

High Carbon Dioxide Atmospheres Affect Stored ‘Thompson Seedless’ Table Grapes

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Abstract. Efficacy of controlled atmosphere (CA) conditions for decay control in ‘Thompson Seedless’ table grapes was evaluated during the 1998–2000 seasons. During the 1998 season, early (16.5% soluble solids concentration = SSC) and late harvested (19% SSC) grapes were exposed to 5%, 10%, 15%, 20%, or 25% CO₂ combined with 3%, 6%, and 12% O₂. In 1999 and 2000, 10% or 15% CO₂ combined with 3%, 6%, or 12% O₂ were used. In all trials, fruit were initially SO₂ fumigated and air-stored grapes were used as controls. Storage atmospheres did not affect SSC, titratable acidity (TA), or sugar-to-acid ratio (SSC : TA). The main storage limitations for early harvested ‘Thompson Seedless’ table grapes were “off flavor” and rachis and berry browning development, which resulted from exposure to >10% CO₂. However, ≥15% CO₂ was needed to control total decay and nesting development independent of O₂ concentrations. High carbon dioxide atmospheres (15% to 25%) were more effective in decay control without detrimental effects on quality when late harvested grapes were used. The combination of 15% CO₂ with 3%, 6%, or 12% O₂ is suggested for up to 3 months storage only for late harvested ‘Thompson Seedless’ table grapes; it should not be used for early harvested grapes.

The most important table grape cultivar worldwide is ‘Thompson Seedless’ and it is marketed nearly year-round throughout the world. For example, in 2000, California produced ≈224 t, and in 2001, Chile produced ≈176 t (Chilean Fresh Fruit Association, personal communication). Controlled atmospheres (CA) with elevated CO₂ concentrations have been shown to control decay development on some commodities without affecting quality attributes during transport, storage, or both. Optimal CA combinations have been developed for different species, and even cultivars within the same species (Kader, 1997; Lidster et al., 1990; Smock, 1979).

Preliminary CA work has been done on other table grape cultivars such as ‘Ribier’ and ‘Razaki’ (Eris et al., 1993), South African cultivars (Laszio, 1985), ‘Emperor’ (Uota, 1957), and some Italian cultivars (Cimino et al., 1987). A few studies have been done on ‘Thompson Seedless’ (Berry and Aked, 1997; Nelson, 1969; Yahia et al., 1983). Nelson (1969) found that berry internal browning incidence overcame the potential benefits of CA in his early trials with ‘Thompson Seedless’ table grapes from the Coachella Valley of California. Yahia et al. (1983) using 5% CO₂ + 2% O₂, reported that *Botrytis cinerea* decay (gray mold) and internal berry browning were the main deterioration symptoms beyond 8 weeks storage. In research carried out in En-

gland with ‘Thompson Seedless’ table grapes from Egypt, Berry and Aked (1997) observed an inhibition of *Botrytis cinerea* by exposure to a CA of 15% CO₂ + 5% O₂.

Botrytis cinerea (gray mold) is the primary postharvest pathogen of table grapes in California. *Botrytis* rot on grapes can be diagnosed by its characteristic “slipskin” that develops on the surface of infected berries. Areas of the berry skin infected with *Botrytis* are brown in color and slip freely when rubbed with the fingers, leaving the firm underlying pulp exposed (Luvisi et al., 1992). Uncontrolled infections result in the development of aerial mycelium that spreads to adjacent berries. These pockets of decay that develop are called “nests”. *Rhizopus* sp. and *Aspergillus niger* can infect table grapes, but they do not develop during storage at low temperatures. *Penicillium* sp. do not infect healthy grapes, but may develop on wounds. *Cladosporium herbarum*, *Alternaria* sp., and *Stemphylium* sp. infections develop slowly in cold storage and can be distinguished from *Botrytis* by the black lesions they form.

Our objective was to determine optimal CO₂ and O₂ levels to control gray mold without affecting soluble solids concentration (SSC), titratable acidity (TA), flesh firmness, rachis or berry condition, or flavor in ‘Thompson Seedless’ table grapes.

