nova Zelândia e Austrália, utilizando-se monóxido de carbono como agente inseticida (comunicação pessoal).

3.5.5 Cloreto de cálcio

A aplicação de solução com 50% de etanol + 1% $CaCl_2$ em pré-colheita reduziu as perdas devido à podridão cinzenta em 50%, em comparação com controles não tratados (CHERVIN, LAVIGNE e WESTERCAMP, 2009).

Em testes em larga escala, simulando condições práticas comerciais, duas aplicações de sal (30 e 90 d antes da colheita) de cloreto de cálcio reduziu significativamente a incidência pós-colheita de mofo cinzento. Estas aplicações reduziram a infecção de 64% entre os controles não tratados para 22%, após 30 d de armazenamento a 0 ^{o}C, apresentando melhor controle do mofo cinzento do que a aplicação da mistura dos fungicidas ciprodinil e fludioxonil (NIGRO *et al.*, 2006).

A maioria das pesquisas sobre o uso de carbonatos e sais de cálcio para controlar podridões envolvem aplicações pós-colheita, porém independentemente dos resultados, eles não são comercialmente aplicáveis, devido ao impacto negativo na aparência dos cachos de uva (IPPOLITO e NIGRO, 2000; NIGRO *et al.*, 2006). O uso do cloreto de cálcio em pré-colheita não afeta a aparência, possui baixo custo e pode ser aplicado com os mesmos equipamentos usados na aplicação de pesticidas (NIGRO *et al.*, 2006).

A imersão de uvas ‘Isabel’ em doses de $CaCl_2$ entre 0,5 e 2,0% reduziram a incidência de podridão, e a aplicação de $CaCl_2$ associado à AM reduziu a degrana durante o armazenamento refrigerado (DA SILVA *et al.*, 2012). Outro efeito positivo da imersão de uvas em $CaCl_2$ foi o aumento da concentração de frutose, que pode melhorar o sabor dos frutos (DE CARVALHO *et al.*, 2008).

NIGRO *et al.* (2006) sugerem que a inibição da atividade de poligalacturonase pelo $CaCl_2$, seja o principal mecanismo de ação sobre o *B. cinerea*. Acredita-se que a aplicação de cloreto de cálcio no início da formação do fruto favoreça a sua penetração através da epiderme, e que uma segunda aplicação aumentaria o nível de cálcio no interior dos frutos, resultando em melhor proteção do que tratamentos realizados próximos a colheita. A penetração do cloreto de cálcio na epiderme dos frutos ocorre através das aquaporinas (LIND *et al.*, 2003). Em uvas cultivadas no Chile, a aplicação de $CaCl_2$ no solo ou via foliar não alterou a concentração final de cálcio nas bagas de uva. A produção e a concentração de açúcar nos frutos também não foram alteradas (BONOMELLI e RUIZ, 2010).

O mecanismo em que o cálcio melhora a resistência dos tecidos de plantas aos patógenos não é completamente compreendido. A maior parte do cálcio, que penetra no tecido de fruta, acumula-se na região da lamela média da célula, onde exerce um efeito estabilizador, formando pontes iônicas entre e dentro dos polissacarídeos pécticos, conferindo rigidez à parede celular. A formação de ligações cruzadas entre o cálcio e polímeros de pectina tornaria a parede celular mais resistente às enzimas hidrolíticas produzidas por organismos causadores de podridões (TOBIAS *et al.*, 1993).

Referências:

ARTÉS-HERNÁNDEZ, F.; AGUAYO, E.; ARTÉS, F. Alternative atmosphere treatments for keeping quality of 'Autumn seedless' table grapes during long-term cold storage. **Postharvest Biology and Technology,** v. 31, n. 1, p. 59-67, 2004.

ARTÉS-HERNÁNDEZ, F.; ARTÉS, F.; TOMAS-BARBERÁN, F. A. Quality and enhancement of bioactive phenolics in Cv. Napoleon table grapes exposed to different postharvest gaseous treatments. **Journal of Agricultural and Food Chemistry,** v. 51, n. 18, p. 5290-5295, Aug 27 2003.

ARTÉS-HERNÁNDEZ, F.; TOMÁS-BARBERÁN, F. A.; ARTÉS, F. Modified atmosphere packaging preserves quality of SO2-free 'Superior seedless' table grapes. **Postharvest Biology and Technology,** v. 39, n. 2, p. 146-154, 2006.

BENARDE, M. A.; ISRAEL, B. M.; OLIVIERI, V. P.; GRANSTRO.ML. Efficiency of Chlorine Dioxide as a Bactericide. **Applied Microbiology,** v. 13, n. 5, p. 776-780, 1965.

BENATO, E. A.; SIGRIST, J. M. M.; OLIVEIRA, J. J. V.; DIAS, M. S. C.; CORRÊA, A. C. C. Controle de doenças pós-colheita de uva 'Itália' e avaliação dos níveis residuais de SO2 e thiabendazol. **Brazilian Journal of Food Technology,** v. 1, n. 1-2, p. 107-112, 1998.

BODELÓN, O. G.; BLANCH, M.; SANCHEZ-BALLESTA, M. T.; ESCRIBANO, M. I.; MERODIO, C. The effects of high CO2 levels on anthocyanin composition, antioxidant activity and soluble sugar content of strawberries stored at low non-freezing temperature. **Food Chemistry,** v. 122, n. 3, p. 673-678, 2010.

BONOMELLI, C.; RUIZ, R. Effects of foliar and soil calcium application on yield and quality of table grape cv. 'Thompson Seedless'. **Journal of Plant Nutrition,** v. 33, n. 3, p. 299-314, 2010.

CAMARGO, R. B.; PEIXOTO, A. R.; TERAO, D.; ONO, E. O.; CAVALCANTI, L. S. Survey of Fungi Causing Postharvest Rot in Seedless Grapes in Agricultal Polo of Juazeiro-Ba and Petrolina-Pe. **Revista Caatinga,** v. 24, n. 1, p. 15-19, 2011.

CASTRO, J. V. D. **RESFRIAMENTO, EMBALAGENS E USO DE DIÓXIDO DE ENXOFRE NA CONSERVAÇÃO E NA QUALIDADE DE UVAS (Vitis vinifera L.) 'ITÁLIA' E 'RED GLOBE'**. 1999. 109 Doutorado (Doutorado). FACULDADE DE ENGENHARIA AGRÍCOLA, UNICAMP, Campinas-SP.

CASTRO, J. V. D.; PARK, K. J.; HONÓRIO, S. L. Emprego de geradores de dióxido de enxofre na conservação de uvas 'Red Globe'. **Engenharia Agrícola,** v. 18, p. 66-75, 1998.

CENCI, S. A.; CHITARRA, M. I. F. Controle da abscisão pós-colheita de uva ´Niágara Rosada´ (Vitis Labrusca L.x vinifera L.): mecanismos decorrentes da aplicação de ANA e cálcio no campo. **Revista Brasileira de Fruticultura,** v. 16, n. 1, p. 146-155, 1994.

CHITARRA, M.I.F.; CHITARRA, A.B.. **Pós-colheita de frutas e hortaliças: fisologia e manuseio, 2 ed. rev. e amp.** ed. EDITORA UFLA, LAVRAS-MG, 2005. ISBN: 85.87692-27-5

CHERVIN, C.; AKED, J.; CRISOSTO, C. H. Grapes. In: REES, D.;FARRELL, G.*, et al* (Ed.). **Crop Post-Harvest: Science and Technology**: Blackwell Publishing Ltd, 2012. p.187-211. ISBN 9781444354652.

CHERVIN, C.; LAVIGNE, D.; WESTERCAMP, P. Reduction of gray mold development in table grapes by preharvest sprays with ethanol and calcium chloride. **Postharvest Biology and Technology,** v. 54, n. 2, p. 115-117, Nov 2009.

CHERVIN, C.; WESTERCAMP, P.; MONTEILS, G. Ethanol vapours limit Botrytis development over the postharvest life of table grapes. **Postharvest Biology and Technology,** v. 36, n. 3, p. 319-322, Jun 2005.

CHOUDHURY, M. M.; COSTA, T. S. D. Cultivo da Videira. **Sistemas de Produção, 1**, Petrolina-PE, 2004. ISSN 1807-0027. Disponível em: <

http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Uva/CultivodaVideira/colheita.htm >. Acesso em: 10/05/2013.

CHOUDHURY, M. M.; RESENDE, J. M.; COSTA, T. S. Deteriorações pós-colheita. In: CHOUDHURY, M. M. (Ed.). **Uva de mesa: pós-colheita (Frutas do Brasil, 12).** Brasília: Embrapa Informação Tecnológica, 2001. p.45-55.

CIA, P.; BENATO, E. A.; VALENTINI, S. R. D.; SANCHES, J.; PONZO, F. S.; FLORES, D.; TERRA, M. M. Modified atmosphere and cold storage for postharvest conservation of 'Niagara Rosada' table grape. **Pesquisa Agropecuária Brasileira,** v. 45, n. 10, p. 1058-1065, Oct 2010.

CONDE, C.; SILVA, P.; FONTES, N.; DIAS, A. C. P.; TAVARES, R. M.; SOUSA, M. J.; AGASSE, A.; DELROT, S.; GERÓS, H. Biochemical changes throughout grape berry development and fruit and wine quality. **Food,** v. 1, n. 1, p. 22, June 2007 2007.

CRISOSTO, C. H.; CRISOSTO, G. M. Understanding American and Chinese consumer acceptance of 'Redglobe' table grapes. **Postharvest Biology and Technology,** v. 24, n. 2, p. 155-162, Mar 2002.

CRISOSTO, C. H.; GARNER, D.; CRISOSTO, G. Carbon dioxide-enriched atmospheres during cold storage limit losses from Botrytis but accelerate rachis browning of 'Redglobe' table grapes. **Postharvest Biology and Technology,** v. 26, n. 2, p. 181-189, Sep 2002a.

_____. High carbon dioxide atmospheres affect stored 'Thompson seedless' table grapes. **Hortscience,** v. 37, n. 7, p. 1074-1078, Dec 2002b.

CRISOSTO, C. H.; MITCHELL, F. G. Postharvest handling systems: small fruits. Table grapes. In: (Ed.). **Postharvest Technology of Horticulture Crops.** Oakland, University of California, Agriculture and Natural Resources: Kader, A., 2002. p.357−363.

CRISOSTO, C. H.; SMILANICK, J. L.; DOKOOZLIAN, N. K. Table grapes suffer water loss, stem browning during cooling delays. **California Agriculture,** v. 55, n. 1, p. 39-42, 2001.

DA SILVA, R. S.; SILVA, S. D.; DANTAS, A. L.; MENDONÇA, R. M. N.; GUIMARÃES, G. H. C. Quality of 'Isabel' Grape Treated with Calcium Chloride in Postharvest and Stored under Modified Atmosphere. **Revista Brasileira de Fruticultura,** v. 34, n. 1, p. 50-56, Mar 2012.

DE CARVALHO, G. L.; LIMA, L. C. D.; SILVA, J. D.; SIQUEIRA, H. H.; MORAIS, E. C. Calcium chloride concentrations and storage time on reducing sugar contents of grape cv red globe (Vitis vinifera L). **Ciencia E Agrotecnologia,** v. 32, n. 3, p. 894-899, May-Jun 2008.

DENG, Y.; WU, Y.; LI, Y. Physiological responses and quality attributes of ‘Kyoho’ grapes to controlled atmosphere storage. **LWT - Food Science and Technology,** v. 39, n. 6, p. 584-590, 2006.

DENG, Y.; WU, Y.; LI, Y. F. Effects of high O-2 levels on post-harvest quality and shelf life of table grapes during long-term storage. **European Food Research and Technology,** v. 221, n. 3-4, p. 392-397, Aug 2005.

ELMER, P. A. G.; MICHAILIDES, T. J. Epidemiology of Botrytis cinerea in Orchard and Vine Crops. In: ELAD, Y.;WILLIAMSON, B.*, et al* (Ed.). **Botrytis: Biology, Pathology and Control**: Springer Netherlands, 2004. p.243-272. ISBN 978-1-4020-2626-3.

FOURIE, J. F. Harvesting, handling and storage of table grapes (with focus on pre- and post-harvest pathological aspects). **Acta Horticulturae,** v. 785, p. 421-424, 2008.

FRANCK, J.; LATORRE, B. A.; TORRES, R.; ZOFFOH, J. P. The effect of preharvest fungicide and postharvest sulfur dioxide use on postharvest decay of table grapes caused by Penicillium expansum. **Postharvest Biology and Technology,** v. 37, n. 1, p. 20-30, Jul 2005.

GARDEA, A. A.; CARVALLO, T.; SASTRÉ, B.; MARTÍNEZ-TÉLLEZ, M. A.; YÉPIZ-PLASCENCIA, G. M.; DÍAZ-CINCO, M.; OROZCO, J. A. TABLE GRAPE POSTHARVEST MANAGEMENT AND SAFETY ISSUES. In: DRIS, R. e JAIN, S. M. (Ed.). **Production Practices and Quality Assessment of Food Crops, "Quality Handling and Evaluation"**. Netherlands: Kluwer Academic Publishers, v.3, 2004.

GLATZ, B. A.; WISNIEWSKY, M. A.; GLEASON, M. L.; REITMEIER, C. A. Reduction of Escherichia coli O157 : H7 counts on whole fresh apples by treatment with sanitizers. **Journal of Food Protection,** v. 63, n. 6, p. 703-708, Jun 2000.

IBRAF. Comparativo das Exportações Brasileiras de Frutas Frescas - 2010/2009. **Estatísticas/Frutas Frescas**, 2013. Disponível em: < http://www.ibraf.org.br/estatisticas/Exporta%C3%A7%C3%A3o/Comparativo_das_Exporta%C3%A7%C3%B5es_Brasileiras_de_Frutas_frescas_2010-2009.pdf >. Acesso em: 02/08/2013.

IPPOLITO, A.; NIGRO, F. Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. **Crop Protection,** v. 19, n. 8-10, p. 715-723, Sep-Dec 2000.

KADER, A. A. Modified atmosphere during transport and storage. In: KADER, A. A. (Ed.). **Postharvest Technology of Horticultural Crops**. Oakland, CA: University of California, Agriculture and Natural Resources Communication Services, 2002a. cap. 14, p.135-144. ISBN 9781879906518.

KADER, A. A. Postharvest Biology and Technology: An Overview. In: KADER, A. A. (Ed.). **Postharvest Technology of Horticultural Crops**. Oakland, CA: University of California, Agriculture and Natural Resources Communication Services, 2002b. p.39-47. ISBN 9781879906518.

KADER, A. A. **Physiology of CA Treated Produce**. Proc. 8th Int. CA Conference. OOSTERHAVEN, J. e PEPPELENBOS, H. W.: Acta Hort, ISHS. 600**:** 349-356 p. 2003.

KARABULUT, O. A.; ILHAN, K.; ARSLAN, U.; VARDAR, C. Evaluation of the use of chlorine dioxide by fogging for decreasing postharvest decay of fig. **Postharvest Biology and Technology,** v. 52, n. 3, p. 313-315, Jun 2009.

KARACA, H.; SMILANICK, J. L. The influence of plastic composition and ventilation area on ozone diffusion through some food packaging materials. **Postharvest Biology and Technology,** v. 62, n. 1, p. 85-88, Oct 2011.

KASSEMEYER, H.-H.; BERKELMANN-LÖHNERTZ, B. Fungi of Grapes. In: KÖNIG, H.;UNDEN, G.*, et al* (Ed.). **Biology of Microorganisms on Grapes, in Must and in Wine**: Springer Berlin Heidelberg, 2009. cap. 4, p.61-87. ISBN 978-3-540-85462-3.

KRETSCHMER, M.; KASSEMEYER, H.-H.; HAHN, M. Age-dependent Grey Mould Susceptibility and Tissue-specific Defence Gene Activation of Grapevine Berry Skins after Infection by Botrytis cinerea. **J. Phytopathology** v. 155, p. 258-263, 2007.

LICHTER, A.; MLIKOTA GABLER, F.; SMILANICK, J. L. Control of spoilage in table grapes. **Stewart Postharvest Review,** v. 2, p. 1-10, 2006.

LIND, K.; G. LAFER; K. SCHLOFFER; INNERHOFER, G.; MEISTER, H. **Organic Fruit Growing**. CAB International 2003. 304 ISBN 0 85199 640 X.

LIU, Y.-B. Controlled atmosphere treatment for control of grape mealybug, Pseudococcus maritimus (Ehrhorn) (Hemiptera: Pseudococcidae), on harvested table grapes. **Postharvest Biology and Technology,** v. 86, n. 0, p. 113-117, 12// 2013.

LURIE, S.; PESIS, E.; GADIYEVA, O.; FEYGENBERG, O.; BENARIE, R.; KAPLUNOV, T.; ZUTAHY, Y.; LICHTER, A. Modified ethanol atmosphere to control decay of table grapes during storage. **Postharvest Biology and Technology,** v. 42, n. 3, p. 222-227, 2006.

LUVISI, D.; SHOREY, H.; SMILANICK, J.; THOMPSON, J.; GUMP, B.; KNUTSON, J. **Sulfur Dioxide Fumigation of Table Grapes**. Universty of California, DANR, Bulletin 1932**:** 22 p. 1992.

LUVISI, D. A.; SHOREY, H. H.; THOMPSON, J. F.; HINSCH, T.; SLAUGHTER, D. C. **Packaging California Table Grapes**. Division of Agriculture and Natural Resources, University of California, 1995, 1995.

LYDAKIS, D.; AKED, J. Vapour heat treatment of Sultanina table grapes. II: Effects on postharvest quality. **Postharvest Biology and Technology,** v. 27, n. 2, p. 117-126, Feb 2003.

MAPA. Anexo II: Regulamento técnico de identidade e de qualidade para a classificação da uva fina de mesa. . **D.O.U., 04/02/2002**, 2002.

MARI, M.; BERTOLINI, P.; PRATELLA, G. C. Non-conventional methods for the control of post-harvest pear diseases. **Journal of Applied Microbiology,** v. 94, n. 5, p. 761-766, 2003.

MARTÍNEZ-ROMERO, D.; GUILLÉN, F.; CASTILLO, S.; VALERO, D.; SERRANO, M. Modified atmosphere packaging maintains quality of table grapes. **Journal of Food Science,** v. 68, n. 5, p. 1838-1843, Jun-Jul 2003.

MELLO, L. M. R. D. **Vitivinicultura brasileira: Panorama 2009**. Bento Gonçalves, RS: Embrapa Uva e Vinho**:** 4 p. 2010.

_____. **Atuação do Brasil no Mercado Vitivinícola Mundial – panorama 2010**. Bento Gonçalves, RS: Embrapa Uva e Vinho**:** 3 p. 2011.

_____. **Vitivinicultura brasileira: Panorama 2011**. Comunicado Técnico. Bento Gonçalves, RS: Embrapa Uva e Vinho. 115**:** 4 p. 2012a.

_____. **Vitivinicultura mundial: principais países e posição do Brasil**. Comunicado Técnico. Bento Gonçalves-RS. 121 2012b.

MLIKOTA GABLER, F.; SMILANICK, J. L.; MANSOUR, M. F.; KARACA, H. Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes. **Postharvest Biology and Technology,** v. 55, n. 2, p. 85-90, 2010.

NELSON, K. E. **Harvesting and handling California table grapes for market**. [Berkeley]: University of California, 1985. 72 p.

NEVES, L. C.; DA SILVA, V. X.; BENEDETTE-MARCOS, R. M.; PRILL, A. D.; VIEITES, R. L.; ROBERTO, S. R. Conservation of grapes "Crimson seedless" and "Itália", submitted to different types of packings and sulfur dioxide (SO2). **Revista Brasileira de Fruticultura,** v. 30, n. 1, p. 65-73, Mar 2008.

NIGRO, F.; SCHENA, L.; LIGORIO, A.; PENTIMONE, I.; IPPOLITO, A.; SALERNO, M. G. Control of table grape storage rots by pre-harvest applications of salts. **Postharvest Biology and Technology,** v. 42, n. 2, p. 142-149, Nov 2006.

