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Carbon dioxide-enriched atmospheres during cold storage limit losses from Botrytis but accelerate rachis browning of 'Redglobe' table grapes

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Abstract

We evaluated a range of CO_2 and O_2 concentrations as a potential substitute to SO_2 treatments for controlling decay development on 'Redglobe' table grape during storage. During the 1998 season, early (14% soluble solids concentration, SSC) and late harvested (17% SSC) 'Redglobe' grapes were stored in 16 controlled atmospheres of 5, 10, 15, 20, and 25 kPa CO_2 combined with 3, 6, and 12 kPa O_2. During the 1999 season, 10 or 15 kPa CO_2 combined with 3, 6, and 12 kPa O_2 were tested in comparison with air stored grapes as control. In the 2000 season, late harvested 'Redglobe' grapes were kept in 12 kPa $CO_2 + 6$ kPa O_2 in comparison with standard commercial storage practices of SO_2 fumigation. None of the atmospheres tested for up to 12 weeks at 0 °C influenced SSC, titratable acidity (TA), SSC:TA, or berry shatter and browning. Rachis browning was accelerated and trained judges perceived 'off-flavor' in grapes exposed to CO_2 levels above 10 and 15 kPa for early and late harvested 'Redglobe' grapes, respectively. Atmospheres including above 10 kPa CO_2 controlled decay incidence and spread among berries (nesting) independent of O_2 concentrations during storage at 1 °C for up to 8 weeks and after 3 days at 20 °C, simulating a retail display period. Based on these data, a combination of 10 kPa CO_2 with 3, 6 or 12 kPa O_2 is suggested for up to 12 weeks storage for late harvested 'Redglobe' grapes. An atmosphere of 10 kPa $CO_2 + 6$ kPa O_2 is suggested for early harvested 'Redglobe' grapes, but not to exceed 4 weeks.

Keywords: Controlled atmosphere; *Vitis vinifera*; SO_2 alternative; Maturity; Off-flavor; Storage potential

1. Introduction

Optimal controlled atmosphere (CA) combinations of low O_2 and high CO_2 levels have been developed for different fruit species and even cultivars within the same species (Kader, 1997), but CA is not recommended for commercial use on table grapes (Nelson, 1969; Laszio, 1985; Cimino et al., 1987; Kader, 1997). The influence of CA conditions with an emphasis on botrytis decay development has been evaluated for 'Emperor' grapes (Uota, 1957), 'Alphonse Lavallee', and

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'Rakazi' grapes (Eris et al., 1993). Yahia et al. (1983) and Berry and Aked (1997) included the following among CA benefits for grapes: delaying senescence, decreasing stem and berry respiration, reducing stem browning, maintaining berry firmness, and retarding decay development. However, formation of 'off-flavors' and berry browning are a concern (Uota, 1957; Nelson, 1969). In early harvested 'Thompson Seedless' grapes from the Coachella Valley, Nelson (1969) found that berry internal browning overshadowed the potential benefits of CA. Insecticidal controlled atmospheres including up to 45 kPa CO_2 for up to 2 weeks are being developed for insect control in table grapes (Ahumada et al., 1996).

Our objective was to identify the optimum CO_2 and O_2 levels to control gray mold without affecting quality attributes for 'Redglobe' table grapes.

2. Materials and methods

During the 1998–2000 seasons, commercially harvested 'Redglobe' were field packaged in cluster bags in corrugated cartons, then fumigated with sulfur dioxide (150 ppm-h) during precooling (Luvisi et al., 1992) before transport. Fruit were then transported to the F. Gordon Mitchell Postharvest Laboratory at the Kearney Agricultural Center, Parlier, California and forced air-cooled to a berry temperature of approximately 0 °C to remove heat gained during transportation. After cooling, grapes were divided into 16 and seven treatments for the 1998 and 1999 seasons, respectively. Grapes were stored at 0 °C for up to 12 weeks in 338-l sealed aluminum tanks under a continuous flow of either air or the desired mix of CO_2 and O_2 concentrations.