Materials and Methods

Commercially harvested ‘Thompson Seedless’ table grapes were field packaged in cluster bags that were placed in corrugated

cartons, and then fumigated with sulfur dioxide (250 ppm-hour) during precooling (Luvisi et al., 1992). Grapes were then transported to the F. Gordon Mitchell Postharvest Laboratory at the Kearney Agricultural Center in Parlier, Calif. and forced air-cooled again to a berry temperature of ≈0 °C. After cooling, grapes were divided into storage treatments. Grapes were stored at 0 °C in 338-L sealed aluminum tanks under a continuous flow of either air or the desired mixture of CO₂ and O₂.

During the 1998 season, ‘Thompson Seedless’ table grapes were harvested according to their SSC at early (16.5%) or late (19%) commercial maturity. Grape samples were stored in air and 15 CA combinations for up to 3 months at 0 °C. The 15 CA combinations were 5%, 10%, 15%, 20%, or 25% CO₂ combined with 3%, 6%, or 12% O₂. In the 1999 season, ‘Thompson Seedless’ table grapes harvested at 20% SSC were commercially packed and stored at 0 °C in 338-L sealed aluminum tanks under a continuous flow of either air or combinations of 10% or 15% CO₂ combined with 3%, 6%, or 12% O₂. In the 2000 season, ‘Thompson Seedless’ table grapes with 20% SSC were harvested from six different vineyards having historically “high” incidence of decay. Grapes were packaged and handled as previously described. After cooling, the fruit were stored at 0 °C in 338-L sealed aluminum tanks under a continuous flow of either 10% CO₂ + 3% O₂, 15% CO₂ + 3% O₂, 15% CO₂ + 12% O₂, or air as previously described.

In the three seasons, air storage was used as a control for all of the experiments. Flow rates and gas mixtures were established using a mixing board with micro-metering valves (Post Harvest Research, Davis, Calif.). Supply and exhaust gas O₂ and CO₂ composition was monitored using an paramagnetic oxygen analyzer (model S-3A/II, Ametek Thermox, Pittsburgh) and an infrared gas analyzer (model VIA-510; Horiba, Irvine, Calif.) for CO₂.

Quality evaluations. In the three seasons, five clusters (replications) per treatment were removed every month (4 weeks) for quality evaluations, including rachis browning, berry browning, *Botrytis* development, berry firmness, SSC, and TA. Rachis browning development was evaluated using the following subjective 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* rot was identified visually by its characteristic brown lesions and “slipskin.” For each cluster, the number of nests of *Botrytis* present was noted. Clusters were considered to have formed a nest if mycelia actively grew from one berry to at least one other adjacent berry. In addition, all infected berries were removed, weighed, and decay expressed as a percentage of cluster weight. In the 1999 season, nesting and total decay were evaluated immediately after cold

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storage, and then again after 3 d at 20 °C, simulating a retail display period.

Berry firmness was measured on 10 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 (Western Industrial Supply, San Francisco) with a 3-mm tip. Ten berries from each replication were pooled, pressed through cheesecloth to extract the juice, and SSC was measured with a temperature compensating refractometer (model ATC-1, Atago Co., Tokyo). The TA was measured with an automatic titrator (Radiometer, Copenhagen, Denmark) and reported as percent tartaric acid.

A taste evaluation focusing on “off flavor” development was carried out by six trained judges only during the first season. Judges were screened for their acuity in perceiving “off flavor” using a triangle test (O’Mahony, 1986). The “off flavor” of 12 berries per treatment was evaluated using a binary response of “yes” or “no.” Each grape sample consisted of a whole berry.

In the 1998 experiment, we used a factorial design, using CO₂ and O₂ levels as factors, with three replications. In the 1999 and 2000 experiments, 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 program. The SAS statistical software (SAS Institute, Cary, N.C.) was used for these analyses.