OLMEZ, H.; KRETZSCHMAR, U. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. **Lwt-Food Science and Technology,** v. 42, n. 3, p. 686-693, Apr 2009.

PALOU, L.; CRISOSTO, C. H.; GARNER, D.; BASINAL, L. M. Effect of continuous exposure to exogenous ethylene during cold storage on postharvest decay development and quality attributes of stone fruits and table grapes. **Postharvest Biology and Technology,** v. 27, n. 3, p. 243-254, Mar 2003.

PALOU, L.; CRISOSTO, C. H.; GARNER, D.; BASINAL, L. M.; SMILANICK, J. L.; ZOFFOLI, J. P. Minimum constant sulfur dioxide emission rates to control gray mold of cold-stored table grapes. **American Journal of Enology and Viticulture,** v. 53, n. 2, p. 110-115, 2002.

PAO, S.; KELSEY, D. F.; KHALID, M. F.; ETTINGER, M. R. Using aqueous chlorine dioxide to prevent contamination of tomatoes with Salmonella enterica and Erwinia carotovora during fruit washing. **Journal of Food Protection,** v. 70, n. 3, p. 629-634, Mar 2007.

PEARSON, R. C.; GOHEEN, A. C. **Compendium of grape diseases**. 1988.

RETAMALES, J.; DEFILIPPI, B. G.; ARIAS, M.; CASTILLO, P.; MANRIQUEZ, D. High-CO2 controlled atmospheres reduce decay incidence in Thompson Seedless and Red Globe table grapes. **Postharvest Biology and Technology,** v. 29, n. 2, p. 177-182, Aug 2003.

ROMERO, I.; SANCHEZ-BALLESTA, M. T.; MALDONADO, R.; ESCRIBANO, M. I.; MERODIO, C. Expression of class I chitinase and β-1,3-glucanase genes and postharvest fungal decay control of table grapes by high CO2 pretreatment. **Postharvest Biology and Technology,** v. 41, n. 1, p. 9-15, 2006.

ROMERO, I.; TERESA SANCHEZ-BALLESTA, M.; MALDONADO, R.; ISABEL ESCRIBANO, M.; MERODIO, C. Anthocyanin, antioxidant activity and stress-induced gene expression in high CO2-treated table grapes stored at low temperature. **J Plant Physiol,** v. 165, n. 5, p. 522-30, 2008.

ROSALES, R.; FERNANDEZ-CABALLERO, C.; ROMERO, I.; ESCRIBANO, M. I.; MERODIO, C.; SANCHEZ-BALLESTA, M. T. Molecular analysis of the improvement in rachis quality by high CO2 levels in table grapes stored at low temperature. **Postharvest Biology and Technology,** v. 77, n. 0, p. 50-58, 3// 2013.

SANCHEZ-BALLESTA, M. T.; JIMÉNEZ, J. B.; ROMERO, I.; OREA, J. M.; MALDONADO, R.; UREÑA, Á. G.; ESCRIBANO, M. I.; MERODIO, C. Effect of high CO2 pretreatment on quality, fungal decay and molecular regulation of stilbene phytoalexin biosynthesis in stored table grapes. **Postharvest Biology and Technology,** v. 42, n. 3, p. 209-216, 2006.

SANCHEZ-BALLESTA, M. T.; ROMERO, I.; JIMÉNEZ, J. B.; OREA, J. M.; GONZÁLEZ-UREÑA, Á.; ESCRIBANO, M. I.; MERODIO, C. Involvement of the phenylpropanoid pathway in the response of table grapes to low temperature and high CO2 levels. **Postharvest Biology and Technology,** v. 46, n. 1, p. 29-35, 2007.

SMILANICK, J. L.; MANSOUR, M. F.; GABLER, F. M.; MARGOSAN, D. A.; HASHIM-BUCKEY, J. Control of Postharvest Gray Mold of Table Grapes in the San Joaquin Valley of California by Fungicides Applied During the Growing Season. **Plant Disease,** v. 94, n. 2, p. 250-257, Feb 2010.

TOBIAS, R. B.; CONWAY, W. S.; SAMS, C. E.; GROSS, K. C.; WHITAKER, B. D. Cell-Wall Composition of Calcium-Treated Apples Inoculated with Botrytis-Cinerea. **Phytochemistry,** v. 32, n. 1, p. 35-39, Jan 1993.

VARDAR, C.; ILHAN, K.; KARABULUT, O. A. The application of various disinfectants by fogging for decreasing postharvest diseases of strawberry. **Postharvest Biology and Technology,** v. 66, p. 30-34, Apr 2012.

WU, B.; LI, X. P.; HU, H. G.; LIU, A. Y.; CHEN, W. X. Effect of chlorine dioxide on the control of postharvest diseases and quality of litchi fruit. **African Journal of Biotechnology,** v. 10, n. 32, p. 6030-6039, Jul 4 2011.

ZAHAVI, T.; COHEN, L.; WEISS, B.; SCHENA, L.; DAUS, A.; KAPLUNOV, T.; ZUTKHI, J.; BEN-ARIE, R.; DROBY, S. Biological control of Botrytis, Aspergillus and Rhizopus rots on table and wine grapes in Israel. **Postharvest Biology and Technology,** v. 20, n. 2, p. 115-124, Sep 2000.

ZOFFOLI, J. P.; LATORRE, B. A.; DAIRE, N.; VIERTEL, S. Effectiveness of chlorine dioxide as influenced by concentration, pH, and exposure time on spore germination of *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer*. **Ciencia e Investigacion Agraria,** v. 32, n. 3, p. 181-188, 2005.

ZOFFOLI, J. P.; LATORRE, B. A.; NARANJO, P. Hairline, a postharvest cracking disorder in table grapes induced by sulfur dioxide. **Postharvest Biology and Technology,** v. 47, n. 1, p. 90-97, 2008.

ZOFFOLI, J. P.; LATORRE, B. A.; RODRIGUEZ, J.; AGUILERA, J. M. Biological indicators to estimate the prevalence of gray mold and hairline cracks on table grapes cv. Thompson Seedless after cold storage. **Postharvest Biology and Technology,** v. 52, n. 1, p. 126-133, 2009.

4. CAPÍTULO 2:

Prestorage application of high carbon dioxide combined with controlled atmosphere storage as a dual approach to control *Botrytis cinerea* in organic 'Flame Seedless' and 'Crimson Seedless' table grapes[1]

Aplicação de alta concentração de CO_2 em pré-estocagem combinada com atmosfera controlada como uma alternativa de tratamento orgânico para o controle de Botrytis cinerea em uvas de mesa, ´Flame Seedless´ e 'Crimson Seedless'

[1] Artigo submetido ao periódico científico: Postharvest Biology and Technology em 13/05/2013.

Prestorage application of high carbon dioxide combined with controlled atmosphere storage as a dual approach to control *Botrytis cinerea* in organic 'Flame Seedless' and 'Crimson Seedless' table grapes

Cesar S Teles[ac]; Benedito C. Benedetti[a], W. Douglas Gubler[b], Carlos H. Crisosto[c*]

[a] Universidade Estadual de Campinas-SP, Faculdade de Engenharia Agrícola, FEAGRI, Av. Cândido Rondon 501, Campinas-SP, Brazil.

[b] Department of Plant Pathology, University of California, Davis, One Shields Avenue, 95616 Davis, CA, United States.

[c] Department of Plant Sciences, University of California, Davis, One Shields Avenue, 95616 Davis, CA, United States.

*Corresponding author at: University of California Davis, Department of Plant Sciences Mail Stop 2 One Shields Avenue, Davis, CA 95616 USA. Phone: +1 530.752.7549, fax: 530.752.8502. Email address: chcrisosto@ucdavis.edu (Carlos H. Crisosto).

Key words: : Gray mold; quality; flavor; SO_2 alternative; conidia germination; mycelium growth; sulfite free.

Abstract:

Pre-storage application of 40% CO_2 at 0 ^oC for 24 or 48 h and controlled atmosphere (12% O_2 + 12% CO_2) storage at 0 ^oC for up to eight weeks on decay control and quality of organic 'Flame Seedless' and 'Crimson Seedless' table grapes were studied as a postharvest disease control alternative. To simulate different potential field conditions, these organic treatments were applied to organic-grown grapes that were naturally infected (without inoculation), surface inoculated (berries inoculated by spraying with a conidia suspension), and nesting

inoculated (clusters inoculated by placing in the middle an artificially infected berry) with the pathogen *Botrytis cinerea*, the cause of grape gray mold. Under these three conditions, a 40% CO_2 for 48 h pre-storage treatment followed by controlled atmosphere reduced the gray mold incidence from 22% to 0.6% and from 100% to 7.4% after four and seven weeks, respectively. High CO_2 pre-storage alone limited botrytis incidence in both naturally and artificially infected grapes, but was more effective when combined with CA. These treatments did not affect visual or sensory fruit quality. Exposure to high CO_2 for 24 or 48 h effectively inhibited mycelial growth of *B. cinerea* in PDA plates incubated at 22 ^{o}C for up to 72 h. Conidia germination in PDA plates was reduced ~ 60% after 12 h incubation. *In vitro* studies demonstrated a fungistatic effect, but further studies on the mechanism of action could improve treatment performance. This novel high CO_2 initial fumigation followed by controlled atmosphere during storage or transportation could be a commercially feasible alternative for postharvest handling of organic and conventional table grapes. Our results encourage validating this combined physical treatment in other cultivars and under commercial conditions.

Highlights:

- A short, high CO_2 exposure followed by CA controls gray mold in table grapes.
- This new, organic approach controls gray mold without affecting sensory quality.
- In *in vitro* experiments, application of 40% CO_2 significantly reduced mycelium growth.

4.1 Introduction

Fungal decay is the primary cause of rapid and extensive postharvest deterioration in table grapes. The major disease is gray mold caused by *Botrytis cinerea*, which can grow at temperatures as low as – 0.5 ^{o}C and spreads rapidly by means of aerial mycelium among the berries (Crisosto and Mitchell, 2002). If grapes are not treated after harvest and/or during storage, gray mold infection can affect the majority of the berries, leading to substantial losses.

The three main mechanisms of infection that cause postharvest losses to botrytis in table grapes are: latent infection during growth and development before harvest; infection from conidia present in the air and/or on the surface of the berries; and nesting infection from visibly infected berries that have escaped removal during packaging (Luvisi et al., 1992; Lichter et al., 2006). The contribution from each type of infection varies, depending on the incidence and severity of field infections, storage conditions, and inoculum amounts. However, maturity and length of storage increase a berry's susceptibility to infection and decay symptoms during postharvest handling. Decay is visible as "slip-skin" (separation of the skin from the flesh upon touch) accompanied by red-brown coloration (easily scored on white varieties), resulting in formation of "nests" (Luvisi et al., 1992; Chervin et al., 2012).

Efficient postharvest control of gray mold is commonly achieved using weekly applications of sulfur dioxide (SO_2) gas in storage rooms, following an initial SO_2 application prior to cold storage (Luvisi et al., 1992), and/or by packing the grapes with a pad or generator containing sodium metabisulfite, which releases SO_2 when hydrated by water vapor inside the boxes (Droby and Lichter, 2004). A series of SO_2 fumigations are the commercial practice applied in California and the SO_2 generating pad is used in Chile, Israel, South Africa, and by most California shippers who transport long distance (Droby and Lichter, 2004). Despite its efficacy at controlling gray mold, the SO_2 technology may compromise fruit taste, cause damage to berries (evident as hairline cracks and bleaching), contribute to air pollution, and potentially be corrosive to metal equipment within storage facilities. Sulfite residues cause reactions in people allergic to sulfite. Thus, a tolerance limit of 10 $\mu L\ L^{-1}$ for sulfite residues in table grapes was established by the U.S. Environmental Protection Agency (Anonymous, 1989). Human health and worker exposure concerns imposed dramatic changes to the amount of SO_2 applied by improving exposure efficiency without creating greater loss (Luvisi et al., 1992; Crisosto and Mitchell, 2002). Neither synthetic fungicides nor SO_2 can be classified as organic, and therefore, these are not allowed on organic grapes (Romanazzi et al., 2012). There are also strict regulations regarding synthetic fungicides and SO_2 residues in important European markets (Nigro et al., 2006). Some alternatives to SO_2 have been studied: application of ethanol (Karabulut et al., 2003), ethanol combined with chitosan or calcium chloride (Romanazzi et al., 2007; Chervin et al., 2009), organic salts (Nigro et al., 2006), controlled atmosphere (Crisosto et al., 2002a, 2002b; Retamales et al., 2003; Artés-Hernández

et al., 2004; Chen et al., 2011), or ozone (Palou et al., 2002; Smilanick et al., 2010). These methods lack enough support to replace SO_2 as a commercial practice.

The organic vegetables and fruits market has expanded due to the increasing number of consumers demanding healthy food that is free of pesticide residues. In 2009, U.S. sales of organic fruits and vegetables were $ 7.8 billion (USDA, 2012). The organic system focuses on sustainable production and the use of readily soluble mineral fertilizers and synthetic chemical pesticides is forbidden (Lind et al., 2003). Options for organic technologies controlling gray mold have been evaluated: dipping berries in an ethanol solution (Lichter et al., 2002), heated water or ethanol solutions (Mlikota Gabler et al., 2005), or organic salts (Nigro et al., 2006). The use of a high carbon dioxide (CO_2) atmosphere to control *B. cinerea* in table grapes has been studied (Crisosto et al., 2002a, 2002b; Sanchez-Ballesta et al., 2006; Chen et al., 2011). Although high CO_2 effectively delays decay development, it also causes rachis browning and off flavors, particularly in early-harvested fruits with low soluble solids concentrations (Crisosto et al., 2002a, 2002b; Retamales et al., 2003). Therefore, organic growers only have two promising commercial treatments currently available to them: pre-harvest application of $CaCl_2$, and postharvest ozone atmosphere during storage and transportation (Romanazzi et al., 2012).

In preliminary studies (Teles, unpublished results), we applied various CO_2 concentrations for 24 or 48 h and examined the effect on nesting formation on inoculated grapes. These results led to the selection of 40% CO_2 applied for 24 or 48 h to replace the SO_2 initial fumigation. Thus, our objective was to test our combined organic approach that consisted of an initial high CO_2 application prior to cold storage, followed by controlled atmosphere (12% O_2 + 12% CO_2) during storage and/or transportation, for their effects on decay incidence and quality of 'Flame Seedless' and 'Crimson Seedless' table grapes. This combined approach can be a reliable alternative to SO_2 applications for organic table grape growers and shippers.

4.2 Materials and methods

4.2.1 Plant material

'Crimson Seedless' and 'Flame Seedless' certified organic table grapes (*Vitis vinifera*) were commercially harvested in Delano, CA, immediately transported to the Postharvest Laboratory at UC Davis, and air-cooled to a berry temperature of 1 °C.

4.2.2 Inoculation

Prior to the treatments, 'Flame Seedless' grape clusters were labeled and randomly divided into three groups: 1. naturally infected (without inoculation), 2. surface inoculated (berries inoculated by spraying with a conidia suspension) and 3. nesting inoculation (inoculated with an infected berry). After inoculation, grape clusters (800 to 1000 g) were placed inside perforated plastic cluster bags and packed into expanded polystyrene (EPS) boxes (nine cluster bags with three bags from each inoculation group). 'Crimson Seedless' clusters were not inoculated because they were harvested in a vineyard with a historic high incidence of gray mold.

B. cinerea (isolate IC 08, from T.J. Michailides, UC Davis) was incubated on PDA in 85 mm Petri dishes, the conidia were extracted, and the suspension was adjusted to a concentration of 2×10^4 conidia/mL and sprayed on the surface of the clusters (Palou et al., 2002). Grapes were air-dried for an hour before the treatment applications. For nesting inoculated fruits, a 10 µL suspension of 2×10^6 *B. cinerea* conidia/mL was injected 10 mm deep into the flesh of individual berries using a Hamilton syringe (needle 1 mm external diameter) and incubated at 20 °C for 5 d. One inoculated berry was placed in the middle of the bagged cluster prior to treatment applications.

4.2.3 Treatment

After inoculation, fruits were weighed, packed, and grape boxes were stored at 0 ± 0.5 °C with 95 to 98% relative humidity (RH) inside 338 L sealed metal tanks with a continuous flow of either air (atmospheric air) for 48 h, or 40% CO_2 (40% CO_2 + 60% N_2) applied for 24 h or 48 h (initial fumigation). After these pre-cooled storage treatments, grapes were again cold stored in air or controlled atmosphere (CA), which consisted of 12% O_2 + 12% CO_2 + 76% N_2 (Crisosto et al., 2002a). Flow rates and gas mixtures were established using a mixing board with micro-metering valves. Supply and exhaust gas composition was monitored using a

CO_2/O_2 gas analyzer (model 900141, Bridge Analyzers Inc., Alameda, CA). 'Flame Seedless' grapes were stored at 0 ± 0.5 °C with 95 to 98% RH for four or seven weeks, then removed from the tanks and stored for 48 h at 20 ± 1.0 °C to simulate shelf life (SL). 'Crimson Seedless' grapes were stored for eight weeks under the same conditions.

4.2.4 Decay incidence

Decay incidence in 'Flame Seedless' grapes was measured in naturally infected, surface inoculated, and nesting inoculated grapes after four weeks storage at 0 °C, four weeks at 0 °C + SL, seven weeks at 0 °C, and seven weeks at 0 °C + SL. In naturally infected 'Crimson Seedless' grapes, decay incidence was evaluated after three weeks storage at 0 °C, three weeks at 0 °C + SL, six weeks at 0 °C, six weeks at 0 °C + SL, and eight weeks at 0 °C. Decay incidence was measured as the weight of the decayed berries after removal and expressed as percentage total cluster weight. After each evaluation, all fruits were discarded.

For the evaluation at eight weeks 0 ± 0.5 °C + 1, 2, or 3 days, the decayed berries were removed, weighed, and then the remaining healthy berries were stored at 20 °C until the next day's evaluation.

4.2.5 Quality evaluation

Evaluation of grape quality included measurements of weight loss, rachis browning, color, berry firmness (maximum force and percent deformation), soluble solids concentration (SSC), and titratable acidity (TA) in naturally infected fruits. Six cluster bags (replicates) per treatment were evaluated before the treatments and after four and seven weeks storage at 1 °C. Weight loss was determined with a scale (LC 22016, Sartorius, Elk Grove, IL), accurate to 0.01 g, and expressed as the percentage of initial weight. Rachis browning was evaluated using a subjective scoring in which 1 = green and fresh, 2 = green and partially dry, 3 = dry and brownish green, 4 = dry and brown, and 5 = very dry, brown, and brittle (Mlikota Gabler et al., 2005). Fifteen berries from each replicate were randomly detached from the cluster for color, firmness, SSC, and TA measurements. The surface color of the berries was measured with a Chroma meter (CR400 model, Konica Minolta Optics, Japan) using the CIELAB color system. Color index for red grapes (CIRG) (Carreño et al., 1995) was calculated as CIRG = $(180 - h^o)/(C^* + L^*)$, where L^* is the lightness corresponding to a black-white scale (0, black; 100, white), h^o is the hue angle on the color wheel, and C^* is the Chroma (measuring the

intensity of color, beginning from zero (achromatic) as it increases in intensity. Berry firmness was measured using a texture analyzer (TA XT, Stable Micro Systems Ltd, UK) with a 2 mm probe that penetrated to a depth of 6 mm at a speed of 1 mm/s. The maximum force (N) necessary to puncture the skin of an individual berry was measured and berry deformation (%) was determined.

Fifteen berries per replicate were filtered through two layers of cheesecloth to extract the juice. SSC was measured with a refractometer (PR-32α, Atago, Japan) and the titratable acidity (TA) was determined from the same juice sample by titrating with 0.1 N NaOH to pH 8.2, and expressed as percentage tartaric acid.