During the 1998 season, 'Redglobe' table grapes were harvested at early (14% SSC) and late (17% SSC) commercial maturity stages. The 16 CA combinations included 5, 10, 15, 20, and 25 kPa CO_2 combined with 3, 6, and 12 kPa O_2. For the 1999 season, 'Redglobe' grapes harvested at 16.7% SSC were commercially packed and stored at 0 °C in 338-l sealed aluminum tanks under a continuous flow of either air or 10 and 15 kPa CO_2 combined with 3, 6, and 12 kPa O_2. In both seasons, air storage was used as a control for all treatments. Flow rates and gas mixtures were established using a mixing board with micro-metering valves. Supply and exhaust gas composition was monitored using an Ametek paramagnetic oxygen analyzer (S-3A/II) and a Horiba infrared gas analyzer (VIA-510 for CO_2).

2.1. Quality attribute evaluations

In the 1998 and 1999 seasons, five clusters (replications) per CA treatment were removed from the storage tanks every 4 weeks for quality evaluation and 'off-flavor' development determinations. Quality evaluations included SSC, TA, SSC:TA, rachis browning, berry shatter, skin and berry browning, and botrytis-induced decay development. Ten berries from each replication were pooled, pressed through cheesecloth to extract the juice, and the SSC was measured with a temperature compensated refractometer (Atago model ATC-1). TA was measured with an automatic titrator (Radiometer, Copenhagen, Denmark) and reported as percent tartaric acid. Berry firmness was measured on ten healthy berries per replication by first removing the skin from the cheek with a razor blade, then measuring the force of penetration in grams using a U.C. firmness tester with a 3-mm tip.

Rachis browning development was evaluated using the following scoring system: (1) healthy, entire rachis including the cap stems (merging point between berries and rachis) green; (2) slight, only cap stems showing browning; (3) moderate, cap stems and secondary rachis showing browning, and (4) severe, cap stems, secondary and primary rachis completely brown (Crisosto et al., 2001). All brown berries were removed and weighed, and berry browning was expressed as a percentage of cluster weight.

Botrytis decay was evaluated as nesting formation and total decay. Clusters were considered to have formed a nest, if mycelia actively grew from one berry to at least one other adjacent berry. The number of nests was then counted on each cluster. Total decay was calculated by removing and weighing healthy berries. Then, decayed berry weight was calculated by subtracting total cluster

weight minus the weight of the healthy berries. Thus, total decay was expressed as a percentage of decayed berries based on the original cluster weight. In the 1999 season, nesting and total decay were evaluated immediately after cold storage, and after 3 days at 20 °C simulating a retail display period.

2.2. 'Off- flavor' sensory evaluation and statistical analysis

A flavor evaluation focusing on 'off-flavor' development was carried out by a trained panel of six judges. Judges were screened for their acuity in perceiving 'off-flavor' using a triangle test (O'Mahony, 1986). The flavor of 12 berries per treatment was evaluated using a binary response of 'yes' or 'no'. Each grape sample consisted of a whole berry with seed. Each judge was instructed to cleanse his or her mouth with distilled water, chew the grape sample and mark a yes or a no on the scorecard for 'off-flavor', then cleanse again before proceeding to the next sample.

In the 1998 experiment, we used a factorial design, using CO_2 and O_2 as factors, with three replications. In the 1999 experiment, we used a completely randomized design with nine replications. The data were subjected to analysis of variance (ANOVA) prior to a least significant differences (LSD) means separation using the SAS statistical software (SAS Institute, Cary, NC).