Results and Discussion

Quality evaluations. During the 1998 season, high CO₂ treatment suppressed gray mold growth on early (16.5% SSC) and late (19% SSC) harvested ‘Thompson Seedless’ table grapes. *Botrytis* infection in the first month ranged from none to 5.5% for air-stored early harvested grapes. For late harvested grapes, *Botrytis* infection ranged from 1% to 32.8%. In all cases, decay incidence was not related to O₂ or the O₂-CO₂ interaction (Table 1). In all of the evaluations, the beneficial effect of ≥10% CO₂ on suppression of *Botrytis* decay (total berries infected) was evident (Fig. 1). For both harvest dates, there were no significant differences in decay incidence between grapes stored at 10% to 25% CO₂, except after 2 months in storage when decay was better controlled by 15% CO₂ than 10% CO₂ on late harvested grapes. Since *Botrytis* decay was only affected by CO₂ concentrations, ANOVA and LSD analysis were carried out excluding O₂ as a treatment. For both early and late harvested grapes, *Botrytis* development was not

visible until the second month of storage, when it was significantly higher on the air-stored grapes than any of the CO₂ storage treatments. In all of the cases, the weight of decayed berries was significantly reduced when CO₂ concentrations were ≥15% during storage (Fig. 1). Similar results were reported for *Monilinia fructicola* decay on sweet cherries (Tian et al., 2001).

For early and late harvested grapes, the CO₂ and O₂ combinations tested did not significantly affect SSC, TA or SSC : TA (data not shown). Berry browning became commercially important (>5.0%) by the second month on early (16.5% SSC) harvested grapes. This browning was also only related to CO₂ concentrations (Table 2). Air-stored early and late harvested grapes did not develop commercially important berry browning even after 3 months storage. However, berry browning was accelerated by ≥15% CO₂ after 2 months in storage (Fig. 2). None or very slight berry browning was observed on late harvested (19% SSC) grapes, except those exposed to 25% CO₂, which exhibited ≈11% berry browning after 2 months in storage.

For early harvested grapes, rachis browning became commercially important (score ≥2.0) by the first month (Fig. 3). For grapes at both maturity stages, O₂ and the interaction between O₂ and CO₂ was not related to the onset of rachis browning (Table 3). At all evaluation times, ≥10% CO₂ increased rachis browning for early harvested grapes. Late harvested grapes tolerated high CO₂ very well. Only late harvested grapes stored in ≥20% CO₂ showed signs of rachis browning (score ≥2.0) after 2 months. Because of a high incidence of decay, rachis condition of late harvested grapes was not evaluated after 3 months storage.

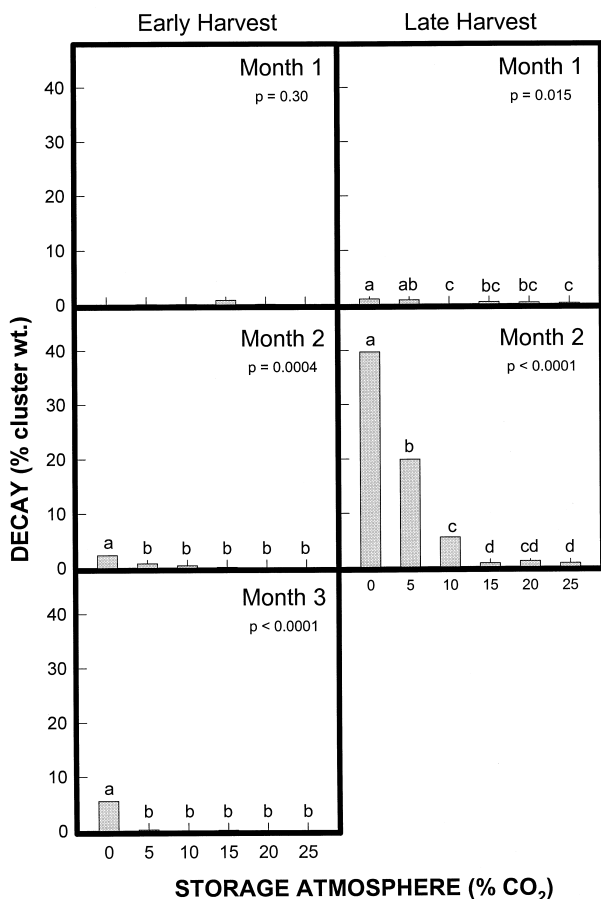


Fig. 1. Decayed berries, expressed as a percentage of cluster fresh weight, of early (16.5% SSC) and late (19% SSC) 1998 harvested ‘Thompson Seedless’ table grapes after 1, 2, and 3 months storage in different CO₂-enriched atmospheres. Different letters indicate a significant difference between storage atmospheres by LSD_{0.05}.