4.2.6 Acetaldehyde and ethanol

Acetaldehyde and ethanol concentrations were determined after 'Flame Seedless' grapes were treated with Air, 40% CO_2 for 24 h + Air, and 40% CO_2 for 48 h + Air, following one or two weeks storage at 0 ^{o}C. Samples of all treatments (Air and CA stored) were taken at four and seven weeks storage at 0 ^{o}C. Five mL juice was incubated for 1 h at 65 ^{o}C in a septum-capped tube (Ke et al., 1991). The acetaldehyde and ethanol concentrations in 1 mL headspace gas were determined using a gas chromatograph (model GC-9 AM; Shimadzu, Kyoto, Japan) with a flame ionization detector (250 ^{o}C) and a 5% carbowax on 60/80 Carbopack column (Supelco, Bellefonte, PA). Volatiles were quantified through comparison to known standards and the juice sample concentration was expressed as μL L^{-1} (Ke et al., 1991). Six replicates per treatment were analyzed.

4.2.7 Sensory triangle test

To test whether high CO_2 followed by CA affected the flavor of 'Flame Seedless' table grapes, grapes cold stored for seven weeks in air or treated with 40% CO_2 for 48 h followed by CA were compared. Twenty untrained panelists participated in a triangle test with three randomized samples of three berries each, one different sample and two alike. The panelists were instructed to eat the berries and identify the odd sample (Meilgaard et al., 1999).

4.2.8 Effect of high CO_2 on in vitro mycelial development of *B. cinerea*

The effect of high CO_2 on mycelial growth of *B. cinerea* was assayed in PDA. An 8 mm diameter plug of agar containing mycelia was obtained from the growing edge of 3 d old *B. cinerea* cultures and placed in the center of an 85 mm diameter Petri dish of PDA medium.

The Petri dishes were exposed to a 200 mL/min flow of 40% CO_2 for 24 or 48 h and the controls were exposed to air at the same flow for 24 or 48 h. The treatments were applied inside 7.8 L tanks stored in a cold room at 0 ^{o}C with a RH of 95 to 98%. Radial growth of *B. cinerea* was recorded at 0, 24, 48, and 72 h after incubation at 22 °C in the dark. To determine whether the effects observed during *in vitro* studies were influenced by the direct impact of CO_2 on the culture medium, dishes with PDA were exposed to 40% CO_2 as above, prior to inoculation. Relative growth (RG) was estimated for each interval based on the ratio of the mean of the diameter, less 8 mm at each interval, divided by the mean diameter, less 8 mm, of colonies that were not exposed to CO_2 for the same time interval. Relative inhibition growth (RIG) was estimated using the equation RIG% = 100 − RG. For each treatment, six replicate dishes were prepared and the experiment was repeated once.

4.2.9 High CO_2 effects on conidial viability in vitro

Conidia from 10 to 11 d old colonies of *B. cinerea* were collected by adding 10 mL sterile deionized water to each Petri dish. The density of the conidia suspension was measured with a haemacytometer (Brightline, NY, USA) and adjusted to 2×10^5 conidia/mL, then 100 µL suspension was placed onto 85 mm Petri dishes containing 20 mL PDA. The Petri dishes were exposed to a 200 mL/min^{-1} flow of 40% CO_2 for 24 or 48 h, and controls were exposed to air at the same flow for 24 or 48 h. The treatments were applied inside 7.8 L tanks stored in a cold room at 0 ± 0.5 ^{o}C and 95% RH. After treatments, the dishes were transferred to an incubation chamber at 22 ^{o}C in the dark for 24 h. The extent of germination of 100 conidia per dish was assessed by microscopic observation (200x). Conidia with germ tubes longer than the conidial diameter were considered germinated. For each treatment, six replicate dishes were prepared and the experiment was repeated once.

4.2.10 Statistical analysis

A factorial design was used, with three pre-storage and two storage conditions as factors for six treatments (3x2), and six replicates at each evaluation date of 'Flame Seedless'. Each of the six replicates per treatment and evaluation date consisted of one grape cluster of 800 to 1000 g. Decay incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before the analysis. For mycelial growth and conidial germination, six replicate dishes were prepared for each treatment, the experiment was repeated once, and the results

presented are the average of both experiments.

The data were subjected to an analysis of variance (ANOVA) and the means were separated using Tukey's test ($p \leq 0.01$ or $p \leq 0.05$) or an unpaired *t* test ($p \leq 0.05$).

4.3 Results

4.3.1 Decay Incidence

Some two-way interactions between the pre-storage and storage factors were significant; these are indicated in the tables. When the interactions were not significant, the factors effects are discussed in the text. There were no significant differences between untreated (control) and 40% CO_2 for 24 h + CA on naturally infected 'Crimson Seedless' until six weeks cold storage. Decay incidence became important after six weeks, when control grapes had 4.1% decay and treated grapes, only 0.4% decay. After six weeks + SL, treated grapes had ~3% decay while the control reached ~27% (Table 1). The 40% CO_2 for 24 h + CA still reduced decay incidence after 3 d SL at 20 ± 1 ◦C, simulating commercial marketing after eight weeks cold storage.

During cold storage, the natural incidence of gray mold decay in untreated 'Flame Seedless' grapes increased from 22% to 100% over the seven-week evaluation (Table 2). At four weeks, gray mold was significantly controlled by pre-storage and storage treatments. Grapes stored under CA exhibited approximately seven times less decay (2%) than did grapes stored under air (16%) (data not shown). By 4 weeks + SL, the significantly effect on the reduction of decay became evident with the pre-storage treatment of high CO_2, as well as the storage conditions. At this time, the decay incidence for berries under air was again significantly higher (72%) than under CA (16%). A similar effect was observed for pre-storage in 40% CO_2 for 48 h alone (40%) compared with the control (56%) (data not shown). By seven weeks, untreated grapes reached 100% decay and interaction between pre-storage and storage treatments was significant ($p \leq 0.0009$). At this time, the best gray mold control treatment was pre-storage with 40% CO_2 combined with CA, followed by Air + CA. Grapes from the other treatments were completely decayed by gray mold. After 7 weeks + SL, the decay incidence in all treatments was > 90% (Table 2).

In surface inoculated grapes, the decay incidence in control grapes increased from 26% at four weeks cold storage to 99% at seven weeks (Table 3). A significant interaction between pre-storage high CO_2 and storage conditions was observed up to seven weeks cold storage. After 4 weeks, fruits treated with high CO_2 + CA did not exhibit any decay, while the treatments combined with air yielded a decay incidence of 10-34%. At four weeks + SL, the order of decay control, from greatest to least, was 40% CO_2 for 48 h + CA (4%), 40% CO_2 for 24 h + CA (7%), and Air + CA storage (25%). At the same time, grapes from the remaining treatments had gray mold incidence > 93%. After seven weeks cold storage, the 40% CO_2 for 48 h + CA, 40% CO_2 for 24 h + CA and Air + CA treatments again yielded the highest decay control at 27%, 50%, and 75% infection, respectively, while the other treatments had > 95% decay (Table 3). At seven weeks + SL, all treatments exhibited very high botrytis infection rates. Surface inoculated and naturally infected both yielded > 95% gray mold incidence.

In nesting inoculated table grapes, the interaction between high CO_2 pre-storage and storage conditions significantly affected decay incidence (Table 4). By four weeks, grapes treated with 40% CO_2 for 48 h + CA and 40% CO_2 for 24 h + CA exhibited 38 times less decay (0.5%) than did grapes stored under air (19%). At four weeks + SL, the decay spread very quickly in grapes stored in air, covering 98% of the clusters. Once more, treatments with 40% CO_2 for 48 h + CA and 40% CO_2 for 24 h + CA had the lowest infection rates at 10% and 11%, respectively. After seven weeks cold storage, the order of decay control, from greatest to least, was 40% CO_2 for 48 h + CA, 40% CO_2 for 24 h + CA, and Air + CA storage, with decay incidence of 11%, 25%, and 32%, respectively. At this time, clusters stored in air exhibited > 98% decay incidence (Table 4). After seven weeks + SL, the 40% CO_2 for 48 h + CA clusters still had less decay, at 84%, than clusters from the other treatments, which were completely covered by decay. Throughout storage, irregardless of inoculation method, CA storage significantly reduced decay incidence below that of clusters stored in air (data not shown).

4.3.2 Quality evaluation

At harvest, ‘Flame Seedless’ grapes had 18.2% SSC, 0.52% tartaric acid, a rachis browning rating of 2.4, 3.7 N maximum penetration force, and 19.5% deformation, while ‘Crimson Seedless’ had 20.4% SSC, 0.65% tartaric acid, 4.5 N maximum penetration force, and 17.0% deformation.

The quality of 'Flame Seedless' grapes was evaluated during storage. The treatments did not significantly affect rachis browning, SSC, TA, weight loss, or color, these values remained similar to those at harvest (data not shown). The TA after seven weeks cold storage (0.53%) was similar to the TA before storage (0.52%). The grapes retained a CIRG of 4.4 (red) from harvest through storage. After four weeks cold storage, fruits stored in air required 12% less maximum force to penetrate. The 40% CO_2 for 24 h + CA and 40% CO_2 for 48 h + CA treatments maintained fruit firmness during four weeks storage. At seven weeks, no significant difference between treatments was detected, but the maximum penetration force was ~20% lower than that required to penetrate the fruits prior to storage (Table 5). After four weeks cold storage, berry deformation was more pronounced in the control (13.6%) than in fruits pre-stored with 40% CO_2 for 24 h (16.7%) or 40% CO_2 for 48 h (15.8%). Storage also affected berry deformation, as grapes stored in air had less deformation (14.7%) than fruits stored in CA (16.0%). After seven weeks storage, the treatments stored in air could not be analyzed due to the high decay incidence. Grapes stored in CA had berry deformation values similar to that at harvest: 19.2% and 19.5%, respectively. A similar situation occurred in 'Crimson Seedless', where quality was not affected by the combined treatment (data not shown).

4.3.3 Acetaldehyde (AA) and ethanol

The AA concentration in fruits stored in air increased from 2.1 μL L^{-1} before storage to 4.0 μL L^{-1} by week four (Table 6). After pre-storage treatment, grapes treated with 40% CO_2 yielded about four times more AA than did grapes pre-stored under air: 8.8 to 9.5 and 2.1 μL L^{-1}, respectively. After one week cold storage, 40% CO_2 for 48 h + Air had the most AA at 18.9 μL L^{-1} and 40% CO_2 for 24 h + Air had 8.6 μL L^{-1}. To measure the effect of 40% CO_2 pre-storage alone, concentrations of acetaldehyde and ethanol were analyzed weekly for four weeks in fruits stored in air. The AA concentration in these grapes dropped with pre-storage at 40% CO_2, with no significant difference at four weeks among treatments of fruits stored in Air (Table 6). For fruits stored in CA for four weeks, those treated with 40% CO_2 for 48 h + CA had 12.4 μL L^{-1} AA, but the concentration fell to 3.0 μL L^{-1} after seven weeks cold storage, with no significant difference with 40% CO_2 for 24 h + CA and Air + CA. Ethanol was not detected in 'Flame Seedless' organic table grapes before treatment. During four weeks cold storage, the ethanol concentration in Air stored fruits increased from 0 to 100 μL L^{-1} (Table 6).

Pre-storage with 40% CO_2 for 24 h increased the ethanol concentration from 0 to 79.7 µL L^{-1} and for 48 h, from 0 to 228 µL L^{-1}. After four weeks storage, 40% CO_2 + Air fruit did not have a significantly different ethanol concentration. At this time, 40% CO_2 for 48 h + CA yielded the highest ethanol concentration (486 µL L^{-1}). By seven weeks, fruits stored in air could not be analyzed because of extensive decay and the treatments of fruits stored in CA had no significant differences in ethanol concentrations ($p \leq 0.1124$).

4.3.4 Sensory triangle test

There was no significant difference between the taste of samples stored in Air and CA ($p \leq 0.01$). Untrained panelists could not perceive flavor differences between air and treated 'Flame Seedless' table grapes (40% CO_2 for 48 h + CA) stored seven weeks.

4.3.5 Effect of high CO_2 on *in vitro* mycelium development and conidial viability of *B. cinerea*

40% CO_2 for 24 or 48 h significantly inhibited *B. cinerea* mycelial growth up to 72 h after treatment ($p \leq 0.01$). The relative inhibition of growth (RIG) was enhanced by increased time at 40% CO_2 ($p < 0.01$). At 24 h, *B. cinerea* treated with 40% CO_2 for 48 h had a RIG of 92% and for 24 h, 69% (Fig. 1). After 72 h incubation, *B. cinerea* treated with 40% CO_2 for 48 h had a RIG of 39%, with 18% for fungi treated for 24 h. Pre-treating the culture dishes with 40% CO_2 before adding mycelial plugs did not affect *B. cinerea* growth (data not shown). After 12 h dark incubation at 22 ^{o}C, *B. cinerea* conidia germination was reduced significantly by 40% CO_2 ($p \leq 0.05$), from 93% in the control to 39% or 37% in conidia treated with 40% CO_2 for 24 or 48 h, respectively. After 24 h incubation, no significant differences among treatments were found. Germination in treated conidia was 95% or 96% of the control (data not shown).

4.4 Discussion

Under three postharvest infection conditions (naturally infected, surface inoculated, or nesting inoculated grapes), 40% CO_2 pre-storage + CA limited decay incidence in 'Flame seedless' organic table grapes throughout seven weeks cold storage and throughout eight weeks in 'Crimson Seedless'. We assume that surface inoculated and naturally infected fruits represent

a wide range of potential field conditions. Placing infected berries into the middle of the cluster simulated a situation where fast packing and/or lack of sanitation occurs. In addition, decay suppression from the combined treatment carried over to the simulated shelf life. According to current U.S. marketing regulations, 0.5% is the maximum decay rate accepted at the shipping point and 1.0% at the receiving point for U.S. N^{o} 1 grade California grapes (Anonymous, 1999). Across three inoculation conditions, 40% CO_2 for 24 h + CA and 40% CO_2 for 48 h + CA yielded decay incidence below this maximum: 0.5% in 'Flame Seedless' and 'Crimson Seedless' after four and eight weeks cold storage, respectively, reaching the minimal quality standards for commercial table grapes. Furthermore, at 4 weeks + SL, Flame Seedless' grapes treated with 40% CO_2 for 48 h + CA still had the lowest decay incidence.

The mode of action of the combined treatment on decay control in table grapes is not fully understood. CO_2 at high concentrations reduced decay through direct action against *B. cinerea*, including partial inhibition of conidia germination and suppression of mycelial nesting development on grapes with natural and inoculated infections. In our study, the mode of action by which 40% CO_2 pre-storage + CA induces natural resistance to disease was not evaluated. In grapes and kiwifruit, the efficacy of high CO_2 or ozone (O_3) treatments in controlling decay resulted from formation of reactive oxygen species associated with stilbene synthase gene expression and resveratrol accumulation (Sanchez-Ballesta et al., 2006; Romero et al. , 2008; Minas et al., 2010), or increased internal ethanol and acethaldehyde to fungal-toxic concentrations (Pesis et al., 1989). Ethanol and acethaldehyde have fungicidal properties and act by damaging membranes and reducing fungal growth (Pesis, 2005). Application of ethanol has allowed good control of gray mold incidence in table grapes (Lichter et al., 2002; Mlikota Gabler et al., 2005; Candir et al., 2012). Latent infections caused by conidia are very difficult to control, even when non-systemic fungicides are applied. Thus, decay will eventually develop in cold storage (Smilanick et al., 1990). Weekly SO_2 fumigation can kill conidia on the berry surface and prevent spread through nesting on the fruit's surface, but it cannot control latent infection underneath the skin (Luvisi et al., 1992). Therefore, grapes from lots with high latent infection rates are difficult to store successfully for long periods.

Although high CO_2 pre-treatments prior to cold storage reduced decay early in the cold storage period, decay continued to occur if the fruits were stored or transported in Air. This result emphasizes the synergistic benefit of applying both treatments.

Application of 40% CO_2 exhibited a direct control over fungal infection. By 24 h, pre-treatment with 40% CO_2 for 24 or 48 h reduced *in vitro* mycelial growth by 70% or 92%, respectively. When 40% CO_2 was applied and fungal development occurred in the air, the fungistatic effect of 40% CO_2 was observed in treated mycelia and conidia, indicating a carry-over effect. A carry-over effect of pre-storage high CO_2 on disease incidence under controlled and commercial conditions has not previously been reported to our knowledge. Dipping grapes in 33% or higher ethanol solutions before storage controlled conidia germination of *B. cinerea in vitro* and gray mold in bunches, but did not control mycelial infection. This implies that once germination is established, ethanol is not effective (Lichter et al., 2002). In agreement with our findings, 20% CO_2 reduced *B. cinerea* mycelial growth by 50% and growth decreased linearly at increased CO_2 concentrations (Lichter et al., 2002). High CO_2 application associated with low O_2 concentration yielded stronger control of *B. cinerea* and *Rhizopus stolonifer* spore germination than high CO_2 applied with 21% O_2 (Wells and Uota, 1970). In our *in vitro* test, after 12 h incubation conidia germination was reduced from 93% in the control to 37% in cultures treated with 40% CO_2 for 48 h. However, germination in both control and treated cultures was ~95% at 24 h.

Pre-storage with 40% CO_2 followed by CA reduced loss of firmness, expressed as maximum force to penetrate the fruit, maintaining berry deformation throughout seven weeks cold storage. This treatment did not increase rachis browning or affect color, SSC, or TA, in agreement with previous semi-commercial CA work in which 10 to 15% CO_2 balanced with air or nitrogen reduced *B. cinerea* decay during long storage periods (Crisosto et al., 2002b; Retamales et al., 2003, Droby and Lichter, 2004), while CO_2 concentrations ≥15% reduced immature grape flavor and appearance while accelerating rachis browning. Thus, this technology should be limited to mature grapes (Crisosto et al., 2002a, 2002b). A short application of 20% CO_2 + 20% O_2 for 72 h in 'Cardinal' table grapes maintained the visual appearance of the rachis, reducing browning and water loss, after 33 d storage at 0 ^{o}C (Sanchez-Ballesta et al., 2006). An atmosphere of 4% O_2 + 30% CO_2 reduced the activities of cellulase, polygalacturonase, and peroxidase, reducing berry drop of 'Kyoho' grapes (Deng et al., 2007). CA using low O_2 and high CO_2 has been tested for control of quarantined insects on exported Californian table grapes as a potential alternative to methyl bromide fumigation (Ahumada et al., 1996; Liu, 2013) and CA and carbon monoxide are actually applied in

California as a quarantine treatment in table grapes exported to Australia and New Zealand (Crisosto, personal communication), showing that the use of a short high CO_2 application and CA in table grapes could be economic feasible.

Postharvest application of high CO_2 and/or low O_2 can induce accumulation of fermentative volatiles like ethanol and AA and produce off flavors in fruits. In our study, after seven weeks cold storage, untrained panelists could not identify flavor differences between fruits stored in air and fruits pre-treated with 40% CO_2 for 48 h and stored in CA. Similarly, five trained panelists detected no off flavors in 'Kyoho' grapes stored in high barrier film for two weeks, at which time the ethanol concentration in the fruits had reached ~550 μL L^{-1} (Chen et al., 2011). Ethanol vapor applied in a package with low permeability increased the ethanol concentration of 'Red Globe' table grapes from ~280 μL L^{-1} at harvest to ~700 μL L^{-1} after one month storage at 0 ^{o}C, while reducing decay from 51.5% to 4.5%; a trained panel classified the appearance and taste as "like very much" on a hedonic scale (Candir et al., 2012). No off flavors were reported for 'Thompson Seedless' treated with 0.5% O_2 + 45% CO_2 (144 h at 5 ^{o}C) and then transferred to air (72 h at 0 ^{o}C). In these grapes, ~10 μL L^{-1} of AA and ~ 900 μL L^{-1} of ethanol were measured, but consumers were unable to detect flavor differences (Ahumada et al., 1996).