2.3. Commercial trial

In the 2000 season, three 66.5 m^3 (2348 ft^3) refrigerated containers were commercially loaded for export. Each contained 20 pallets (1560 boxes) of 'Redglobe' table grapes from the same lot. A similar number of pallets (control) were stored under standard commercial conditions at the packer/shipper facility. All grapes were packaged in cluster bags in 7.2 kg Styrofoam boxes. The container temperature was set at 1.1 °C and the three containers were parked and operated off of a 440 V power supply at the Kearney Agricultural Center in Parlier, California. On the day of arrival, a controlled atmosphere of 12 kPa $CO_2 + 8$ kPa O_2 was established in all three containers. Container atmosphere was monitored as previously described, and CO_2 introduced as necessary to compensate for container leakage. At the end of the 8-week trial, ethylene levels were measured in each container with a Carle gas chromatograph equipped with a FID detector. The containers were taken back to the shipper and the grapes were repackaged before sale. Repackaging losses for CA-stored and commercially air-stored grapes were calculated as the percentage of the product by weight that had to be discarded due to decay, breakdown or otherwise poor quality.

3. Results and discussion

3.1. Quality attribute evaluations

During the 1998 season, in general, high CO_2 treatment suppressed gray mold growth on early (14% SSC) and late (17% SSC) harvested 'Redglobe' during the CA storage test. Botrytis infection ranged from 13.0% at 1 month to 84.5% at 3 months for air stored 'Redglobe'. By the second month, for early harvested 'Redglobe' grapes, the level of natural infection was low (0.7–10.1%), the CO_2, O_2 and the interaction between them did not affect decay incidence. By the third month, decay on early harvested 'Redglobe' grapes reached levels between 9.4 and 40%, depending on CO_2 levels. The beneficial effect of high CO_2 on botrytis decay suppression (total berries infected) during 3 months of storage was clearly demonstrated in the early picked 'Redglobe' grapes with a high level of quiescent gray mold infection (Table 1); $CO_2 \geq 10$ kPa significantly reduced incidences of botrytis while O_2 concentration did not have an effect. A similar situation occurred for late harvested 'Redglobe' (Table 1). For late harvested 'Redglobe', natural Botrytis infection became important by the second month of storage (3.7–13.8%) and its control was only related to CO_2 concentrations. Similarly as in the early harvested 'Redglobe', $CO_2 \geq 10$ kPa controlled botrytis development. On both harvest dates, there were no significant differences in 'Redglobe' decay incidence between grapes stored at 10 or 25 kPa CO_2.

For early and late harvested 'Redglobe', these CA treatments did not significantly affect berry shatter, SSC, TA or SSC:TA (data not shown) after 3 months at 0 °C. 'Redglobe' berry browning was not observed during these 3 years of CA studies.

CO_2 concentrations independent of O_2 levels affected stem browning. There was no significant interaction between CO_2 and O_2 on early and late harvested 'Redglobe' stem browning development (Table 2); stem browning became commercially important (score ≥ 2.0) after 1 and 2 months. At 1 and 2 months storage, CO_2 above 10 kPa increased stem browning for early harvested 'Redglobe' (Table 2). Late harvested 'Redglobe' grapes tolerated >15 kPa CO_2 before they started to show stem browning by the second month of storage (Table 2).

As botrytis and stem browning incidence were only affected by CO_2 concentrations, ANOVA and LSD analyses were carried out according to CO_2 and air treatment. Botrytis development was significantly higher in the air-stored, early harvested 'Redglobe' grapes than any of the CO_2 storage treatments. However, grapes stored under 5 kPa CO_2 had higher botrytis incidence than grapes stored under 10 kPa or higher CO_2 (Fig. 1). On the three evaluation dates, botrytis incidence was significantly reduced when CO_2 concentrations were 10 kPa or higher during storage (Fig. 1). There were no significant differences in decay among the 10–25 kPa CO_2 treatments. Since decay incidence was very low in late harvested 'Redglobe' the data are not shown. 'Redglobe' stem browning was significantly lower on the air-stored grapes than for some of the CA storage treatments (Fig. 2). For early harvested 'Redglobe' grapes, the use of CO_2 levels >10 kPa significantly increased stem browning by 1 and 2 months storage. For late harvested 'Redglobe' grapes, stem browning became a commercial problem by the second month of storage; early and late harvested grapes stored at CO_2 levels ≤ 10 kPa had the same level of rachis browning