Table 1. Probabilities for significance in the analysis of variance for the effects of carbon dioxide (CO₂ = 0%, 5%, 10%, 15%, 20%, and 25%) and oxygen (O₂ = 0%, 3%, 6%, 12%, and 21%) concentrations on the percentage *Botrytis* rot of early (16.5% SSC) and late (19% SSC) harvested ‘Thompson Seedless’ table grapes during cold storage at 0 °C, 1998.

Treatment	Storage time				
	1 month		2 months		3 months
	16.5% SSC	19% SSC	16.5% SSC	19% SSC	16.5% SSC
CO ₂	0.27	0.047	0.013	<0.0001	0.009
O ₂	0.26	0.60	0.30	0.62	0.31
CO ₂ × O ₂	0.72	0.54	0.16	0.80	0.42

Table 2. Probabilities for significance in the analysis of variance for the effects of carbon dioxide (CO₂ = 0%, 5%, 10%, 15%, 20%, and 25%) and oxygen (O₂ = 0%, 3%, 6%, 12%, and 21%) concentrations on berry browning of early harvested (16.5% SSC) ‘Thompson Seedless’ table grapes during cold storage at 0 °C, 1998.

Treatment	Storage time		
	1 month	2 months	3 months
	Berry browning (% by weight)		
CO ₂	0.29	0.017	<0.0001
O ₂	0.15	0.47	0.39
CO ₂ × O ₂	0.40	0.11	0.32

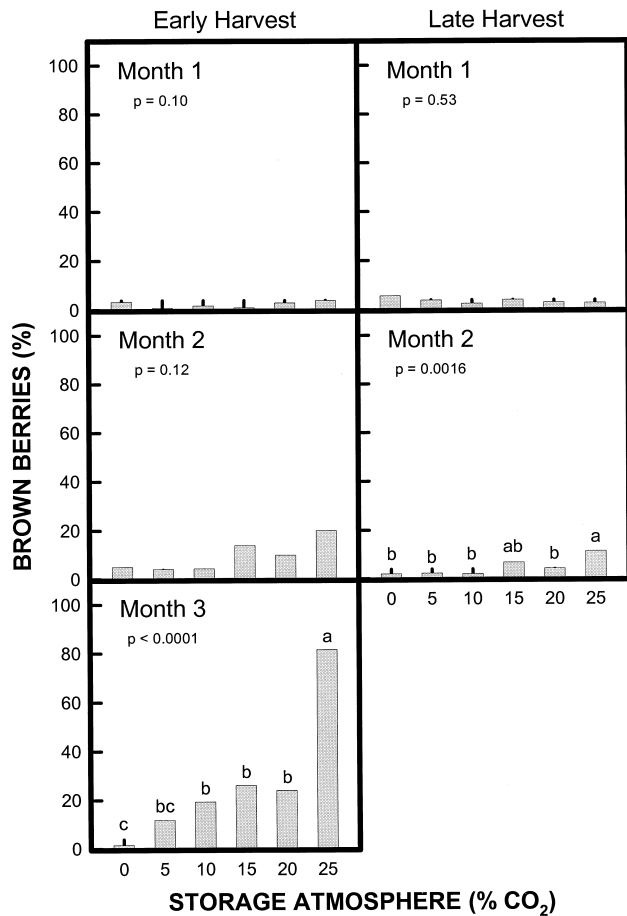


Fig. 2. Brown berries, expressed as a percentage of cluster fresh weight, of early (16.5% SSC) and late (19% SSC) 1998 harvested 'Thompson Seedless' table grapes after 1, 2, and 3 months storage in different CO₂-enriched atmospheres. Different letters indicate a significant difference between storage atmospheres by LSD_{0.05}.