The 40% CO_2 pre-storage for 24 or 48 h, followed by CA, applied to naturally infected 'Flame Seedless' organic table grapes reduced the incidence of decay 55-fold, maintaining USDA commercial standards until four weeks cold storage. Similar, 49-fold reduction, was found in naturally infected 'Crimson Seedless', which maintained commercial standards during eight weeks cold storage. This organic alternative combination treatment provided similar control in conidia- and mycelium-inoculated grapes. High CO_2 pre-storage alone limited incidence of gray mold decay in naturally infected and artificially inoculated 'Flame Seedless' early during storage, but was less effective than the combined treatments. These treatments did not affect visual or sensory quality. The results of *in vitro* experiments and analysis of acetaldehyde and ethanol concentration in stored grapes suggested that high CO_2 acts both directly on *B. cinerea* and also on the grapes, where it increased the acetaldehyde and ethanol concentrations. Thus, high CO_2 short application followed by CA during storage or transportation could be a commercially feasible alternative for postharvest handling of organic grapes. Our results

encourage validating this combined organic treatment in other cultivars and studying the mechanism of action to improve performance.

Acknowledgments

We would like to recognize financial support from the CAPES Foundation, a federal agency under the Ministry of Education of Brazil, covering senior author residence at the University of California, Davis. Authors thank Sunview Cold Storage (Delano, CA) for providing organic table grapes, and Gayle Crisosto, Megan Haug, and Palma Lower for their assistance with this work and for reviewing the manuscript.

References

1- Ahumada, M.H., Mitcham, E.J., Moore, D.G., 1996. Postharvest quality of 'Thompson Seedless' grapes after insecticidal controlled-atmosphere treatments. HortScience 31, 833-836.

2- Anonymous, 1989. Pesticide tolerance for sulfur dioxide. Federal Register 1989:40:20, 125−20, 126.

3- Anonymous, 1999. United States standards for grades of table grapes (European or Vinifera type). USDA, Agricultural Marketing Service, USA, 14 pp.

4- Artés-Hernández, F., Aguayo, E., Artés, F., 2004. Alternative atmosphere treatments for keeping quality of 'Autumn seedless' table grapes during long-term cold storage. Postharvest Biol. Technol. 31, 59-67.

5- Candir, E., Ozdemir, A.E., Kamiloglu, O., Soylu, E.M., Dilbaz, R., Ustun, D., 2012. Modified atmosphere packaging and ethanol vapor to control decay of 'Red Globe' table grapes during storage. Postharvest Biol. and Technol. 63, 98-106.

6- Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1995. Proposal of an Index for the objective evaluation of the color of red table grapes. Food Res. Int. 28, 373-377.

7- Chen, S.J., Zhang, M., Wang, S.J., 2011. Effect of initial hermetic sealing on quality of 'Kyoho' grapes during storage. Postharvest Biol. Technol. 59, 194-199.

8- Chervin, C., Aked, J., Crisosto, C.H., 2012. Grapes, In: Rees, D., Farrell, G., Orchard, J. (Eds.), Crop Post-Harvest: Science and Technology. Blackwell Publishing Ltd, pp. 187-211.

9- Chervin, C., Lavigne, D., Westercamp, P., 2009. Reduction of gray mold development in table grapes by preharvest sprays with ethanol and calcium chloride. Postharvest Biol. Technol. 54, 115-117.

10- Crisosto, C.H., Garner, D., Crisosto, G., 2002a. Carbon dioxide-enriched atmospheres during cold storage limit losses from Botrytis but accelerate rachis browning of 'Redglobe' table grapes. Postharvest Biol. Technol. 26, 181-189.

11- Crisosto, C.H., Garner, D., Crisosto, G., 2002b. High carbon dioxide atmospheres affect stored 'Thompson Seedless' table grapes. Hortscience 37, 1074-1078.

12- Crisosto, C.H., Mitchell, F.G., 2002. Postharvest handling systems: small fruits. Table grapes., Postharvest Technology of Horticulture Crops. Kader, A., Oakland, University of California, Agriculture and Natural Resources, p. 357−363.

13- Deng, Y., Wu, Y., Li, Y.F., Yang, M.D., Shi, C.B., Zheng, C.J., 2007. Studies of postharvest berry abscission of 'Kyoho' table grapes during cold storage and high oxygen atmospheres. Postharvest Biol. Technol. 43, 95-101.

14- Droby, S., Lichter, A., 2004. Post-Harvest Botrytis Infection: Etiology, Development and Management, In: Elad, Y., Williamson, B., Tudzynski, P., Delen, N. (Eds.), Botrytis: Biology, Pathology and Control. Kluwer Academic Publishers, London, UK, pp. 349-367.

15- Mlikota Gabler, F, Smilanick, J.L., Ghosoph, J.M., Margosan, D.A., 2005. Impact of postharvest hot water or ethanol treatment of table grapes on gray mold incidence, quality, and ethanol content. Plant Dis. 89, 309-316.

16- Karabulut, O.A., Smilanick, J.L., Mlikota Gabler, F., Mansour, M., Droby, S., 2003. Near-harvest applications of *Metschnikowia fructicola*, ethanol, and sodium bicarbonate to control postharvest diseases of grape in central California. Plant Dis. 87, 1384-1389.

17- Ke, D.Y., Goldstein, L., O'Mahony, M., Kader, A.A., 1991. Effects of short-term exposure of low O_2 and high CO_2 atmospheres on quality attributes of strawberries. J. Food Sci. 56, 50-54.

18- Lichter, A., Mlikota Gabler, F., Smilanick, J.L., 2006. Control of spoilage in table grapes. Stewart Postharvest Rev. 2, 1-10.

19- Lichter, A., Zutkhy, Y., Sonego, L., Dvir, O., Kaplunov, T., Sarig, P., Ben-Arie, R., 2002. Ethanol controls postharvest decay of table grapes. Postharvest Biol. Technol. 24, 301-308.

20- Lind, K., G. Lafer, K. Schloffer, Innerhofer, G., Meister, H., 2003. Organic Fruit Growing. CAB International.

21- Liu, Y.-B., 2013. Controlled atmosphere treatment for control of grape mealybug, *Pseudococcus maritimus* (Ehrhorn) (Hemiptera: Pseudococcidae), on harvested table grapes. Postharvest Biol. Technol. 86: 113-117.

22- Luvisi, D., Shorey, H., Smilanick, J., Thompson, J., Gump, B., Knutson, J., 1992. Sulfur Dioxide Fumigation of Table Grapes, University of California, DANR, Bulletin 1932, p. 22.

23- Meilgaard, M., Civille, G.V., Carr, B.T., 1999. Sensory Evaluation Techniques, 3rd ed. CRC Press, Boca Raton, Florida.

24- Minas, I.S., Karaoglanidis, G.S., Manganaris, G.A., Vasilakakis, M., 2010. Effect of ozone application during cold storage of kiwifruit on the development of stern-end rot caused by *Botrytis cinerea*. Postharvest Biol. Technol. 58, 203-210.

25- Nigro, F., Schena, L., Ligorio, A., Pentimone, I., Ippolito, A., Salerno, M.G., 2006. Control of table grape storage rots by pre-harvest applications of salts. Postharvest Biol. Technol. 42, 142-149.

26- Palou, L., Crisosto, C.H., Smilanick, J.L., Adaskaveg, J.E., Zoffoli, J.P., 2002. Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. Postharvest Biol. Technol. 24, 39-48.

27- Pesis, E., Marinansky, R., Avissar, I., 1989. Effect of prestorage treatments with acetaldehyde vapors or anaerobic conditions on volatiles accumulation during storage of various fruits. Acta Hortic. 258, 661-667.

28- Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biology and Technology 37, 1-19.

29- Retamales, J., Defilippi, B.G., Arias, M., Castillo, P., Manriquez, D., 2003. High-CO_2 controlled atmospheres reduce decay incidence in Thompson Seedless and Red Globe table grapes. Postharvest Biol. Technol. 29, 177-182.

30- Romanazzi, G., Karabulut, O.A., Smilanick, J.L., 2007. Combination of chitosan and ethanol to control postharvest gray mold of table grapes. Postharvest Biol. Technol. 45, 134-140.

31- Romanazzi, G., Lichter, A., Mlikota Gabler, F., Smilanick, J.L., 2012. Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. Postharvest Biol. Technol. 63, 141-147.

32- Romero, I., Sanchez-Ballesta, M.T., Maldonado, R., Escribano, M.I., Merodio, C., 2008. Anthocyanin, antioxidant activity and stress-induced gene expression in high CO_2-treated table grapes stored at tow temperature. J. Plant Physiol. 165, 522-530.

33- Sanchez-Ballesta, M.T., Jimenez, J.B., Romero, I., Orea, J.M., Maldonado, R., Urena, A.G., Escribano, M.I., Merodio, C., 2006. Effect of high CO_2 pretreatment on quality, fungal decay and molecular regulation of stilbene phytoalexin biosynthesis in stored table grapes. Postharvest Biol. Technol. 42, 209-216.

34- Smilanick, J.L., Hartsell, P.I., Henson, D., Fouse, D.C., Assemi, M., Harris, C.M., 1990. Inhibitory activity of sulfur-dioxide on the germination of spores of *Botrytis cinerea*. Phytopathology 80, 217-220.

35- Smilanick, J.L., Mlikota Gabler, F., Margosan, D., 2010. Influence of continuous, low concentration ozone during cold storage on postharvest decay and quality of table grapes, 6th International Table grape Symposium, Davis-CA, USA, pp. 85-86.

36- USDA, E.R.C., 2012. Organic Market Overview, http://www.ers.usda.gov/topics/natural-resources-environment/organic-agriculture/organic-market-overview.aspx#.UWmwFZFHCX8.

37- Wells, J.M., Uota, M., 1970. Germination and growth of five fungi in low-oxygen and high-carbon dioxide atmospheres. Phytopathology 60, 50-53

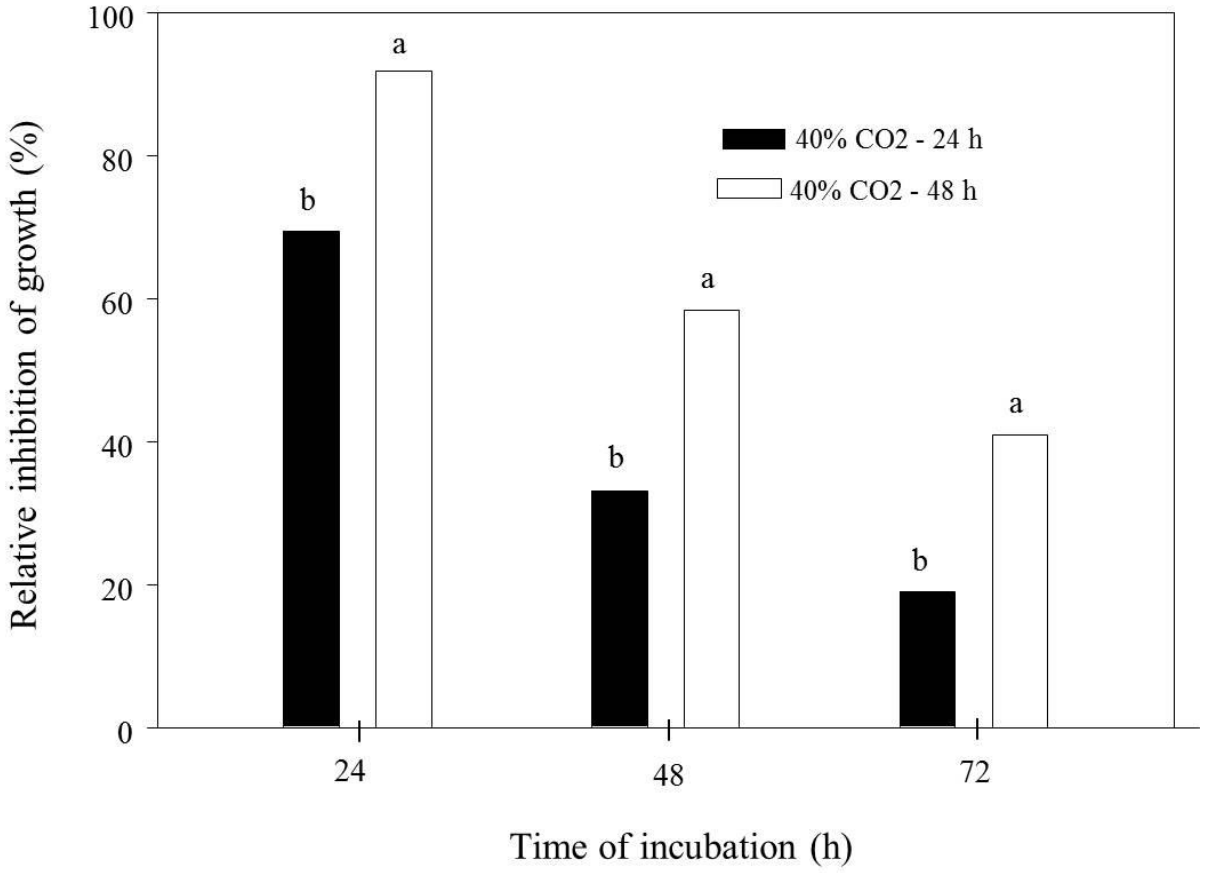

Fig. 1. Relative inhibition growth (%) of in vitro mycelial growth of *Botrytis cinerea* treated with 40% CO_2 (0 ^{o}C, RH 95%) for 24 or 48 h and incubated at 22 ^{o}C for up to 72 h. Different letters indicate a significant difference between treatments by Tukey's test ($p \leq 0.01$), n = 12.

Table 1 Natural decay incidence (%) in 'Crimson Seedless' table grapes clusters stored at 0.5 ± 1 °C, 95 to 98% RH and subjected to an atmosphere of air (Control) or pre-stored in 40% CO_2 for 24 h and then stored in a controlled atmosphere of 12% CO_2 + 12% O_2 (40% CO_2 for 24 h + CA) for eight weeks cold storage. Finally, to simulate commercial shelf life, the grapes were stored in air for 2 d at 20 ± 1 °C (SL) or kept at 20 ± 1 °C from 1 to 3 days to evaluate decay evolution when decayed berries were removed.

Treatments	Storage time							
	3 weeks	3 weeks +SL	6 weeks	6 weeks +SL	8 weeks	8weeks + 1d	8 weeks + 2d	8 weeks + 3d
Control	0.0	1.7	4.1 a	27.2 a	13.3 a	22.0 a	29.1 a	47.2 a
40% CO_2 for 24 h + CA	0.4	0.6	0.4 b	3.3 b	0.2 b	0.4 b	1.8 b	7.5 b
P value	0.3409	0.2697	0.0165	0.0003	0.0001	0.0000	0.000	0.0051

Incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before analysis of variance and Student's t-test.

Values followed by the same letters for each assessment time did not differ significantly according to pairwise Student's t-test ($p \leq 0.05$), n = 6.

Table 2 Natural decay incidence (%) in 'Flame Seedless' table grapes affected by the interaction of high CO_2 pre-storage (Air; 40% CO_2 for 24 h; 40% CO_2 for 48 h) and storage (Air; CA= 12% CO_2 + 12% O_2). The fruits were stored at 1.0 ± 0.5 ^{o}C, 95 to 98% RH, and then stored in air for 2 d at 20 ± 1 ^{o}C (SL) to simulate commercial shelf life.

Treatment	Storage time			
	4 weeks	4 weeks + SL	7 weeks	7 weeks + SL
Air	22.0	79.0	100.0 a	100.0 a
40% CO_2 for 24 h + Air	16.3	59.3	100.0 a	100.0 a
40% CO_2 for 48 h + Air	9.0	76.7	100 a	98.7 a
Air + CA	6.3	32.8	46.0 b	98.4 a
40% CO_2 for 24 h + CA	0.2	12.4	23.1 c	94.0 b
40% CO_2 for 48 h + CA	0.6	3.7	7.4 c	90.7 b
Pre-storage *P*-value	0.0084	0.0451	0.0009	0.0006
Storage *P*-value	0.0000	0.000	0.0000	0.0000
Pre-storage x Storage *P* value	0.3636	0.0827	0.0009	0.0338

Statistical analysis was performed with arcsine of the square root transformed data. Values presented are non-transformed means. Values in the column followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$), n= 6.

Table 3 Decay incidence (%) in surface-inoculated 'Flame Seedless' table grapes affected by the interaction of high CO_2 pre-storage (Air; 40% CO_2 for 24 h; 40% CO_2 for 48 h) and storage (Air; CA= 12% CO_2 + 12% O_2). The fruits were stored at 1.0 ± 0.5 ^oC, 95 to 98% RH, and finally, to simulate commercial shelf life, the grapes were stored in air for 2 d at 20 ± 1 ^oC (SL).

Treatment	Storage time			
	4 weeks	4 weeks + 2 days	7 weeks	7 weeks + 2 days
Air	25.6 a	98.9 a	99.2 a	100.0
40% CO_2 for 24 h + Air	33.8 a	93.6 a	95.0 ab	100.0
40% CO_2 for 48 h + Air	10.1 b	100.0 a	95.8 a	100.0
Air + CA	0.0 c	24.7 b	75.4 bc	100.0
40% CO_2 for 24 h + CA	0.0 c	6.9 c	50.1 cd	97.4
40% CO_2 for 48 h + CA	0.0 c	4.0 c	27.2 d	95.2
Pre-storage P-value	0.0037	0.0250	0.0002	0.2465
Storage P-value	0.0000	0.0000	0.0000	0.0229
Pre-storage x Storage P value	0.0037	0.0258	0.0197	0.2465

Statistical analysis was performed with arcsine of the square root transformed data. Values presented are non-transformed means. Values in the column followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$), n= 6.

Table 4 Decay incidence (%) in nesting inoculated 'Flame Seedless' table grapes affected by the interaction of high CO_2 pre-storage (Air; 40% CO_2 for 24 h; 40% CO_2 for 48 h) and storage (Air; CA= 12% CO_2 + 12% O_2). The fruits were stored at 1.0 ± 0.5 ^oC, 95 to 98% RH, and finally, to simulate commercial shelf life, the grapes were stored in air for 2 d at 20 ± 1 ^oC (SL).

Treatment	Storage time			
	4 weeks	4 weeks + 2 days	7 weeks	7 weeks + 2 days
Air	18.3 a	94.4 a	100.0 a	100.0 a
40% CO_2 for 24 h + Air	22.7 a	100.0 a	98.3 a	98.8 a
40% CO_2 for 48 h + Air	16.7 a	100.0 a	98.3 a	100.0 a
Air + CA	4.1 b	22.2 b	32.3 b	98.3 a
40% CO_2 for 24 h + CA	0.5 c	11.1 bc	25.3 bc	100.0 a
40% CO_2 for 48 h + CA	0.4 c	10.0 c	10.8 c	84.2 b
Pre-storage P-value	0.0188	0.8101	0.0065	0.0032
Storage P-value	0.0000	0.0000	0.0000	0.0075
Pre-storage x Storage P value	0.0035	0.0000	0.0468	0.0000

[y] Statistical analysis was performed with arcsine of the square root transformed data. Values presented are non-transformed means.

[z] Treatment means followed by the same letter in each column are not significantly different at $P \leq 0.05$, according to the Tukey's test.

Table 5 Maximum force (N) and deformation of berry (%) in 'Flame Seedless' table grapes affected by the interaction of high CO_2 pre-storage (Air; 40% CO_2 for 24 h; 40% CO_2 for 48 h) and storage (Air, CA= 12% CO_2 + 12% O_2). The fruits were stored at 1.0 ± 0.5 ^{o}C, 95 to 98% RH.

	Maximum force (N)		Deformation (%)	
Initial quality	3.73		19.5	
Treatment	4 weeks	7 weeks	4 weeks	7 weeks
Air	3.28 b	NE	12.9	NE
40% CO_2 for 24 h + Air	3.43 b	NE	15.8	NE
40% CO_2 for 48 h + Air	3.51 b	NE	15.3	NE
Air + CA	3.30 b	2.95	14.2	18.4
40% CO_2 for 24 h + CA	3.99 a	3.09	17.6	18.9
40% CO_2 for 48 h + CA	3.89 a	2.83	16.4	20.4
Pre-storage P-value	0.0000	—	0.0000	—
Storage P value	0.0000	—	0.0008	—
Pre-storage x Storage P value	0.0000	0.1582	0.7521	0.4063

NE= Not evaluated because of high decay incidence. Values in the column that are followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$), n= 6.