Table 1
Effect of atmospheric composition on *Botrytis cinerea* incidence of early (14% SSC) and late (17% SSC) harvested 'Redglobe' table grapes kept at 0 °C (1998)

Treatment	Decay (wt.%)				
	Storage time				
	1 month		2 months		3 months
	14% SSC	17% SSC	14% SSC	17% SSC	14% SSC
CO_2 (kPa)					
5	3.5	6.8	10.1	13.8a	40.0a
10	1.7	7.4	4.6	3.7b	15.3b
15	2.6	4.4	5.6	6.3b	9.4b
20	0.7	5.9	5.0	7.7b	12.3b
25	0.2	7.0	0.9	4.2b	12.5b
P-value	0.12	0.91	0.27	0.0076	0.0001
LSD 0.05%	NS	NS	NS	6.5	11.7
O_2 (kPa)					
3	2.3	6.1	4.5	9.5	12.0
6	1.8	7.3	6.8	11.6	20.3
12	1.2	5.5	4.4	6.3	17.7
P-value	0.62	0.80	0.68	0.12	0.18
LSD 0.05%	NS	NS	NS	NS	NS
$CO_2 \times O_2$	NS	NS	NS	NS	NS
P-value	(0.23)	(0.76)	(0.18)	(0.46)	(0.10)

Table 2
Effect of atmospheric composition on stem browning development of early (14% SSC) and late (17% SSC) harvested 'Redglobe' table grapes kept at 0 °C (1998)

Treatment	Stem browning score (1–4)[a]			
	Storage time			
	1 month		2 months	
	14% SSC	17% SSC	14% SSC	17% SSC
CO_2 (kPa)				
5	1.0a	1.3a	1.6a	2.8a
10	1.2a	1.4a	1.7a	2.8a
15	2.4b	1.7a	3.9b	2.9a
20	3.0b	2.0b	4.0b	3.6b
25	4.0cb	1.8b	4.0b	3.6b
P-value	0.0001	0.017	0.0001	0.0010
LSD 0.05%	0.8	0.4	0.5	0.5
O_2 (kPa)				
3	2.7	1.5	3.1	2.9
6	2.1	1.8	3.0	3.1
12	2.3	1.6	2.9	3.3
P-value	0.17	0.21	0.38	0.095
LSD 0.05%	NS	NS	NS	NS
$CO_2 \times O_2$ *P*-value	NS (0.68)	0.022	NS (0.60)	NS (0.49)

[a] Stem browning score: 1, healthy; 2, browning of the cap stems; 3, browning of the cap stems and lateral stems; and 4, browning of the cap stems, lateral stems and main rachis.

as those kept in air. At that time, CO_2 levels ≥ 20 kPa significantly increased rachis-browning, while CO_2 ≤ 15 kPa did not affect rachis browning development. By 3 months, CO_2 levels ≥ 15 kPa significantly accelerated rachis-browning development. Similar stem browning and 'off-flavor' development by short exposure to high CO_2 has been reported in grapes (Ahumada and Mitcham, 1996; Kader, 1997). A moderately high CO_2 concentration may interfere with the oxidation of succinic acid that can accumulate to toxic levels. The difference in stem browning development due to CO_2 according to harvest maturity may be explained by changes in the tissue phenolics composition of the tissue during maturation. It has been reported that hydroxycinnamic acid-tartaric acid esters decrease during maturation and that rachises of early harvested 'Flame Seedless' are more susceptible to browning than those of late harvested grapes (Peynaud and Ribereau-Gayon, 1971; Romeyer et al., 1983).