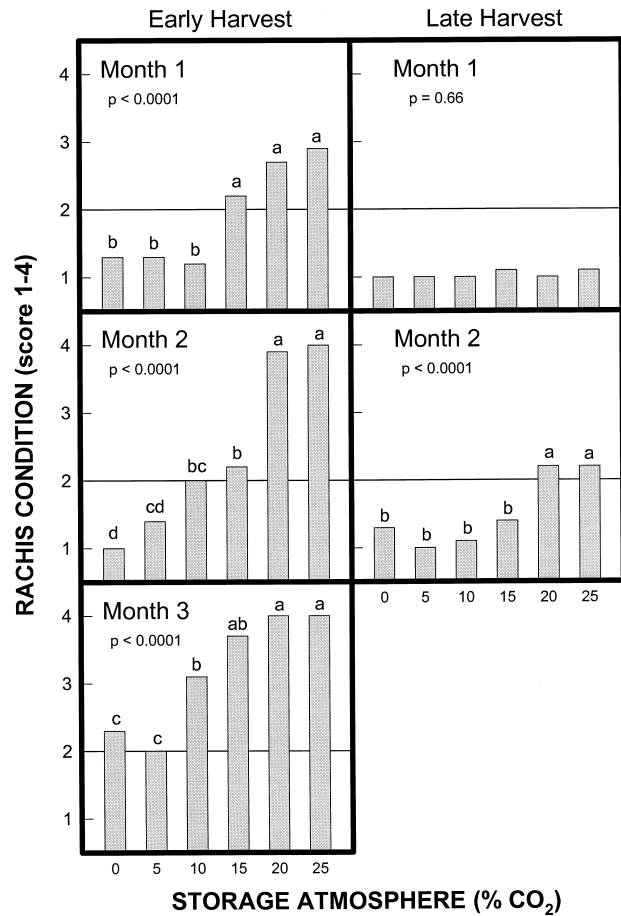


Fig. 3. Rachis browning score of early (16.5% SSC) and late (19% SSC) 1998 harvested 'Thompson Seedless' table grapes after 1, 2, and 3 months storage in different CO₂-enriched atmospheres where 1 = healthy stems with no browning; 2 = brown cap stems, laterals and rachis green; 3 = brown cap stems and laterals, rachis green; 4 = cap stems, laterals and main rachis brown. Different letters indicate a significant difference between storage atmospheres by LSD_{0.05}.

Judges on a trained panel perceived development of "off flavor" in 'Thompson Seedless' table grapes, but its presence was not related to O₂ concentration. The development of "off flavor" evaluated on early and late harvested grapes after one month at 0 °C followed by 2 d at 20 °C was only related to CO₂ levels (Fig. 4). Early harvested grapes had more "off flavors" than late harvested grapes. In the evaluation carried out after 2 months at 0 °C followed by 2 d at 20 °C, ≈35% of the judges detected "off flavor" in

air- and 10% CO₂-stored grapes, while ≈60% to 80% of the judges detected "off flavor" in early harvested grapes stored in ≥15% CO₂. None of the judges detected "off flavor" in grapes stored in 5% CO₂. In all of the evaluations, grapes from the 5% CO₂ treatments had similar or less "off flavor" development than air-stored grapes.

"Off flavor" was induced by CA treatments when grapes at both maturities were stored in ≥15% CO₂. Differences in "off flavor" development between air-stored early and late har-

vested grapes can be explained by differences in chemical composition (such as aroma compounds and hydroxycinnamoyl esters) due to their maturity stages (Peynaud and Ribereau-Gayon, 1971; Robredo et al., 1991; Romeyer et al., 1983). 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% CO₂ storage treatment on reducing "off flavor" development over air storage is more apparent for early harvested grapes and 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 the percentage of consumers in a consumer test detecting "off flavor" at these levels. Based on previously published information (Ke et al., 1991; O'Mahony, 1986), 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 rachis browning development were induced in early and late harvested grapes by >10% CO₂ and >15% CO₂, respectively, while decay was limited at ≥10% CO₂.

Table 3. Probabilities for significance in the analysis of variance for the effects of carbon dioxide (CO₂ = 0%, 5%, 10%, 15%, 20%, and 25%) and oxygen (O₂ = 0%, 3%, 6%, 12%, and 21%) concentrations on rachis browning of early harvested (16.5% SSC) 'Thompson Seedless' table grapes during cold storage at 0 °C, 1998.