Table 6 Concentration of acetaldehyde and ethanol (μL L^{-1}) in 'Flame Seedless' table grapes affected by the interaction of high CO_2 pre-storage (Air; 40% CO_2 for 24 h; 40% CO_2 for 48 h) and storage (Air, CA= 12% CO_2 + 12% O_2). The fruits were stored at 1.0 ± 0.5 ^{o}C, 95 to 98% RH.

	Acetaldehyde (μL.L^{-1})					Ethanol (μL.L^{-1})				
Initial quality	2.1					0				
Treatments	After treatment	1 week	2 weeks	4 weeks	7 weeks	After treatment	1 week	2 weeks	4 weeks	7 weeks
Air	2.1 b	2.1 c	3.5 b	4.0 bc	NE	0.0 c	0.0 c	69.4 b	100.4 b	NE
40% CO_2 for 24 h + Air	8.8 a	8.6 b	4.7 b	4.4 bc	NE	79.7 b	37.8 b	129.5 b	129.0 b	NE
40% CO_2 for 48 h + Air	9.5 a	18.9 a	16.0 a	5.9 b	NE	228.4 a	187.6 a	255.0 a	75.8 b	NE
Air + CA	NA	NA	NA	2.8 c	2.4	NA	NA	NA	110.2 b	179.3
40% CO_2 for 24 h + CA	NA	NA	NA	5.2 bc	2.4	NA	NA	NA	211.5 b	182.9
40% CO_2 for 48 h + CA	NA	NA	NA	12.4 a	3.0	NA	NA	NA	486.3 a	260.0
Pre-storage P-value	—	—	—	0.0000	—	—	—	—	0.0000	—
Storage P value	—	—	—	0.0002	—	—	—	—	0.0000	—
Pre-storage x Storage P value	0.0000	0.0000	0.0000	0.0001	0.1988	0.0000	0.0000	0.0003	0.0000	0.1124

NA= Not analyzed. NE= Not evaluated because of high decay incidence. Values in the column followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$), n= 6.

5. CAPÍTULO 3:

Pre-harvest applications of calcium chloride and chlorine dioxide, postharvest controlled atmosphere treatments, and the quality of 'Crimson Seedless' table grapes

Efeito de aplicações de $CaCl_2$ ou ClO_2 em pré-colheita, e tratamentos com Atmosfera Controlada na qualidade de uvas de mesa 'Crimson Seedless'

[1] Artigo a ser submetido ao periódico científico: American Journal of Enology and Viticulture.

Pre-harvest applications of calcium chloride and chlorine dioxide, postharvest controlled atmosphere treatments, and the quality of 'Crimson Seedless' table grapes

Cesar S. Teles[1], Matthew W. Fidelibus[2], Kimberley A. Cathline[2], Benedito C. Benedetti[1], Carlos H. Crisosto[3*]

[1]Faculdade de Engenharia Agrícola (FEAGRI), Universidade Estadual de Campinas, Av. Cândido Rondon 501, Campinas, SP 13083-875, Brazil

[2]Department of Viticulture and Enology, University of California, Davis, Kearney Agricultural Center, 9240 S. Riverbend Avenue, Parlier, CA 93648, USA

[3] Department of Plant Sciences, University of California, Davis, One Shields Avenue, 95616 Davis, CA, United States.

* Corresponding author at: University of California Davis, Department of Plant Sciences Mail Stop 2 One Shields Avenue, Davis, CA 95616 USA. Phone: +1 530.752.7549, fax: 530.752.8502. Email address: chcrisosto@ucdavis.edu (Carlos H. Crisosto)

[d] Financing: Cesar S. Teles scholarship was funded from CAPES Foundation, a federal agency under the Ministry of Education of Brazil. Additional financial support and calcium chloride were provided by Tetra Technologies, Houston, TX.

- **Abstract**

A single alternative approach might not effectively reduce decay, so the integration of two or more alternatives, may result in an additive or synergistic effect in the control of *Botrytis cinerea*. It was evaluated the field pre-harvest application of $CaCl_2$ or ClO_2 combined with storage in 40% CO_2 for 24 h + CA (12% O_2 + 12% CO_2) as an organic approach to control *B. cinerea* in 'Crimson Seedless'. The grapes were inoculated on the berry surface (inoculated by spraying with a conidia solution) and nesting inoculated (inoculated with an infected berry) and stored at 0.5 ^{o}C. After 6 weeks of cold storage in air, the $CaCl_2$ and ClO_2 treatments reduced the decay incidence in surface inoculated grapes from 45% to 23%

and 15.6%, respectively. On the other hand, these pre-harvest applications did not control the decay in nesting inoculated fruits, moreover in some evaluations the decay in treated fruits were higher than in the control. High CO_2 storage treatment reduced the decay incidence in nesting and surface inoculated fruits during the whole storage. By 6 weeks of storage at 0.5 ^oC, the high CO_2 yielded a decay incidence reduction of 15-fold in nesting inoculated, and 70-fold in surface inoculated. In surface inoculated grapes, there was a significant interaction between field and storage treatments. By 6 weeks of cold storage, the combination of field treatments and high CO_2 kept the infection lower than 0.5%. The storage in High CO_2 did not compromise the visual or chemical quality or the flavor. Our results show that it is possible to reach the US standards for table grapes up to 6 weeks of cold storage in high CO_2, for surface inoculated grapes.

Key words: Gray mold, *Botrytis cinerea*, quality, flavor, SO_2 alternative, sulfite free.

5.1 Introduction

Gray mold, caused by the fungus *Botrytis cinerea* Pers, is the primary cause of postharvest decay of table grapes. Botrytis may grow at temperatures ≥ -0.5 ^oC and rapidly colonize fruits via aerial mycelia Efficient postharvest control of gray mold is achieved with weekly application of sulfur dioxide (SO_2) gas in storage rooms (Luvisi et al., 1992). However alternatives to SO_2 are desired to reduce some of the potential risks associated with SO_2 use including the safety of workers at packing plants, residue on grapes which could lead to allergic reactions in some people, and damage to the grapes, thus reducing their quality (Lichter et al., 2006; Luvisi et al., 1992). Moreover, SO_2 may not be applied to organic grapes, which are an increasingly important product (Romanazzi et al., 2012). Thus, there is considerable interest in the development of alternatives to SO_2 and other synthetic fungicides.

A recent review summarized more than 50 studies, published from 2006 to 2010, on the testing of alternative pre and post-harvest methods for controlling postharvest decay in table grapes (Romanazzi et al., 2012). Most of the treatments tested have not yet been commercially adopted, but the pre-harvest application of calcium chloride ($CaCl_2$) is used

in conventional and organic vineyards in Italy (Romanazzi et al., 2012). The pre-harvest application of a 1% $CaCl_2$ solution reduced decay incidence at harvest, and after postharvest storage, of Italy (Nigro et al., 2006) and Chasselas (Chervin et al., 2009) table grapes, with the best efficacy achieved with the earliest and most numerous applications (Nigro et al., 2006). Another pre-harvest treatment having potential as an alternative decay control method is chlorine dioxide (ClO_2), a compound included in the US Food and Drug Administration (FDA) list of products 'generally recognized as safe' (GRAS) (Vardar et al., 2012). Chlorine dioxide is a powerful oxidant which is more stable and has more oxidizing power than chlorine. Moreover, the effects of ClO_2 remain constant over a wide pH range (Olmez and Kretzschmar, 2009). The potential for ClO_2 to control decay of fruits has been evaluated in litchi (Wu et al., 2011), figs (Karabulut et al., 2009) and strawberries (Vardar et al., 2012), but not grapes.

Among the postharvest treatment alternatives to SO_2 is controlled atmosphere (CA). For example, a CA treatment of >15 kPa CO_2 provided good control of gray mold in 'Thompson Seedless' and 'Red Globe' table grapes (Crisosto et al., 2002a, 2002b), with results similar to those obtained using metabisulphite pads (Retamales et al., 2003). Though high CO_2 effectively controls decay, there is a narrow threshold between efficacy and damage expressed as browning of the berries or rachis and development of off-flavor (Chen et al., 2011; Crisosto et al., 2002a; Retamales et al., 2003). However, evidence suggests that subjecting the grapes to high concentrations CO_2 for a short period of time could provide rot control without excessive berry damage. For example, 'Cardinal' grapes subjected to 20% CO_2 for 3 d had low levels of rot and good berry and rachis appearance (Romero et al., 2006; Sanchez-Ballesta et al., 2006). We have also found that a post-harvest treatment combining an initial fumigation with 40% CO_2 for 24 h followed by storage in CA (12% O_2 + 12% CO_2) reduced the decay incidence in Flame Seedless grapes (Crisosto, unpublished data). A single alternative approach might not effectively reduce decay, so the integration of two or more alternatives, in a multifaceted approach, may be worthwhile and may result in an additive or synergistic effect. This approach can reduce the decay, following the "multiple hurdle concept", which involves of the reduction of decay by presenting to the pathogen with several consecutive hurdles, with each one contributing to a portion of the reduction, possible resulting in additive or synergistic effects (Ippolito and Sanzani, 2011).

Thus, our objectives were to evaluate the efficacy of pre-harvest $CaCl_2$ or ClO_2 applications alone, or in combination with pre-storage in high CO_2 followed by storage in CA, on fruit quality and on control of rot of 'Crimson Seedless' table grapes at harvest, and after postharvest storage.

5.2 Materials and Methods

5.2.1 Plant material and field treatments

Fifteen year old own rooted 'Crimson Seedless' grapevines of similar capacity and crop load (Kearney Agricultural Center, Parlier, CA) were chosen for this study. The vines were spaced 2.4 m within rows and 3.6 m between rows. The vineyard was drip irrigated at approximately 80% ETc, and standard culturing practices were followed, including berry thinning (2.5g GA_3 per ha at 80% anthesis), girdling for berry sizing (6 mm girdle at fruit set), basal leaf removal, shoot thinning, and the application of abscisic acid at veraison (ProTone; Valent BioSciences, Libertyville, Ill) to promote red color development. Fungicides were applied for powdery mildew, but no fungicides were used to specifically control *B. cinerea*.

Six vine rows were selected for the study, with each row considered a block. Three plots comprised of four consecutive and uniform vines within each block were identified and randomly assigned to one of three different pre-harvest treatments: non-treated control, calcium chloride ($CaCl_2$) or chlorine dioxide (ClO_2) (Figure 1A). The treatments were applied four times (Figures 1B, 1C, 1D and 1E): at late flowering (17 May 2012); berry touch (12 June 2012); veraison (12 July 2012), and one day before harvest (28 August 2012). An aqueous solution of 1% $CaCl_2$ (COR-CLEAR, TETRA Technologies, The Woodlands, TX) with 0.025% (v/v) adjuvant (Latron B-1956, Dow AgroSciences LLC) was used in the first application, as suggested by others (Chervin et al., 2009; Nigro et al., 2006). However, this treatment appeared to cause some phytotoxicity, including marginal leaf necrosis and small necrotic spots on some berries. Therefore, the $CaCl_2$ concentration in the remaining applications was reduced to 0.25%, a concentration which is similar to what the label recommends for grape, in order to reduce the risk of additional phytotoxic responses. The ClO_2 (Oxymer^{TM}, Water Engineers Singapore, Singapore) was applied in

increasing concentrations to avoid phytotoxicity in the early stages of development; in the first application, a solution of 20 µL L^{-1} was used, in the second, 30 µL L^{-1} and in the third and fourth, 150 µL L^{-1}. The concentrations of the ClO_2 solutions were measured using a pocket colorimeter™ II (Hach, Loveland, CO). Each spraying was performed with an air-pulsed sprayer and directed onto the fruit zone until runoff. At least two vines were used as a buffer between each treatment, within rows.

On 28 August, we collected two replicated berry samples from each plot. One sample consisted of 50 berries which were counted, weighed, and then homogenized in a blender. The juice was filtered, total soluble solids (TSS) measured with a temperature compensating digital refractometer (PAL-1, Atago-USA, Bellevue, WA), and pH and titratable acidity (TA) of the filtered juices were determined with an automatic titrator (DL 50, Mettler, Toledo, Columbus, OH), using 0.10 N NaOH to an endpoint of 8.2 pH.

An additional sample consisting of twenty berries per plot were collected, and the surface of each berry in these samples was cleaned, to remove any possible spray residues, by rinsing the berries in 1% HCl followed by three rinses in deionized water. Then, each berry was sectioned into longitudinal quarters, and three quarters of each berry were randomly selected and discarded, retaining one quarter per berry from the 20 berries in each sample. Each sample of quartered berries was then frozen in liquid nitrogen, lyophilized, homogenized, sealed in plastic bags, and held in a desiccator until calcium concentration could be determined by the UC Davis Analytical lab, using nitric acid/hydrogen peroxide microwave digestion and determination by Inductively Coupled Plasma Atomic Emission Spectrometry.

Fruit composition tests confirmed the fruits had exceeded the minimum market requirements of 16.5% TSS and a 20:1 TSS:TA, so commercially-acceptable clusters of grapes were harvested on 29 August 2012. Each cluster was inspected at harvest, and any berries that were green-colored, presented disease symptoms, or exhibited any other quality defects, were removed with shears and discarded as is standard commercial practice. Approximately 600 to 800 g of grapes were field packaged into perforated cluster bags and placed in polypropylene corrugated boxes, with a ten bags/box capacity and internal dimensions of 50.2 x 39.4 x 12.7 cm^3 (Maxplastic™, Maxco, Delano, CA). The boxes of

fruit were loaded into an air-conditioned vehicle, and transported to postharvest facilities at the UC Davis campus.

Because phytotoxicity symptoms were observed after the first application of 1% $CaCl_2$, a separate $CaCl_2$ dose response study was conducted in the same vineyard. Individual clusters of grapes on otherwise untreated vines were sprayed to wetness with solutions containing 0%, 0.25%, 0.50%, or 1.0% $CaCl_2$ and 0.025% Latron B-1956 in a randomized complete block design, where individual vines were considered blocks, and individual clusters, replicates, replicated four times. Clusters were first treated on 30 May 2012, and again on 13 June, 11 July, and 31 August, 2012. All clusters were harvested on 10 October. At harvest, the number of berries on each cluster was determined, and every berry on every cluster was inspected for signs of damage and assigned one of the following classes; none (no visible damage), slight (barely noticeable signs of phytotoxicity such as a few small corky or necrotic spots), readily observable (clearly noticeable damage such as large corky or necrotic spots or rough-textured patches), or shriveled/rotten. The percentage of berries assigned to each class was determined for each cluster, and used for statistical analysis of treatment effects.

5.2.2 Inoculation and postharvest treatments

Botrytis cinerea (isolate IC 08, from T.J. Michailides) was incubated on PDA in 85mm Petri dishes, and the conidia extracted after method of Palou et al. (2002). Bagged grapes were labeled and divided into 3 groups: 1. naturally infected (without inoculation), 2. surface inoculated (berries inoculated by spraying with a conidia suspension) and 3. nesting inoculation (inoculated with an infected berry). For the surface inoculated, the conidia suspension was adjusted to a concentration 2×10^4 conidia/mL and sprayed on the surface of the clusters (Figures 3B, C). Grapes were air-dried for an hour prior to the treatment applications. For the nesting-inoculated fruits, 10 µL of a suspension of 2×10^6 conidia/mL of *B. cinerea* were injected 10 mm deep into the flesh of individual berries with a Hamilton syringe (needle of 1 mm external diameter) and incubated at 20 °C for 5 d. One of these inoculated berries was placed in each nesting inoculated fruit bag in the middle of the cluster inside the perforated plastic bag (Figure 3A).

The field treatments were combined with two postharvest treatments: High CO_2 or Air. A total of six treatments were performed: Control + Air; Control + High CO_2; $CaCl_2$ + Air; $CaCl_2$ + High CO_2; ClO_2 + Air; and ClO_2 + High CO_2. In the High CO_2 storage treatments, 40% CO_2 + 60% N_2 was applied for 24 h, then the gas concentration was changed for 12% CO_2 + 12% O_2 + 76% N_2; in the Air treatment, the fruits were stored in a continuous flow of air. Under both storage conditions, grapes were stored for 8 weeks at 0.5 ± 1 ◦C, 95–98% RH in 338 L sealed metal tanks at a continuous gas flow of 1000 mL/min. When required (3 and 6 weeks), removed from the tanks, and stored for 48 h at 20 ± 1.0 ^{o}C to simulate shelf life (SL). The gas concentration was monitored using a CO_2/O_2 gas analyzer (model 900141, Bridge analyzers Inc., Alameda, CA). Each replicate consisted of one bag with 600-800 g of grapes, and for the evaluation, the treatments were applied to six replications.

5.2.3 Decay incidence

The decay incidence was evaluated after storage for 3 weeks at 0.5 ^{o}C, 3 weeks at 0.5 ^{o}C + SL, 6 weeks at 0.5 ^{o}C, and 6 weeks at 0.5 ^{o}C + SL, 8 weeks at 0.5 ^{o}C. In these evaluations, decay incidence was determined from weight of the decayed berries after removal, and expressed as a percentage of the total weight. For the evaluation at 8 weeks at 0.5 ^{o}C + 1, 2 or 3 days at 20 ^{o}C, the decayed berries were removed, weighted and then the healthy berries were put back in the storage at 20 ^{o}C until the next decay evaluation.

5.2.4 Quality evaluation

Quality analysis was performed in not inoculated fruits. Total soluble solids (TSS), titrable acidity (TA), weight loss, rachis browning, color, berry firmness, acetaldehyde and ethanol content in the berries and a sensory evaluation were performed. For each treatment 6 grape bags were sampled per treatment, before the treatments and after storage for 3 weeks at 0.5 ^{o}C and after 6 weeks at 0.5 ^{o}C, followed in each case by evaluation after 2 days shelf life at 20 ^{o}C (SL).

Fifteen berries per replication for each treatment were juiced and filtered through 4 layers of cheesecloth. TSS were measured by the placement of a few drops of filtered juice onto the refractometer (PR-32α, Atago, Japan), and the TA was determined on the same juice with 0.1 N NaOH up to a pH of 8.2 and expressed as a percentage of tartaric acid (Figure 3D).

Weight loss was determined with a scale accurate to 0.01 g (LC 22016, Sartorius, Elk Grove, IL) and expressed as a percentage of initial weight. Rachis browning development (Figure 3E) was evaluated with the use of the following subjective scoring system: 1 = green and fresh; 2 = green and partially dry; 3 = dry and brownish green; 4 = dry and brown; and 5 = very dry, brown, and brittle (Mlikota Gabler et al., 2005).

Fifteen berries from each replication were detached from the cluster and used for color, firmness, TSS, acidity and pH measurements. The surface color of the berries was measured with a Chroma meter (CR400 model, Konica Minolta Optics, Japan), using the CIELAB color system. From the results, the color index for red grapes (CIRG; Carreño et al. (1995)) was calculated as CIRG = $(180-h^o)/(C^* + L^*)$, where L^* is the lightness corresponding to a black-white scale (0, black; 100, white), h^o is the hue angle on the color wheel, and C^* is the Chroma, a measure of the intensity of color, which begins at zero (achromatic) and increases in intensity. Berry firmness was measured with a texture analyzer (TA XT, Stable Micro Systems Ltd, UK) with a 2 mm probe used to penetrate to a depth of 6 mm at a speed of 1 mm/s. The maximum force (N), the strength necessary to puncture the skin of an individual berry, was measured, and berry deformation was determined.