3.2. 'Off- flavor' sensory evaluation

Judges on a trained panel perceived development of 'off-flavor' in 'Redglobe' table grapes, but its presence was not related to O_2 concentration. The development of 'off-flavor' measured on early and late harvested 'Redglobe' grapes immediately after 1 month storage followed by 2 days at 20 °C was related only to CO_2 levels (Fig. 3). Early harvested grapes had more 'off-flavor' development than late harvested grapes but in most cases

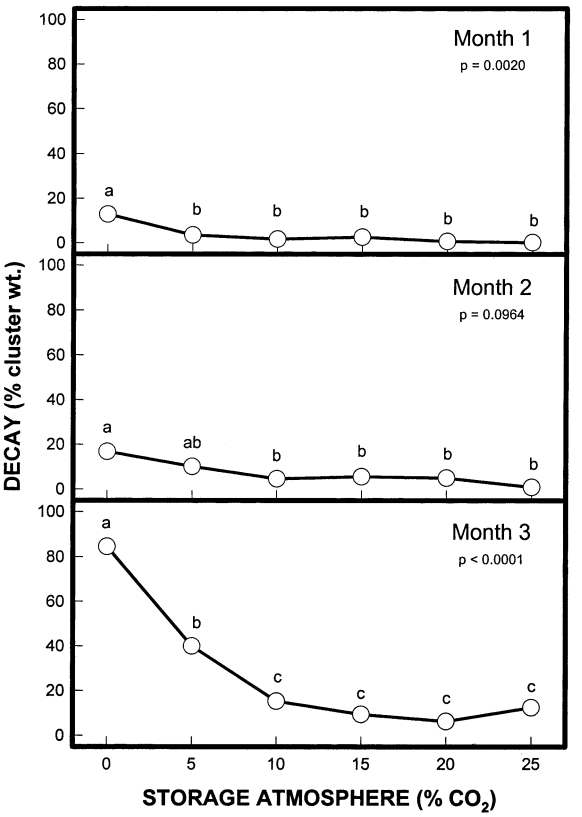

Fig. 1. Decay incidence on early (14% SSC) and late (17% SSC) harvested 'Redglobe' table grapes after 1, 2, and 3 months at 0 °C in different CO_2-enriched atmospheres. Different letters indicate a significant difference between storage atmospheres by $LSD_{0.05}$.