Treatment	Storage time		
	1 month	2 months	3 months
	<i>Rachis browning score²</i>		
CO ₂	<0.0001	<0.0001	<0.0001
O ₂	0.17	0.93	0.63
CO ₂ × O ₂	0.50	0.29	0.48

²Rachis 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.

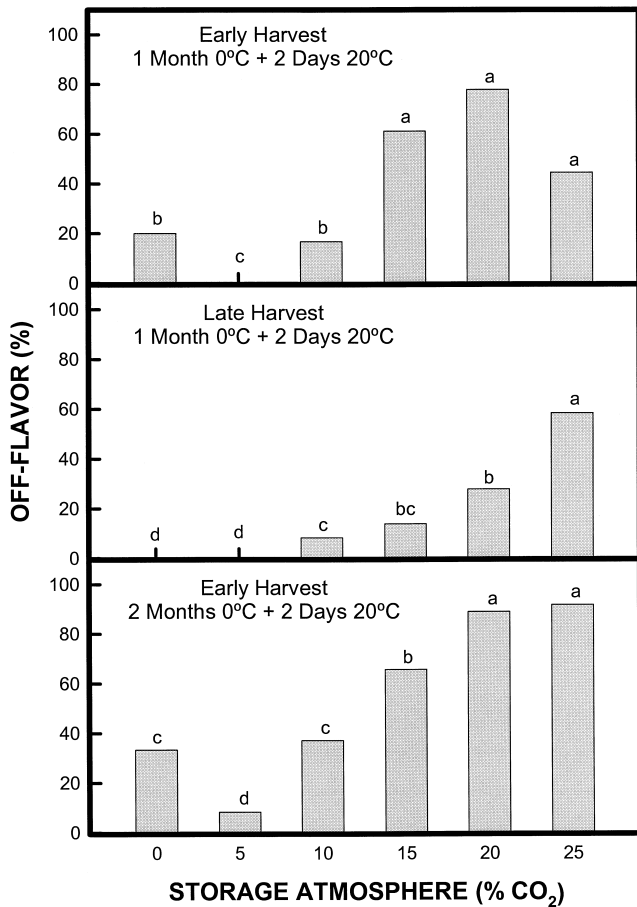


Fig. 4. Off flavor development of early (16.5% SSC) and late (19% SSC) 1998 harvested 'Thompson Seedless' table grapes after 1 or 2 months storage in different CO₂-enriched atmospheres at 0 °C followed by 2 d in air at 20 °C prior to tasting by a trained panel. Different letters represent a statistical difference between storage atmospheres by pair wise binomial analysis (X²) at *P* < 0.05.

During the 1999 season, berry browning of late harvested (20% SSC) grapes was not observed during the 3-month storage period (data not shown). Rachis browning started to develop at the cap stems after the second month of cold storage. After 3 months storage, rachises from all of the treatments showed visible signs of dehydration. On all three evaluation dates, there were no significant differences in rachis browning among any of the treatments (Table 4).

The presence of nests of *Botrytis* after removal from storage and then display (3 d at 20 °C) was not observed until the second month. After 2 months, there was significantly more nesting in air-stored grapes than CA-stored grapes (Table 4). There were no significant differences in nesting development measured after cold storage between the CA treatments, although grapes stored in any of the CA treatments had fewer nests of decay than air-stored grapes. After 2 months storage at 0 °C plus 3 d at 20 °C (display), there were more nests measured on grapes stored in air than in any of the CA treatments. After 3 months, grapes stored in CA had fewer nests at the time of removal than grapes stored in air. After 3 d at 20 °C, nesting developed in all of the treatments with no significant differences between them. There was always less total

decay (% weight) measured after 3 d at 20 °C in grapes from the CA treatments than in air-stored grapes. After three months storage, there was 59% total decay in air-stored grapes and <13% in grapes from the CA treatments.

During the 2000 season, as expected, there were no significant differences in firmness, SSC, TA, or SSC : TA between treatments. Berry browning was not observed in any of the treatments. All three CA treatments effectively controlled decay during the 3-month storage period. The late harvested grapes stored in air developed nests of decay after 2 months at 0 °C (Table 5). After 2 months storage, all of the clusters stored in air had nests of decay, while no nests had developed in any of the CA

treatments. There was 22% and 42% total decay by weight in air-stored fruit after 2 and 3 months storage, respectively. Grapes stored in CA had less than 1.0% total decay.