Ethanol and acetaldehyde concentrations in the grape juice were determined from 5 mL of juice. The juice samples were incubated for 1 h at 65 ^oC in a septum-capped tube. One milliliter sample from the headspace gas was analyzed for acetaldehyde and ethanol concentration using a gas chromatograph (model GC-9 AM; Shimadzu, Kyoto, Japan) with a flame ionization detector (250 ^oC) and a 5% carbowax on 60/80 Carbopack column (Supelco, Bellefonte, PA). Volatiles were quantified through comparisons to known standards, and the juice sample concentration was expressed as µL/L (Ke et al., 1991). Six replications per treatment were analyzed.

To determine whether the postharvest treatments affected berry flavor, fruits without field treatments (Control) were kept in storage for 8 weeks at 0.5 ± 1 ^oC, 95–98% RH in air or High CO_2, as described above. Randomized samples were submitted to a sensory triangle test. Briefly, three samples of two berries each, one different sample and two alike, were presented to 20 untrained panelists. The panelists were instructed to chew the berries and to identify the odd sample and record their answer.

5.2.5 Statistical analysis

A factorial design, with field treatment and storage treatment as factors, with six replications, was used. Decay incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before the analysis. The data were subjected to analysis of variance (ANOVA), and the means were separated by a Tukey's test ($p<0.05$).

5.3 Results

5.3.1 $CaCl_2$ phytotoxicity study

The degree of phytotoxicity caused by $CaCl_2$ depended on the concentration of the spray solution (Table 1). Clusters repeatedly treated with solutions containing 0.25% $CaCl_2$ had a similar proportion of undamaged berries as clusters treated with an adjuvant-only solution (0% $CaCl_2$), and the 0.25% $CaCl_2$ treatment only slightly increased the proportion of berries having slight or readily noticeable damage. However, clusters repeatedly treated with >0.25% $CaCl_2$ had fewer undamaged berries, and more berries with slight or readily observable damage compared with clusters treated with <0.25% $CaCl_2$. Treatments did not affect the proportion of rotten or shriveled berries at harvest.

5.3.2 Effects of pre-harvest treatments on fruit quality at harvest

The preharvest treatments did not affect berry weight, CIRG, or rachis brownness, but the $CaCl_2$ and ClO_2 treatments slightly reduced berry puncture resistance (Table 2). None of the treatments affected the fruit composition variables measured, including TSS, TA, TSS:TA ratio, ethanol, acetaldehyde, or calcium content (Table 3). Rot incidence in the field was <5%, and also not affected by the preharvest treatments (data not shown).

5.3.3 Effects of preharvest and postharvest treatments on fruit quality after postharvest storage

The TSS, TA and TSS/TA ratio were not affected by the treatments (Data not shown), these parameters were stable during all storage, with the mean for TSS/TA at initial quality evaluation being 32, and after 6 weeks + SL, 31.

The weight loss and rachis browning were not affected by field treatments or by postharvest treatments. The score for rachis browning changed during storage from 1 (completely green), at the beginning of the experiment, to 2 (brown color in the cap stem), after 6 weeks at 0.5 ^{o}C + SL (Data not shown).

The color was represented as color index for red grapes (Carreño et al., 1995). By 3 weeks + SL, fruits sprayed with $CaCl_2$ in the field exhibited a significantly stronger red color (3.2) than did Control (3.0) and ClO_2 (3.0) treated, but despite the difference, fruit color was classified as between pink and red. By 6 weeks of cold storage, the $CaCl_2$ exhibited stronger red color than did Control and ClO_2. At this evaluation, $CaCl_2$ treated fruits were classified as red (3.5) and Control (3.2) and ClO_2 (3.2) were classified as between pink and red color (Table 4). By 6 weeks + SL, a significant interaction between field treatment and storage treatments was found. At this time $CaCl_2$ + Air treated fruits exhibited a stronger color (3.3) than did ClO_2 + Air (3.1), but both were classified as being in the same range of color, between pink and red. The CIRG was not affected by storage treatments.

The maximum penetration force (MPF) was measured in order to determine the fruit firmness. No differences were detected after 3 weeks of cold storage, but when the fruits had warmed for two days, the $CaCl_2$ reduced the MPF, at 3.97 N, with the Control and $CaCl_2$ exhibiting values of 3.97 N and 4.41 N, respectively (Table 4). At this evaluation, grapes stored in High CO_2 (4.12 N) exhibited a lower MPF than did in Air (4.51 N). Similar results were obtained after 6 weeks of cold storage; in this evaluation the grapes field treated with $CaCl_2$ and the grapes stored in High CO_2 exhibited lower MPF. After 6 weeks of cold storage + SL, the grapes field treated with $CaCl_2$ (3.71 N) still exhibited a lower MPF than did ClO_2 (4.05 N).

The AA concentration slightly increased during cold storage, from 1.4 µL/L at initial quality to 1.7 µL/L after 6 weeks of cold storage (Table 5). The AA concentration was not affected by the interaction of field treatments and storage treatments or by the field treatments. By 3 weeks + SL the grapes stored in High CO_2 exhibited a higher AA concentration, at 4.4 µL/L, than did Air stored grapes, at 2.9 µL/L. Similarly after 6 weeks of cold storage and after 6 weeks of cold storage + SL, higher AA concentrations were still found in fruits stored in High CO_2. The higher temperature during SL led to a 2-fold increase in the AA concentration (Table 5).

Similar to AA concentration, the ethanol concentration was not affected by the interaction of field treatments and storage treatments or by the field treatments. After 6 weeks of cold storage, the High CO_2 storage significantly affected the ethanol concentration. The ethanol concentration was 3 times greater in the High CO_2, 85.7 µL/L, than in those stored in Air, 29.4 µL/L (Table 5).

A triangle sensory test was performed to determine whether the flavor of the grapes was affected by High CO_2 treatments in long-term cold storage. 20 people were submitted to the triangle test, which asked to them to indicate the odd sample; 16 out of 20, answers were wrong. There was no significant difference between the taste of the samples after 8 weeks cold storage in Air or in these stored in High CO_2 ($p < 0.01$).

5.3.4 Calcium content

Samples collected 5 weeks after the last application was analyzed for calcium content. In fruits treated in the field and no significant differences was detected between $CaCl_2$ (0.075%), Control (0.073%), and ClO_2 (0.064%).

5.3.5 Decay incidence

Natural incidence increased during cold storage from 0% to 13% during the 8 week evaluation period (Table 6). By 3 weeks of cold storage, the disease expression was very low, and the highest decay incidence was presented by fruits field treated with ClO_2 (1.3%). By 3 weeks +SL, the significantly beneficial effect of the High CO_2 storage treatment on the reduction of decay became visible, lasting until the end of experiment (*p-value* = 0.0000). Grapes stored under High CO_2 exhibited about 4 times less decay than did grapes stored under air, with values of 0.6% and 2.8%, respectively. By 6 weeks storage, a significant interaction between field treatments and storage conditions was found, the highest decay control decay being exhibited by the field treatments combined with High CO_2 (0.4%), with Control x Air storage (4.1%) following. By 6 weeks + SL, the ClO_2 field application exhibited a significant effect in the control of *B. cinerea*. In this evaluation, the decay incidence was three times lower in the ClO_2 treated fruits (5.5%) than in the Control (15.3%). At 8 weeks of cold storage, the effect of High CO_2 in the control of decay was

very pronounced. 10.1% fruit stored in Air exhibited decay, while 0.2% of fruits stored in High CO_2 exhibited it (Table 6).

The decay incidence in Control-Air surface inoculated grapes increased from 1.4% to 51.8% during the period from 3 weeks to 8 weeks of cold storage (Table 7 and 7b). A significant interaction between field and storage treatments was found in the grapes inoculated with a conidia solution. By 3 weeks of cold storage, no effect of the treatments could be found due the low decay incidence. By 3 weeks + SL, the gray mold incidence increased very quickly, mainly in grapes stored in air. At this evaluation, a significant interaction between field treatments and storage conditions was found: the best control of decay was obtained by field treatments combined with High CO_2 (~0.6%), with $CaCl_2$+ Air (11.6%) following. Similar outcome was seen at 6 weeks of cold storage: the treatments stored under High CO_2 conditions maintained an infection incidence of 0.5% while 45% of the Control-Air berries had decayed. By 8 weeks of storage at 0.5 ^oC, the decay incidence was over 50% in grapes stored in air, while the infection in fruits stored in High CO_2 was below 8%, representing a 6-fold reduction. In the subsequent warming period, decayed berries were removed from the bag and the fruits without symptoms were kept for the evaluations (Table 7 and 7b). This change in methodology reduced the rate of decay, but it still showing the beneficial effects of High CO_2 storage.

The decay incidence in nesting inoculated fruits increased from 5% to 37% during the 8 weeks of cold storage (Table 8). Storage in High CO_2 reduced the gray mold throughout the experiment. By 3 weeks, grapes stored under High CO_2 exhibited a decay infection (0.3%) 17 times lower than Air stored (5.1%). When the fruits had warmed for two days, the differences between field treatments became visible; at this time Control grapes exhibited a lower incidence of infection than did grapes sprayed with $CaCl_2$, 17% and 35%, respectively. By 8 weeks of cold storage, grapes stored in High CO_2 exhibited 8 times less decay (8%) than did fruits stored in Air (49%). During the warming period, the grapes stored in High CO_2 exhibited a lower decay incidence than did the fruits stored in air (Table 8).

The High CO_2 storage treatment led to a lower incidence of infection in naturally infected berries and artificially inoculated grapes (Tables 6, 7, 7b and 8). The $CaCl_2$ and ClO_2 field treatments provided some protection against conidia infection, but they were not efficient

against nesting infection, moreover, in some evaluations $CaCl_2$ and ClO_2 yielded an increase of the infection.

5.4 Discussion

5.4.1 Quality evaluations

Field and postharvest treatments did not affect the weight loss and rachis browning for the grapes. The rachis appearance changed during the storage from score 1 (completely green) at the beginning of experiment to score 2 (brown color in the cap stem) after 6 weeks at 0.5 ^oC + SL (Data not shown). The absence a difference between weights for the treatments could be a result of the storage in metal tanks, which kept the RH high during the cold storage, reducing transpiration. The application of high CO_2 for 3 days reduced the weight losses and rachis browning in 'Cardinal' table grapes after the storage for 33 days at 0 ^oC (Sanchez-Ballesta et al., 2006). On the other hand, an increase in rachis browning has been reported for several varieties of tables grapes stored in high CO_2 (Chen et al., 2011; Crisosto et al., 2002a, b; Retamales et al., 2003).

The 'Crimson Seedless' table grape was harvested with CIRG between pink and red (~3.1), the field treatments did not affect the CIRG at harvesting. During storage, the grapes treated with $CaCl_2$ exhibited a more red color, but just at 6 weeks of cold storage the fruits were classified in a different range, from pink to red color (Carreño et al., 1995). The high CO_2 did not affect the 'Crimson Seedless' color during storage. The skin color of 'Cardinal' table grape treated with high CO_2 was brighter, with higher C^* value, than that of non-treated grapes (Sanchez-Ballesta et al., 2006).

By harvest, fruits treated with $CaCl_2$ and ClO_2 exhibited lower firmness than did the Control. The foliar application of 1% of $CaCl_2$ did not raise the firmness of 'Thompson Seedless' berries (Bonomelli and Ruiz, 2010). During storage, fruits treated with $CaCl_2$ exhibited a lower MPF, with the exception of the 3 week evaluation. The foliar application of 1% $CaCl_2$ did not raise the firmness of 'Thompson berries', when the treatments were applied to the soil the berries were firmer, but this effect disappeared after 30 days of storage at 0 ^oC (Bonomelli and Ruiz, 2010). The firmness of 'Superior Seedless' grapes

packed in modified atmosphere was not affected by treatments after 1 week of cold storage followed by a shelf life period (Artés-Hernández et al., 2006).

The TSS, TA and TSS/TA ratio were not affected by pre-harvest treatments or by storage treatments. The application of 20% CO_2 + 20% O_2 for 3 d did not affect the SSC and TA in 'Cardinal' tables grapes (Sanchez-Ballesta et al., 2006). Storage in CA with high CO_2 concentrations did not affect the TSS and TA in 'Red Globe' table grapes (Crisosto et al., 2002a) Chervin et al. (2009) reported that the pre-harvest application of $CaCl_2$ solution or combined with 16% ethanol did not affect TA, TSS, visual quality or taste quality.

The storage in High CO_2 did not affect the flavor of 'Crimson Seedless' table grapes after 8 weeks of cold storage. In agreement with our findings, 'Autumn Seedless' table grapes did not yielded off-flavors, and they preserved a good appearance after 60 d of storage at 0 ^oC (Artés-Hernández et al., 2004). On the other hand, off-flavors were detected in 'Thompson Seedless' table grapes stored in CA with concentrations higher than 15% CO_2; it was found that early harvested grapes were more affected than were late harvested (Crisosto et al., 2002b). 'Kyoho' grapes stored at 4% O_2 + 30% CO_2 exhibited off-flavors development too, and the off flavor being associated with high production of ethanol (Deng et al., 2006).

The acetaldehyde (AA) and ethanol concentrations were affected by High CO_2 storage and by the increase in temperature during the shelf life simulation. The high CO_2 increased the concentration of these compounds, but grapes analyzed after the shelf life simulation exhibited 3 times higher concentrations than di those analyzed during the cold storage. The production of AA and ethanol in cherries stored in modified atmosphere increased with the increasing in temperature (Petracek et al., 2002). The low O_2 concentration during the initial fumigation with 40% CO_2, along with the high CO_2 concentration during the cold storage, may have induced anaerobic fermentation in part of the fruit, resulting in the production of AA and ethanol. Pesis (2005) demonstrated that short-duration low O_2 pretreatments, which induce the production of acetaldehyde and ethanol, produces beneficial effects in many subtropical fruits, but treatments of longer duration can lead to production of off-flavors.

An increase in the concentration of AA and ethanol can induce resistance against fungi attack in the fruit (Pesis et al., 1989), and the AA and ethanol it can also act directly against the pathogen. The AA vapor induces the leakage of electrolytes, sugars and amino acids

from the fungi *B. cinerea* and *R. stolonifer*, which suggests that cell membranes are irreversibly disrupted by AA as a first step towards inhibition of the fungus activity (Avissar et al., 1990). In 'Kyoho' grapes stored at 4% O_2 + 30% CO_2, ethanol levels reached 0.326% and the grapes were reject by trained panel because of the off-flavor (Deng et al., 2006); these concentration of ethanol are more than 9 times higher than the highest concentration from our study.

The field treatments did not increase the calcium content in berries. Similar results were obtained in Chile, where foliar application of $CaCl_2$ did not increase the calcium content in the fruits, grape yields, bunch weights and TSS content in two seasons of evaluation (Bonomelli and Ruiz, 2010).

5.4.2 Decay

The association of field treatments and High CO_2 maintaining the decay incidence, in surface inoculated grapes, lower than accepted maximum decay level on commercial stored table grapes at the point of shipping for US n°. 1 grade California grapes (0.5%) up to 6 weeks cold storage. The High CO_2 storage, combination of an initial fumigation with 40% CO_2 for 24 h followed by storage in CA (12% O_2 + 12% CO_2), reduced the decay incidence in conidia and nesting inoculated grapes. The control of gray mold in table grapes stored in CA with CO_2 high concentrations has been reported in grape varieties such as 'Kyoho' (Deng et al., 2006), 'Red Globe' and 'Thompson Seedless' (Crisosto et al., 2002a, b). In agreement with our findings, the pretreatment with 20% CO_2 + 20% O_2 in 'Cardinal' table grapes reduced the incidence of decay from 25% to 5% after 30 days of cold storage (Sanchez-Ballesta et al., 2006). Romero et al. (2008) suggested that the effectiveness of high CO_2 pretreatment in controlling fungal decay and maintaining fruit quality after prolonged low-temperature storage is a result of prevention of the formation of reactive oxygen species rather than a result of their inactivation once formed.

$CaCl_2$ and ClO_2 applications reduced the incidence of decay in surface inoculated fruits up to 6 weeks of cold storage + SL. On the other hand, the field treatments yielded a limited control of gray mold for nesting inoculated grapes, increasing the infection in some evaluations. $CaCl_2$ is one of the alternatives for decay control in commercial use in conventional and organic vineyards in Italy (Romanazzi et al., 2012). The application of

1% $CaCl_2$ solution in pre-harvest reduced the incidence of decay from 64% to 22% in 'Italy' table grapes; the treatment was more effective for earlier applications and higher numbers of applications (Nigro et al., 2006). The $CaCl_2$ also reduced the incidence of decay at harvest from 16% to 11% in 'Chasselas' grapes, and the proportion of commercial grapes after 6 weeks of cold storage was improved from 5% to 45% (Chervin et al., 2009). The mechanism by which calcium increases the resistance of plant tissues to pathogens is not completely understood. The formation of calcium cross-linkages between pectin polymers could make the cell wall more resistant to hydrolytic to enzymes produced by decay causing organisms (Tobias et al., 1993). Another possibility is that the $CaCl_2$ treatments affect pathogens directly, inhibiting the activity of polygalacturonase (Nigro et al., 2006). $CaCl_2$ at a concentration of 1% is used in Europe for pre-treatment of grapes (Chervin et al., 2009; Nigro et al., 2006), but under Central Valley Californian conditions, $CaCl_2$ at this concentration caused phytotoxicity in fruits and leaves; from the second application on the concentration was reduced to 0.25%. Nesting inoculated and $CaCl_2$ treated grapes exhibited a higher decay than did the Control grapes. It was reported that low concentrations of calcium can stimulate *B. cinerea* growth *in vitro* (Chardonnet et al., 2000). After 12 days at 12 ^{o}C, 'Isabel' table grapes treated in postharvest with $CaCl_2$ doses exhibited a higher incidence of decay for concentrations above 1% (da Silva et al., 2012).

Chlorine dioxide (ClO_2) is a powerful oxidant, more stable and stronger than chlorine. The use of ClO_2 in postharvest helped to control anthracnose in litchi (Wu et al., 2011) and decay in figs and strawberries (Karabulut et al., 2009; Vardar et al., 2012). However, to our knowledge, the spraying of ClO_2 aqueous solutions in pre-harvest has not yet been investigated. In our study, ClO_2 control *B. cinerea* when the grapes had been inoculated by conidia. The ClO_2 associated with High CO_2 treatment maintained the decay incidence in naturally infected grapes lower than 0.5% up to 8 weeks of cold storage. ClO_2 affect multiple sites of biochemical activity in microorganisms, affecting the function of cell membranes and interfering in the activity of some proteins and RNA (Zoffoli et al., 2005).

5.5 Conclusion

The storage of 'Crimson Seedless' table grapes treated with 40% CO_2 for 24 h followed by CA of 12% CO_2 + 12% O_2 reduced the decay incidence in naturally infected, surface inoculated and nesting inoculated grapes. The storage in High CO_2 controlled *B cinerea* growth in grapes without compromising the visual or chemical quality or the flavor. Our results show that it is possible to reach the US standards for table grapes up to 6 weeks of cold storage in high CO_2, for naturally infected and surface inoculated grapes. Concentrations of 0.5% $CaCl_2$ or higher caused phytotoxic symptoms, damaging berries and leaves. The $CaCl_2$ and ClO_2 treatments helped reduce the incidence of decay in surface inoculated grapes stored in air. In our conditions the association of $CaCl_2$ or ClO_2 with High CO_2 just improved the control of *B. cinerea* in surface inoculated grapes.

Literature Cited

1. Artés-Hernández, F., Aguayo, E., Artés, F., 2004. Alternative atmosphere treatments for keeping quality of 'Autumn seedless' table grapes during long-term cold storage. Postharvest Biology and Technology 31, 59-67.
2. Artés-Hernández, F., Tomas-Barbéran, F., Artés, F., 2006. Modified atmosphere packaging preserves quality of SO_2-free 'Superior seedless' table grapes. Postharvest Biology and Technology 39, 146-154.
3. Avissar, I., Droby, S., Pesis, E., 1990. Characterization of Acetaldehyde Effects on *Rhizopus stolonifer* and *Botrytis cinerea*. Ann Appl Biol 116, 213-220.
4. Bonomelli, C., Ruiz, R., 2010. Effects of foliar and soil calcium application on yield and quality of table grape cv. 'Thompson Seedless'. Journal of Plant Nutrition 33, 299-314.
5. Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1995. Proposal of an Index for the objective evaluation of the color of red table grapes. Food Res Int 28, 373-377.
6. Chardonnet, C.O., Sams, C.E., Trigiano, R.N., Conway, W.S., 2000. Variability of three isolates of *Botrytis cinerea* affects the inhibitory effects of calcium on this fungus. Phytopathology 90, 769-774.