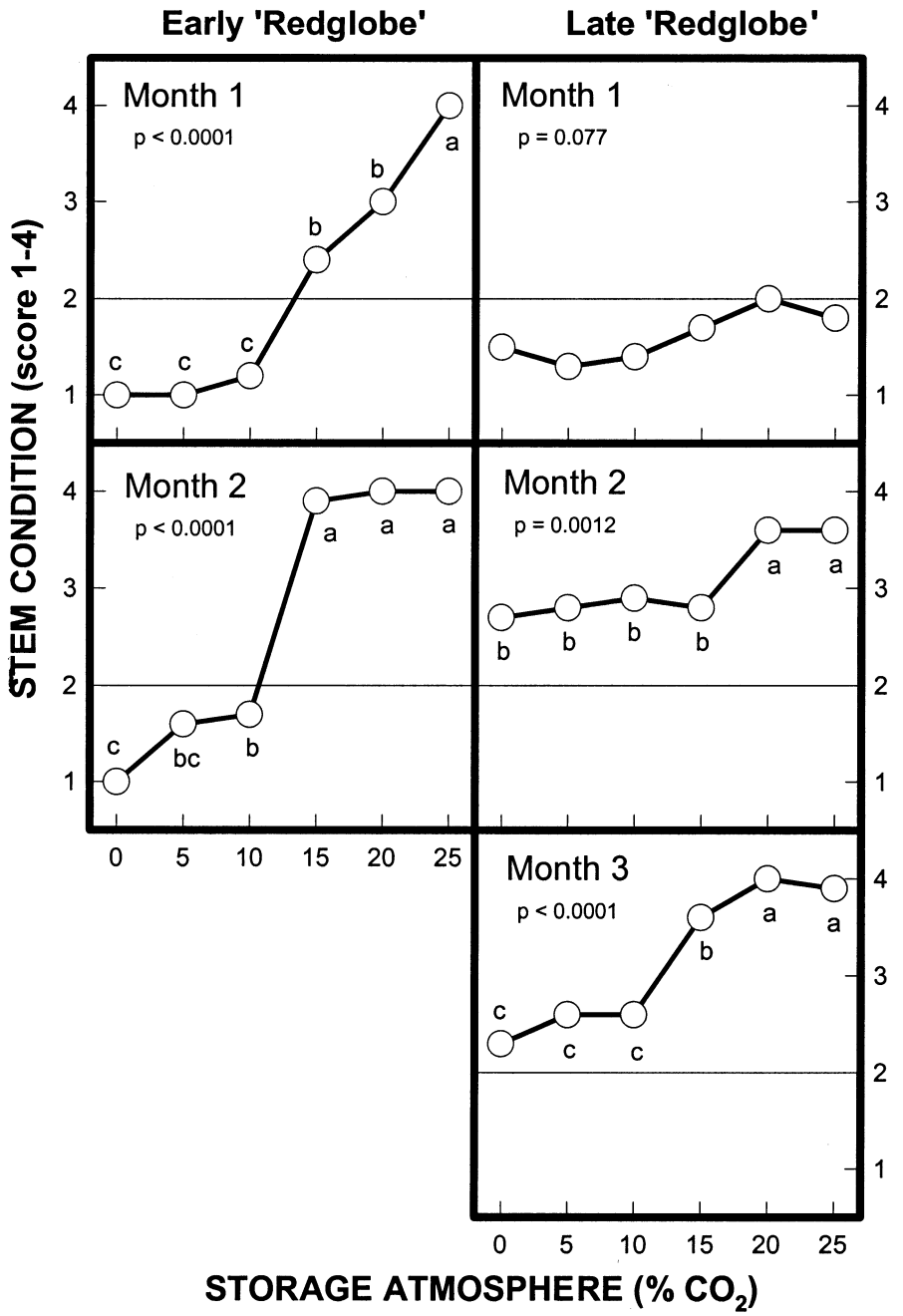

Fig. 2. Stem browning scores of early (14% SSC) and late (17% SSC) harvested 'Redglobe' table grapes after 1, 2 and 3 months at 0 °C in different CO_2-enriched atmospheres where 1, healthy stems with no browning; 2, brown cap stems, laterals and rachis green; 3, brown cap stems and laterals, rachis green; 4, cap stems, laterals and main rachis brown. Different letters indicate a significant difference between storage atmospheres by $LSD_{0.05}$.

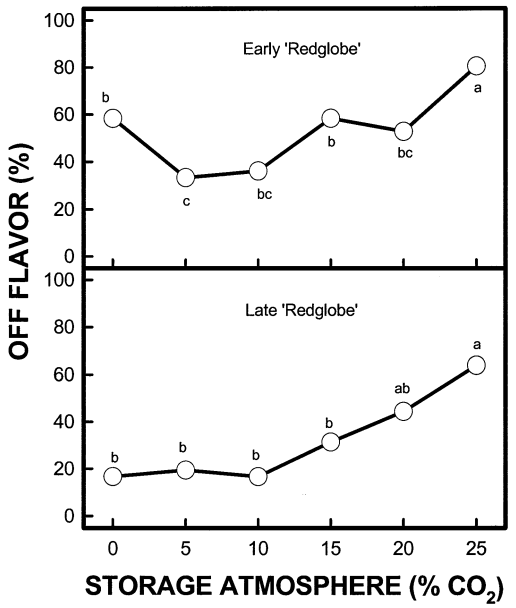

Fig. 3. Relationship between early (14% SSC) and late (17% SSC) harvested 'Redglobe' table grape off-flavor development and CO_2 concentration during 1 month at 0 °C plus 2 days in air at 20 °C prior to tasting by a trained panel. Different letters represent a statistical difference between storage atmospheres by pair-wise binomial analysis (χ^2) at $P < 0.05$.