Based on our three seasons of research, ≥15% CO₂ is necessary to control *Botrytis* decay on 'Thompson Seedless' table grapes. However, grape quality losses due to "off flavor" and rachis and berry browning were induced on early harvested grapes. Because of these potential quality problems, we do not recommend the use of CA for early harvested 'Thompson Seedless' table grapes. In the San Joaquin Valley (California) and Maipo and Aconcagua Valleys (Chile), 'Thompson Seedless' table grapes are harvested with SSC

Table 4. Quality of late harvested (20% SSC) 'Thompson Seedless' table grapes after 1, 2, and 3 months storage at 0 °C in different CA combinations and after 3d display at 20 °C, 1999.

Storage atmosphere		After storage at 0 °C		After storage plus 3 d at 20 °C	
CO ₂ (%)	O ₂ (%)	Rachis score (1-4) ^a	Nests (no. per cluster)	Nests (no. per cluster)	Decay (% wt)
<i>1 month</i>					
10	3	1.0	0.0	0.0	4.0
10	6	1.7	0.0	0.0	6.9
10	12	1.0	0.0	0.0	6.3
15	3	1.3	0.0	0.0	4.4
15	6	1.7	0.0	0.0	4.1
15	12	1.7	0.0	0.0	4.1
Air		1.7	0.0	0.0	25.2
<i>P</i> value		0.33	NS	NS	0.0067
LSD _{0.05}		NS	NS	NS	10.6
<i>2 months</i>					
10	3	2.3	0.3	0.3	1.6
10	6	2.0	0.3	0.3	2.0
10	12	2.0	0.0	0.0	2.4
15	3	2.0	0.0	0.0	0.1
15	6	1.7	0.0	0.0	0.4
15	12	2.0	0.0	0.3	0.6 a
Air		1.3	0.7	1.0	10.3
<i>P</i> value		0.12	0.27	0.038	0.0081
LSD _{0.05}		NS	NS	0.7	4.5
<i>3 months</i>					
10	3	2.3	0.0	0.3	6.4
10	6	2.3	0.3	0.7	9.9
10	12	2.7	0.0	0.7	12.1
15	3	3.0	0.0	0.3	3.2
15	6	3.3	0.0	0.7	1.6
15	12	2.7	0.0	0.7	5.9
Air		3.3	1.0	1.0	59.1
<i>P</i> value		0.34	0.0004	0.33	0.0045
LSD _{0.05}		NS	0.4	NS	26.4

^aRachis 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.

^{NS}Nonsignificant.

Table 5. Effect of controlled atmosphere storage (CA) on decay of 'Thompson Seedless' table grapes after up to 3 months storage at 0 °C, 2000.

Treatment ^a	Nests (no. per cluster)			Decay (% by wt)		
	1 month	2 months	3 months	1 month	2 months	3 months
Air	0.0	0.8	1.0	0.0	21.8	42.4
10% CO ₂ + 3% O ₂	0.0	0.0	0.0	0.0	0.0	1.1
15% CO ₂ + 3% O ₂	0.0	0.0	0.0	0.0	0.0	0.0
15% CO ₂ + 12% O ₂	0.0	0.0	0.0	0.0	0.1	0.2
<i>P</i> value	NS	0.0001	0.0001	0.42	0.0001	0.0001
LSD _{0.05}	NS	0.1	0.8	NS	5.0	5.1

^aAverage of six vineyards with historical records of high decay.

^{NS}Nonsignificant.

ranging from 16% to 21%, with most being harvested at 18% SSC. For our research, we defined early harvested grapes as those with about 16% SSC and late harvested grapes as those nearing 20% SSC. Since late harvested 'Thompson Seedless' table grapes tolerate 15% CO₂ very well, we suggest a CA of 15% CO₂ combined with 3%, 6%, or 12% O₂ to limit *Botrytis* rot development without adversely affecting quality attributes.

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