7. Chen, S.J., Zhang, M., Wang, S.J., 2011. Effect of initial hermetic sealing on quality of 'Kyoho' grapes during storage. Postharvest Biology and Technology 59, 194-199.
8. Chervin, C., Lavigne, D., Westercamp, P., 2009. Reduction of gray mold development in table grapes by preharvest sprays with ethanol and calcium chloride. Postharvest Biology and Technology 54, 115-117.
9. Crisosto, C.H., Garner, D., Crisosto, G., 2002a. Carbon dioxide-enriched atmospheres during cold storage limit losses from Botrytis but accelerate rachis browning of 'Redglobe' table grapes. Postharvest Biology and Technology 26, 181-189.
10. Crisosto, C.H., Garner, D., Crisosto, G., 2002b. High carbon dioxide atmospheres affect stored 'Thompson seedless' table grapes. Hortscience 37, 1074-1078.
11. da Silva, R.S., Silva, S.D., Dantas, A.L., Mendonça, R.M.N., Guimarães, G.H.C., 2012. Quality of 'Isabel' Grape Treated with Calcium Chloride in Postharvest and Stored under Modified Atmosphere. Revista Brasileira de Fruticultura 34, 50-56.
12. Deng, Y., Wu, Y., Li, Y.F., 2006. Physiological responses and quality attributes of 'Kyoho' grapes to controlled atmosphere storage. Lwt-Food Sci Technol 39, 584-590.
13. Hoogerwerf, S.W., Kets, E.P.W., Dijksterhuis, J., 2002. High-oxygen and high-carbon dioxide containing atmospheres inhibit growth of food associated moulds. Lett Appl Microbiol 35, 419-422.
14. Ippolito, A., Sanzani, S.M., 2011. Control of postharvest decay by the Integration of pre- and postharvest application of nonchemical compounds. Acta Hort. (ISHS) 905, 135-143.
15. Karabulut, O.A., Ilhan, K., Arslan, U., Vardar, C., 2009. Evaluation of the use of chlorine dioxide by fogging for decreasing postharvest decay of fig. Postharvest Biology and Technology 52, 313-315.
16. Ke, D.Y., Goldstein, L., O'Mahony, M., Kader, A.A., 1991. Effects of short-term exposure of low O_2 and high CO_2 atmospheres on quality attributes of strawberries. Journal of Food Science 56, 50-54.
17. Lichter, A., Mlikota Gabler, F., Smilanick, J.L., 2006. Control of spoilage in table grapes. Stewart Postharvest Review 2, 1-10.

18. Luvisi, D., Shorey, H., Smilanick, J., Thompson, J., Gump, B., Knutson, J., 1992. Sulfur Dioxide Fumigation of Table Grapes, Universty of California, DANR, Bulletin 1932, p. 22.
19. Mlikota Gabler, F., Smilanick, J.L., Ghosoph, J.M., Margosan, D.A., 2005. Impact of postharvest hot water or ethanol treatment of table grapes on gray mold incidence, quality, and ethanol content. Plant Disease 89, 309-316.
20. Nigro, F., Schena, L., Ligorio, A., Pentimone, I., Ippolito, A., Salerno, M.G., 2006. Control of table grape storage rots by pre-harvest applications of salts. Postharvest Biology and Technology 42, 142-149.
21. Olmez, H., Kretzschmar, U., 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. Lwt-Food Sci Technol 42, 686-693.
22. Palou, L., Crisosto, C.H., Garner, D., Basinal, L.M., Smilanick, J.L., Zoffoli, J.P., 2002. Minimum constant sulfur dioxide emission rates to control gray mold of cold-stored table grapes. American Journal of Enology and Viticulture 53, 110-115.
23. Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biology and Technology 37, 1-19.
24. Pesis, E., Marinansky, R., Avissar, I., 1989. Effect of prestorage treatments with acetaldehyde vapors or anaerobic conditions on volatiles accumulation during storage of various fruits. Acta horticulturae. Dec 258, 661-667.
25. Petracek, P.D., Joles, D.W., Shirazi, A., Cameron, A.C., 2002. Modified atmosphere packaging of sweet cherry (*Prunus avium* L., ev. 'Sams') fruit: metabolic responses to oxygen, carbon dioxide, and temperature. Postharvest Biol Tec 24, 259-270.
26. Retamales, J., Defilippi, B.G., Arias, M., Castillo, P., Manriquez, D., 2003. High-CO2 controlled atmospheres reduce decay incidence in Thompson Seedless and Red Globe table grapes. Postharvest Biology and Technology 29, 177-182.
27. Romanazzi, G., Lichter, A., Gabler, F.M., Smilanick, J.L., 2012. Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. Postharvest Biology and Technology 63, 141-147.

28. Romero, I., Sanchez-Ballesta, M.T., Maldonado, R., Escribano, M.I., Merodio, C., 2006. Expression of class I chitinase and beta-1,3-glucanase genes and postharvest fungal decay control of table grapes by high CO_2 pretreatment. Postharvest Biology and Technology 41, 9-15.
29. Romero, I., Sanchez-Ballesta, M.T., Maldonado, R., Escribano, M.I., Merodio, C., 2008. Anthocyanin, antioxidant activity and stress-induced gene expression in high CO_2-treated table grapes stored at tow temperature. J Plant Physiol 165, 522-530.
30. Sanchez-Ballesta, M.T., Jimenez, J.B., Romero, I., Orea, J.M., Maldonado, R., Urena, A.G., Escribano, M.I., Merodio, C., 2006. Effect of high CO2 pretreatment on quality, fungal decay and molecular regulation of stilbene phytoalexin biosynthesis in stored table grapes. Postharvest Biology and Technology 42, 209-216.
31. Tobias, R.B., Conway, W.S., Sams, C.E., Gross, K.C., Whitaker, B.D., 1993. Cell-Wall Composition of Calcium-Treated Apples Inoculated with *Botrytis cinerea*. Phytochemistry 32, 35-39.
32. Vardar, C., Ilhan, K., Karabulut, O.A., 2012. The application of various disinfectants by fogging for decreasing postharvest diseases of strawberry. Postharvest Biology and Technology 66, 30-34.
33. Wells, J.M., Uota, M., 1970. Germination and growth of five fungi in low-oxygen and high-carbon dioxide atmospheres. Phytopathology 60, 50-53.
34. Wu, B., Li, X.P., Hu, H.G., Liu, A.Y., Chen, W.X., 2011. Effect of chlorine dioxide on the control of postharvest diseases and quality of litchi fruit. Afr J Biotechnol 10, 6030-6039.
35. Zoffoli, J.P., Latorre, B.A., Daire, N., Viertel, S., 2005. Effectiveness of chlorine dioxide as influenced by concentration, pH, and exposure time on spore germination of *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer*. Ciencia e Investigacion Agraria 32, 181-188.

Figure 1: Treatments application in the field: A)- spraying the fruits during the first application; B)- late flowering; C)- berry touch; D)- "veraison"; E)- harvest.

Figure 2: Harvesting the fruits: A)- moving the fruits between rows; B)- cutting clusters; C)- cleaning cluster during the field package.

Figure 3: Inoculation and preparing clusters to the analysis: A) infected berries to nesting inoculation; B)- spraying a conidia solution (surface inoculation); C)- clusters drying after surface inoculation; D)- removing berries to quality analysis; E)- rachis to browning analysis.

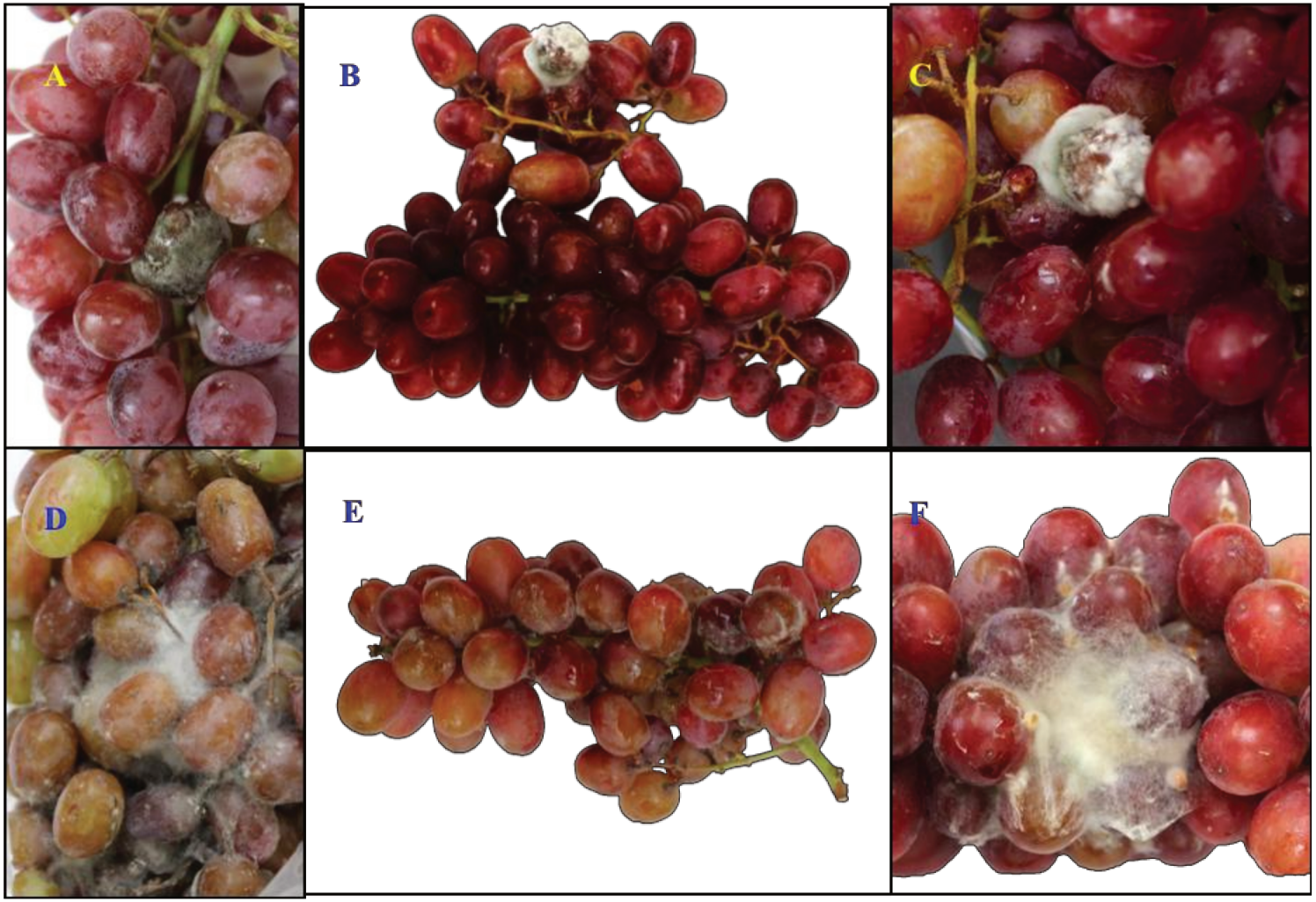

Figure 4: 'Crimson Seedless' grapes nesting inoculated, stored for 6 weeks at 0.5 ^{o}C + 2 days at 20 ^{o}C, RH 95-98% : A)- Detail in nesting, Control + High CO_2 treatment; B)- $CaCl_2$ + High CO_2 treatment; C)- detail in nesting ClO_2 + High CO_2 treatment; D)- detail in nesting , Control + Air treatment; E)- $CaCl_2$ + Air treatment; F)- detail in nesting ClO_2+ Air treatment.

Table 1 Effect of $CaCl_2$ concentration in preharvest spray solutions on phytotoxicity of Crimson Seedless berries, Parlier, CA, 2012.The grapes were sprayed 4 times in the field with $CaCl_2$, and harvested 40 d after the last spray.

$CaCl_2$ concentration (%)	Berries per cluster with observed level of phytotoxicity (%)				Total berries per cluster (N^o)
	None	Slight	Readily observable	Shriveled/Rotten	
0	92.3 a	0.0 c	0.8 b	6.9	124
0.25	72.6 a	15.7 b	5.5 b	6.2	134
0.50	38.7 b	22.7 a	29.8 a	8.8	125
1.00	24.9 b	21.5 a	43.0 a	10.6	167

Treatment means (n = 6) within a column followed by same letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 2 Effect of preharvest treatments on Crimson Seedless berry and rachis physical characteristics at harvest, Parlier, CA, 2012. The grapes were sprayed 4 times in the field with $CaCl_2$ or ClO_2, or not sprayed as control.

Preharvest treatment	Berry			Rachis
	Weight (g)	CIRG	Puncture Force (N)	Browning
Control	6.4	3.09	4.54 a	1.0
$CaCl_2$	6.5	3.06	4.01 b	1.0
ClO_2	6.4	3.12	4.05 b	1.0

Treatment means (n = 6) within a column followed by same letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 3 Effect of preharvest treatments on Crimson Seedless berry composition at harvest, Parlier, CA, 2012. The grapes were sprayed 4 times in the field with $CaCl_2$ or ClO_2, or not sprayed as control.

Preharvest treatment	TSS (Brix)	TA (g/100 ml)	TSS/TA	Ethanol (µL/L)	Acetaldehyde (µL/L)	Calcium content (%)
Control	20.4	0.65	31.7	62.0	1.5 a	0.073
$CaCl_2$	19.5	0.60	32.6	48.3	1.2 a	0.075
ClO_2	19.7	0.63	31.3	51.1	1.5 a	0.064

Treatment means (n = 6) within a column followed by same letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 4 Color index of red grapes (CIRG) and maximum penetration force (MPF) in 'Crimson Seedless' table grapes stored for 3 weeks to 6 weeks at 0.5 ± 1 ◦C, 95–98% RH. The grapes were field treated with $CaCl_2$ or ClO_2 or not treated as control. After harvested, the fruits were stored in metal tanks and submitted to an atmosphere of air (Air) or pre-stored with 40% CO_2 for 24 h and then stored at controlled atmosphere of 12% CO_2 +12% O_2 (High CO_2) until the end of cold storage.

Field treatments[b]	CIRG[a]		MPF (N)	
	Storage time			
	3 weeks	6 weeks	3 weeks	6 weeks
Control	3.19	3.22 b	3.98	3.80 a
$CaCl_2$	3.09	3.49 a	4.28	3.48 b
ClO_2	3.23	3.20 b	4.09	3.82 a
Field p-value	0.8001	0.0001	0.2141	0.0005
Storage treatments[c]				
Air	3.18	3.33	4.03	3.81 A
High CO_2	3.16	3.28	4.20	3.59 B
Storage *P-value*	0.9627	0.3877	0.2367	0.0051
Field x Storage p-value	0.4945	0.5684	0.7216	0.4470

[a] Color classification: yellow (1.4-1.7); Pink (2.2 – 2.8); Red (3.4 – 3.9); Violet (4.5 – 4.9) and dark violet (5.3 – 5.8) (Carreño et al., 1995);

[b] Field treatment means (n = 12) within a column followed by same small letter are not significantly different ($p < 0.05$);

[c] Storage treatments means (n=18) within a column followed by same capital letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 5 Concentration of acetaldehyde and ethanol (µL/L) in 'Crimson Seedless' table grapes stored for 3 weeks to 6 weeks at 0.5 ± 1 °C, 95–98% RH followed or not by a warm up period of 2 days at 20 ± 1 °C (SL). The grapes were field treated with $CaCl_2$ or ClO_2 or not treated as control. After harvested, the fruits were stored in metal tanks and submitted to an atmosphere of air (Air) or pre-stored with 40% CO_2 for 24 h and then stored at controlled atmosphere of 12% CO_2 +12% O_2 (High CO_2) until the end of cold storage.

	Acetaldehyde (µL/L)				Ethanol (µL/L)			
Initial quality	1.4				53.8			
Treatments	3 wks	3 wks + SL	6 wks	6 wks + SL	3 wks	3 wks + SL	6 wks	6 wks + SL
Air	1.0	2.9 b	1.3 b	2.5 b	47.0	182.4	29.4 b	161.9
High CO_2	1.5	4.4 a	2.1 a	3.9 a	88.1	277.3	85.7 a	214.6
Storage P value	0.0667	0.0241	0.0452	0.0178	0.0970	0.1005	0.0080	0.1410
Field P value	0.8940	0.4085	0.5630	0.6869	0.5012	0.0647	0.1952	0.3420
Field x Storage P value	0.9228	0.7830	0.9496	0.5256	0.7429	0.6667	0.3798	0.3390

Storage treatment means within a column followed by the same small letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 6 Natural decay incidence (%) in 'Crimson Seedless' table grapes clusters treated with the combination of field and storage treatments. The grapes were sprayed 4 times in the field with $CaCl_2$ (Calcium) or ClO_2, or not sprayed as control. After harvested, grapes were stored at 0.5 ± 1 ◦C, 95–98% RH and submitted to an atmosphere of air (Air) or pre-stored with 40% CO_2 for 24 h and then stored at controlled atmosphere of 12% CO_2 +12% O_2 (High CO_2) until the end of cold storage, followed or not by a warm up period of 2 days at 20 ± 1 ◦C (SL) or kept at 20 ± 1 ^oC from 1 to 3 days, to evaluate the decay process when decayed berries were removed daily.

Field treatments[a]	Storage time							
	3 weeks	3 weeks + SL	6 weeks	6 weeks + SL	8 weeks	8 weeks + 1 day	8 weeks +2 days	8 weeks +3 days
Control	0.2 b	1.2	2.2	15.3 a	6.8	11.1	14.8	27.2
Calcium	0.5 ab	2.4	5.2	12.7 a	3.3	5.5	11.1	23.7
ClO_2	1.3 a	1.6	6.5	5.5 b	5.3	9.2	14.0	23.5
Field P value	0.0322	0.2688	0.0676	0.0000	0.2141	0.2593	0.8833	0.8915
Storage treatments[b]								
AIR	0.8	2.8 A	8.9 A	20.5 A	10.1 A	16.8 A	25.0 A	40.5 A
High CO_2	0.5	0.6 B	0.3 B	1.9 B	0.2 B	0.5 B	1.6 B	9.1 B
Storage P value	0.4448	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Field x Storage P value	0.1761	0.3033	0.0472	0.7371	0.6496	0.1287	0.1148	0.1403

Incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before analysis of variance and Tukey's test.

[a] Field treatment means (n = 12) within a column followed by same small letter are not significantly different according to Tukey's test ($p < 0.05$);

[b] Storage treatments means (n= 18) within a column followed by same capital letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 7 Decay incidence in surface inoculated 'Crimson Seedless' table grapes clusters treated with the combination of field and storage treatments. The grapes were sprayed 4 times in the field with $CaCl_2$ or ClO_2, or not sprayed as control. After harvested, grapes were stored at 0.5 ± 1 ◦C, 95–98% RH and submitted to an atmosphere of air (Air) or pre-stored with 40% CO_2 for 24 h and then stored at controlled atmosphere of 12% CO_2 +12% O_2 (High CO_2) until the end of cold storage, followed or not by a warm up period of 2 days at 20 ± 1 ◦C (SL) or kept at 20 ± 1 ^oC from 1 to 3 days, to evaluate the decay process when decayed berries were removed daily.