'off-flavor' decreased after 2 days at 20 °C in air. For early harvested grapes, 60% of the judges detected 'off-flavor' presence in air stored grapes immediately after 1-month cold storage plus 2 days display period. A similar percentage of judges (60%) detected 'off-flavor' in early harvested grapes stored in ≥ 15 kPa CO_2-enriched atmospheres. Grapes stored at 5 and 10 kPa CO_2 had the lowest percentage of judges (50%) detecting 'off-flavor' (Fig. 3). For late harvested grapes, approximately 20% of the judges detected 'off-flavor' in grapes from the air stored, 5 and 10 kPa CO_2 treatments after 1 month at 0 °C + 2 days at 20 °C. About 30–60% of the judges detected 'off-flavor' in grapes stored under ≥ 15 kPa CO_2 (Fig. 3). In all of the evaluations, grapes from the 5 and 10 kPa CO_2 treatments had the same or less 'off-flavor' than the air-stored grapes. 'Off-flavor' was induced by CA treatments including > 15 kPa CO_2 in grapes of both maturities. Differences in 'off-flavor' development between early and late harvested 'Redglobe' under air storage can be explained by differences in chemical composition (such as esters, alcohols, aldehydes, and ketones) due to their maturity stages (Peynaud and Ribereau-Gayon, 1971; Robredo et al., 1991). These chemicals may be oxidized during storage, producing an 'off-flavor'. As grapes mature, concentrations of these chemical compounds are reduced; thus, 'off-flavor' development during storage is minimized. The beneficial effect of 5 or 10 kPa CO_2 storage treatment on reducing 'off-flavor' development over air storage is more apparent on early harvested and in grapes prone to 'off-flavor' development. It is important to point out that the same percentage of judges perceiving 'off-flavor' in a trained panel would not be the same as for the percentage of consumers detecting 'off-flavor' at these levels. Based on previously published information (O'Mahony, 1986; Ke et al., 1991), we predict that the percentage of consumers detecting 'off-flavor' from these treatments would be lower than for our trained judges. In general, 'off-flavor' and stem browning development were induced in early and late harvested 'Redglobe' grapes by CO_2 levels > 10 and 15 kPa CO_2, respectively, while decay was limited at ≥ 10 kPa CO_2. Based on these results, 10 or 15 kPa CO_2 combined with 3, 6, or 12 kPa O_2 were tested the following season.

During the 1999 season, 'Redglobe' stems started to show the first signs of stem browning (score > 2.0) by 2 months of cold storage. By 2 months, the stem condition of clusters stored in air and 10 kPa CO_2 looked significantly better than the stem condition of clusters stored under any of the 15 kPa CO_2 combination treatments. After 3 months of storage, rachises from all of the treatments showed visible signs of stem browning. At this point, there were no significant differences in stem condition between treatments (Table 3). SSC, TA, SSC:TA, berry shatter and berry firmness were not affected by storage atmosphere during the 3-month storage period (data not shown).

Decay measured immediately after cold storage was not observed until the third month of storage on any of the CA treated grapes. For air-stored grapes, 30% of clusters had nesting by 1 month and almost 100% had nesting by 2 months. CA-

stored grapes reached 30% nesting in the worst case. Thus, there was significantly higher nesting measured immediately after cold storage on air stored than CA stored 'Redglobe' grapes (Table 3).

After 1 month at 0 °C followed by 3 days at 20 °C, there was more nesting and total decay development in air stored than in CA stored grapes. In the control, 30% of the clusters had nesting, while in the worst performing CA treatment only 10% of the clusters had nesting. After 2 months at 0 °C + 3 days at 20 °C, there was still more nesting and total decay development in air-stored than in CA stored grapes. At this time, 100% of air-stored clusters had nesting, while in the worst performing CA treatment only 30% of the clusters had nesting. In addition, total decay was at least ten-fold higher in the air stored than any of the CA treatments. After 3 months at 0 °C followed by 3 days at 20 °C, total decay reached a commercially-important level but it was always higher in air-stored fruit than CA stored fruit. Grapes stored under any of the CA combinations had signifi-

Table 3
Quality of 'Redglobe' table grapes, harvested at 16.7% SSC and stored under different controlled atmosphere treatments measured during storage at 0 °C and after 3 days at 20 °C (1999)

Storage atmosphere		After storage at 0 °C		After 3 days at 20 °C	
CO_2 (kPa)	O_2 (kPa)	Stem score (1–4)[a]	Nests (#)	Nests (#)	Decay (wt.%)
1 month					
10	3	1.3	0.0	0.0	0.9
10	6	1.3	0.0	0.0	0.3
10	12	1.7	0.0	0.0	2.4
15	3	2.3	0.0	0.0	0.0
15	6	1.7	0.0	0.0	1.3
15	12	1.0	0.0	0.1	3.2
Air		2.0	0.3	0.3	11.3
P-value		0.2	0.46	0.56	0.22
$LSD_{0.05}$		NS	NS	NS	NS
2 months					
10	3	1.7a	0.0a	0.0a	1.1a
10	6	2.3a	0.1a	0.3a	2.1a
10	12	2.0a	0.0a	0.0a	0.0a
15	3	2.7b	0.0a	0.3a	1.3a
15	6	3.3b	0.0a	0.0a	0.0a
15	12	2.7b	0.1a	0.3a	0.3a
Air		1.3a	1.0b	1.0b	14.2b
P-value		0.033	0.011	0.038	0.0009
$LSD_{0.05}$		1.1	0.5	0.7	5.6
3 months					
10	3	2.7	0.0a	0.0a	0.3a
10	6	3.0	0.0a	0.0a	0.6a
10	12	3.7	0.0a	0.0a	0.6a
15	3	4.0	0.0a	0.3a	3.1a
15	6	3.7	0.0a	0.0a	0.7a
15	12	3.7	0.0a	0.0a	1.1a
Air		3.3	0.7b	0.7b	23.3b
P-value		0.20	0.015	0.011	0.0095
$LSD_{0.05}$		NS	0.4	0.5	12.1

[a] Stem score: 1, healthy; 2, slight browning of the cap stems; 3, browning of the cap stems and lateral stems; and 4, severe browning of the cap stems, lateral stems and main rachis.

cantly lower total decay and nesting than air stored grapes. There was 23% total decay in air-stored fruit and less than 3.1% in CA stored fruit. All of the CA combinations tested reduced decay, but none were more effective in controlling decay than the others. However, stem condition measured after 2 months under CA treatments using CO_2 levels ≥ 15 kPa showed high stem browning development.

3.3. Commercial trial

During the 2000 season, the repackaging losses of grapes kept in CA were similar to those of grapes kept under standard commercial conditions. Grapes kept under CA had 3.7, 8.0 and 8.2% repackaging losses for containers 1, 2, and 3, respectively. The mean loss for all three containers was 6.6%. Grapes stored commercially had a mean repackaging loss of 7.8%, with a range of 1.6–14.9%, depending upon the lot. It is important to point out that there were differences other than storage atmosphere to consider when interpreting these results. Grapes stored in CA were held at 1.1–2.2 °C, while fruit stored commercially were kept at -0.6 to 0 °C. Also, grapes stored commercially were fumigated weekly with approximately 150 ppm-h SO_2 (Luvisi et al., 1992), while grapes stored in CA did not receive any weekly SO_2 fumigations.

4. Conclusions

We conclude that ≥ 10 kPa CO_2 combined with 3, 6 or 12 kPa O_2 limits botrytis decay development on 'Redglobe' table grapes during 12-weeks cold storage. However, it is important to point out that above 10 kPa CO_2 accelerates stem browning and 'off-flavor' development. The effect on these two quality attributes was also related to grape maturity. Based on these data, 10 kPa CO_2 combined with 3, 6 or 12 kPa O_2 is suggested for up to 12 weeks storage for late harvested 'Redglobe' grapes. A 10 kPa $CO_2 + 6$ kPa O_2 is suggested only up to 4 weeks for early harvested 'Redglobe' grapes.

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