Treatments	Storage time							
	3 weeks	3 weeks + SL	6 weeks	6 weeks + SL	8 weeks	8 weeks +1 day	8 weeks +2 days	8 weeks +3 days
Control + Air	1.4	46.8 a	45.0 a	79.7	51.8	71.5 a	79.0 a	84.7
$CaCl_2$ + Air	2.1	11.6 b	23.2 b	55.2	42.0	54.3 a	62.6 a	69.7
ClO_2 + Air	0.4	15.7 b	15.6 b	55.0	56.4	66.2 a	74.8 a	84.6
Control + High CO_2	0.0	0.3 c	0.5 c	16.1	0.7	0.9 b	3.3 b	39.3
$CaCl_2$ + High CO_2	1.4	1.3 c	0.5 c	5.5	7.7	10.2 b	14.2 b	33.4
ClO_2 + High CO_2	0.3	0.2 c	0.3 c	10.1	2.7	3.7 b	8.9 b	34.2
Field P value	0.0721	0.0008	0.0036	0.0053	0.6618	0.9856	0.7135	0.1239
Storage P value	0.0582	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Field x Storage P value	0.3776	0.0005	0.0081	0.2092	0.0927	0.0342	0.0199	0.3414

Incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before analysis of variance and Tukey's test. Treatments means (n = 6) within a column followed by same letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 7b Decay incidence in surface inoculated 'Crimson Seedless' table grapes clusters treated with the combination of field and storage treatments. The grapes were sprayed 4 times in the field with $CaCl_2$ or ClO_2, or not sprayed as control. After harvested, grapes were stored at 0.5 ± 1 ◦C, 95–98% RH and submitted to an atmosphere of air (Air) or pre-stored with 40% CO_2 for 24 h and then stored at Controlled Atmosphere of 12% CO_2 +12% O_2 (High CO_2) until the end of cold storage, followed or not by a warm up period of 2 days at 20 ± 1 ◦C (SL) or kept at 20 ± 1 ^{o}C from 1 to 3 days, to evaluate the decay process when decayed berries were removed daily.

Field Treatments[a]	Storage time							
	3 weeks	3 weeks + SL	6 weeks	6 weeks + SL	8 weeks	8 weeks +1 day	8 weeks +2 days	8 weeks +3 days
Control	0.7	23.5 a	22.8 a	47.9 a	26.2	36.2	41.2	62.0
$CaCl_2$	1.7	6.5 b	11.9 b	30.3 b	24.9	32.3	38.4	51.6
ClO_2	0.4	7.9 b	7.9 b	32.5 b	29.5	35.0	41.9	59.4
Field P value	0.0721	0.0008	0.0036	0.0053	0.6618	0.9856	0.7135	0.1239
Storage treatments[b]								
Air	1.3	24.7 A	27.9 A	63.3 A	50.1 A	64.0 A	72.1 A	79.7 A
High CO_2	0.6	0.6 B	0.4 B	10.5 B	3.7 B	4.9 B	8.8 B	35.6 B
Storage P value	0.0582	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Field x Storage P value	0.3776	0.0005	0.0081	0.2092	0.0927	0.0342	0.0199	0.3414

Incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before analysis of variance and Tukey's test.

[a] Field treatment means (n = 12) within a column followed by same small letter are not significantly different ($p < 0.05$);

[b] Storage treatments within a column followed by same capital letter are not significantly different according to Tukey's test ($p < 0.05$).

Table 8 Decay incidence in nesting inoculated 'Crimson Seedless' table grapes clusters treated with the combination of field and storage treatments. The grapes were sprayed 4 times in the field with $CaCl_2$ or ClO_2, or not sprayed as control. After harvested, grapes were stored at 0.5 ± 1 ◦C, 95–98% RH and submitted to an atmosphere of air (Air) or pre-stored with 40% CO_2 for 24 h and then stored at controlled atmosphere of 12% CO_2 +12% O_2 (High CO_2) until the end of cold storage, followed or not by a warm up period of 2 days at 20 ± 1 ◦C (SL) or kept at 20 ± 1 ^{o}C from 1 to 3 days, to evaluate the decay process when decayed berries were removed daily.

Treatments	Storage time							
	3 weeks	3 weeks +2 days	6 weeks	6 weeks +2 days	8 weeks	8 weeks +1 day	8 weeks +2 days	8 weeks +3 days
Control	3.0	17.6 b	19.1	64.1	20.0 b	30.8	40.5	64.5
$CaCl_2$	3.0	34.7 a	16.6	63.8	31.4 a	44.1	55.6	64.4
ClO_2	2.1	16.1 b	24.0	54.4	34.7 a	43.1	52.9	65.8
Field P value	0.7358	0.0003	0.6528	0.0707	0.0365	0.1957	0.2362	0.9070
Storage Treatments								
Air	5.1 A	35.4 A	37.3 A	90.3 A	49.1 A	61.4 A	68.3 A	75.3 A
High CO_2	0.3 B	10.2 B	2.5 B	31.2 B	8.3 B	17.3 B	31.1 B	54.5 B
Storage P value	0.0000	0.0000	0.0000	0.000	0.0000	0.0000	0.0000	0.0128
Field x Storage P value	0.1080	0.4092	0.4379	0.0557	0.8464	0.7807	0.5417	0.2242

[a] Incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before analysis of variance and Tukey's test.

[b] Field treatment means (n = 12) within a column followed by same small letter are not significantly different ($p < 0.05$);

[c] Storage treatments means (n=18) within a column followed by same capital letter are not significantly different according to Tukey's test ($p < 0.05$).

6. CONCLUSÕES

- O uso da associação de concentrações de 40% CO_2 em pré-armazenagem por 24 ou 48 h com atmosfera controlada de 12% O_2 + 12% CO_2 reduz podridões pós-colheita em frutas naturalmente infectadas ou inoculadas artificialmente nas duas variedades testadas, Flame Seedless e Crimson Seedless.
- A aplicação de 40% CO_2 por 24 ou 48 h atua diretamente sobre o *B. cinerea*, inibindo o crescimento radial *in vitro* por 72 h e retardando em 12 h a germinação de conídios.
- A aplicação pré-colheita de $CaCl_2$ e ClO_2 reduz a infecção de *B. cinerea* em uvas 'Crimson Seedless' inoculadas por spray uma solução de conídios.
- A associação de aplicações de $CaCl_2$ e ClO_2 com o armazenamento em alto CO_2, inibe podridões pós-colheita veinculadas por infecções na superfície de uvas 'Crimson Seedless'.
- O uso da associação de concentrações de 40% CO_2 por 24 ou 48 h em pré armazenamento com atmosfera controlada de 12% O_2 + 12% CO_2 não afetou o sabor e aparência dos cachos de uva e preservou os parâmetros físico e químicos analisados.

7. APÊNDICE I :

Capítulo 2 experimentos preliminares para a seleção das concentrações de CO_2 aplicadas durante o pré-armazenamento

Materials and methods

In order to select the best concentration of CO_2, time application and temperature of application two experiments were performed. After these parameters were determined, the combination of high CO_2 pre-storage with storage in controlled atmosphere was evaluated.

Short time exposition to high CO_2 application in 'Thompson Seedless' and 'Flame Seedless' table grapes

Two independent experiments were set up, one with 'Thompson Seedless' and a second one with 'Flame Seedless' table grapes. The table grapes were purchased at the local market and brought to the Postharvest Laboratory at UC Davis, the berries were cut from rachis and then superficially disinfected by immersion for one min in diluted bleach (0.5% sodium hypochlorite), rinsed with fresh water, and allowed to dry at room temperature (20 ± 2 °C).

To evaluate control of gray mold nesting from aerial mycelia growth, 20 μL of a suspension of $1x10^6$ conidia/mL of *B. cinerea* (isolate IC 08, from T.J. Michailides) were injected 10 mm deep into the flesh of individual berries using an automatic pipette (needle of 1 mm external diameter) and incubated at 20 °C for four days until mycelium was visible. One of these previously inoculated berries was placed in the center of a petri dish in contact with six surrounding healthy berries (Palou et al., 2002). The petri dishes were placed in 8.3L polypropylene containers. Each petri dish was considered as an experimental unit.

In 'Thompson seedless was evaluated the effect of application of Air; 100% N_2; 20% CO_2; 40% CO_2 and 60% CO_2 in the control of gray mold nesting from aerial mycelia

growth, it was used four replications per treatment. In 'Flame Seedless' was evaluated the effect of application of Air; 40% CO_2; 60% CO_2 in the control of gray mold nesting from aerial mycelia growth using 6 replications. The gases were balanced with N_2 and applied in constant flow of 200 mL/min, during 24 h or 48 h, at temperature of 0 ^oC or 20 ^oC. Flow rates and gas mixtures were established using a mixing board with micro-metering valves. Supply and exhaust gas composition was monitored using a CO_2/O_2 gas analyzer (model 900141, Bridge analyzers, Inc.).

The berries were stored for 10 days at 0 ^oC and then placed at 20 ^oC. The presence of nesting was evaluated just after the treatment and after one, five, ten days of storage at 0 ^oC; then they were placed at 20 ^oC and evaluated at eleven, twelve and thirteen days. The mycelia growth was expressed as a percentage of decayed berries. It was used a randomized design and analysis of variance was applied to the daily data and means were separated using Tukey's test ($p < 0.05$).

Results and discussion

Decay incidence in 'Thompson Seedless' and 'Flame Seedless' table grapes exposed during a short time to high CO_2 concentrations

It was evaluated the decay incidence in 'Thompson Seedless' table grapes treated with different concentrations of CO_2 for 24 h at temperature of 0 ^oC and 20 ^oC and. It was not verified any significant differences between treatments when the fruits were treated at 0 ^oC (Fig. 1A). When the grapes were treated at 20 ^oC, the 40% CO_2 and 60% CO_2 treatments significantly reduced the decay until ten days at 0 ^oC + two days at 20 ^oC of storage (Fig. 1B). The 100% N_2 did not control the decay incidence in both temperatures (Fig. 1 A, B).

It was evaluated the decay incidence in nesting inoculated 'Flame Seedless' table grapes treated with two high CO_2 concentrations applied for 24 or 48 h in two temperatures 0 and 20 ^oC. Similar to 'Thompson Seedless' results, the effect of high CO_2 treatments was significant just when the treatments were applied at 20 ^oC (Fig. 2A and 2B).

The *B. cinerea* spread from the inoculated berry to healthy berries fast in air treatments and just after the treatments applications at 20 ^oC, it was possible verify a decay

incidence of 18.1%, 58.3% for AIR–24 h and AIR-48 h, respectively (Fig. 2A). 'Flame Seedless' grapes treated with high CO_2 only showed decay at ten days of cold storage and no difference was verified between the high CO_2 treatments. The treatment 60% CO_2 for 48 h at 20 ^oC caused strong off-flavor in 'Flame Seedless', which disappears after ten days of cold storage (Data not shown). Even though the differences between the treatments just expressed when the grapes were treated at 20 ^oC, the final decay incidence was higher than when fruits were treated at 0 ^oC (Fig. 1 and 2).

The application of concentrations of 40% or 60% CO_2 in fruits treated at 20^oC reduced the decay incidence in 'Thompson Seedless' and 'Flame Seedless', without significant difference among these treatments. Similar results were verified for 'Thompson Seedless' treated with concentrations of CO_2 from 15–35%, the high CO_2 promoted a good control of decay but did not present significant differences among the different CO_2 concentrations (Retamales et al., 2003). The application of 20% CO_2 in conidia of *B. cinerea* growing in Petri dishes delayed growth in 11 d and reduced the diameter of the mold to 25% of control values after 17 d (Hoogerwerf et al., 2002). In other hand, our results in grapes treated with 100% N_2 challenges the results verified in vitro by Wells and Uota (1970), when the absence of oxygen inhibit completely the *B. cinerea* spore germination, this difference could be caused by the interaction pathogen x host.

Based in the control of decay in 'Thompson Seedless' and 'Flame Seedless' inoculated and in the absence of off-flavors, it was selected the concentration of 40% CO_2 applied for 24 or 48 h as pre-storage treatment to be applied in 'Flame Seedless' commercially packed. The pre-storage treatments were applied at cold storage temperature, since the application at 20 ^oC accelerated the decay incidence.

References:

Hoogerwerf, S.W., Kets, E.P.W., Dijksterhuis, J., 2002. High-oxygen and high-carbon dioxide containing atmospheres inhibit growth of food associated moulds. Lett Appl Microbiol 35, 419-422.

Palou, L., Crisosto, C.H., Garner, D., Basinal, L.M., Smilanick, J.L., Zoffoli, J.P., 2002. Minimum constant sulfur dioxide emission rates to control gray mold of cold-stored table grapes. American Journal of Enology and Viticulture 53, 110-115.

Retamales, J., Defilippi, B.G., Arias, M., Castillo, P., Manriquez, D., 2003. High-CO2 controlled atmospheres reduce decay incidence in Thompson Seedless and Red Globe table grapes. Postharvest Biology and Technology 29, 177-182.

Wells, J.M., Uota, M., 1970. Germination and growth of five fungi in low-oxygen and high-carbon dioxide atmospheres. Phytopathology 60, 50-53.

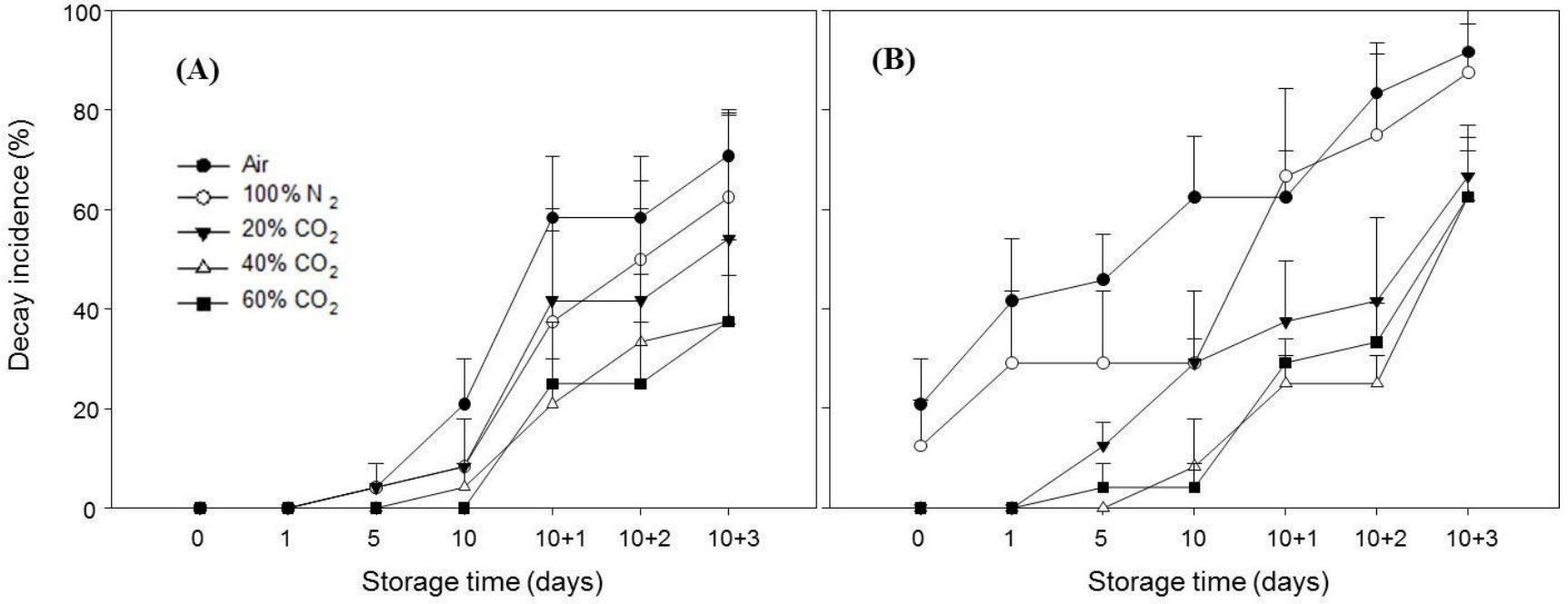

Figure 1: Effect of gas application of 100% N_2; 20% CO_2; 40% CO_2; 60% CO_2 or Air on incidence of *Botrytis cinerea* in berries of 'Thompson Seedless' table grapes surrounding a central berry wounded and inoculated with mycelium. The treatments were applied at 0 ^{o}C (A) or applied at 20^{o}C (B) during 24 h. After the treatment the fruits were stored at 0 ^{o}C day for 10 days and then moved to storage at 20 ^{o}C. Vertical lines represent the standard error of means.

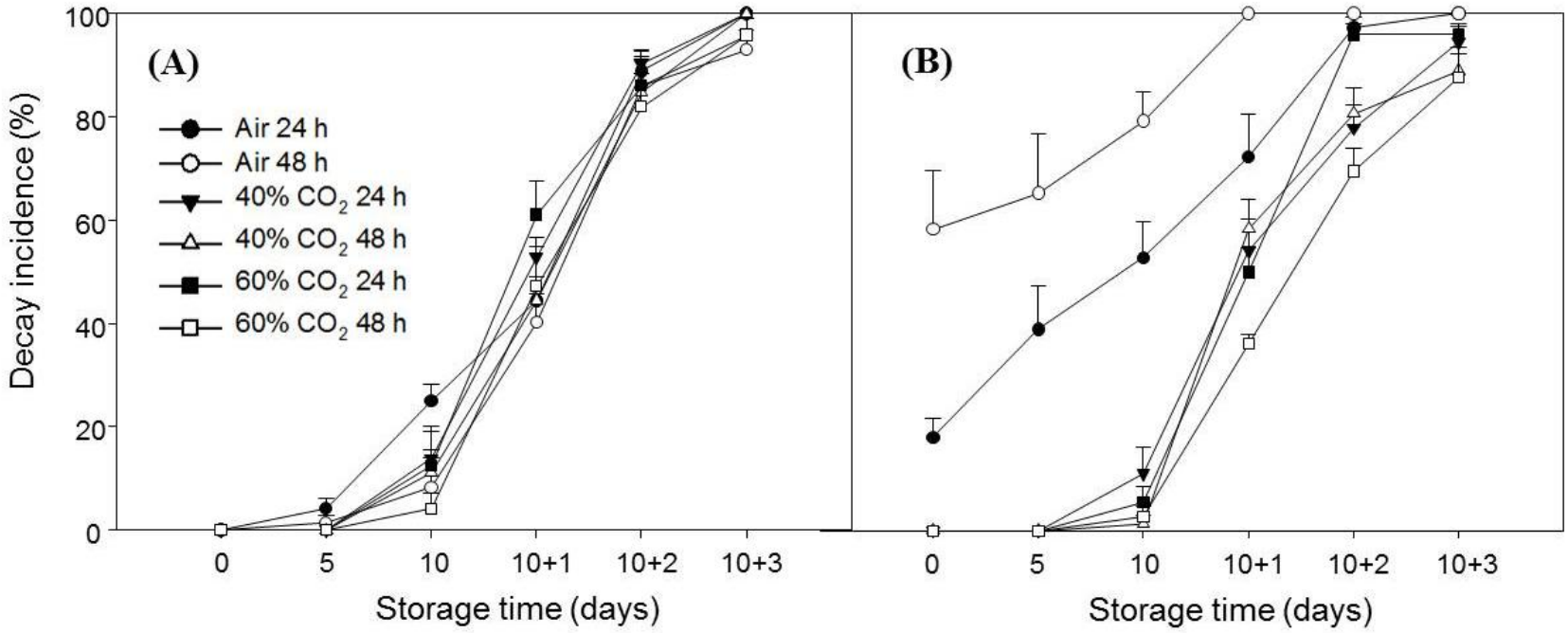

Figure 2: Effect of gas application with Air; 40% CO_2 or 60% CO_2 during 24 or 48 h applied at 0 ^{o}C (A) or at 20 ^{o}C (B), on incidence of *B. cinerea* in berries of 'Flame Seedless' table grapes surrounding a central berry wounded and inoculated with mycelium. After the treatment the fruits were stored at 0 ^{o}C for 10 days and then moved to storage at 20 ^{o}C. Vertical lines represent standard error of the means.