Long-Term Storage Quality of Table Grapes as Influenced by Pre-Harvest Yeast Applications and Post-Harvest Use of Controlled Atmosphere

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The effects of pre-harvest applications of antagonistic yeast and controlled atmosphere storage treatments on inhibiting *Botrytis cinerea* decay and maintaining the quality of table grapes were compared to identify a treatment that could replace the use of sulphur dioxide (SO_2) during post-harvest handling. Treatments for this study included pre-harvest application of antagonistic yeasts *Cryptococcus albidus* (Yieldplus®), *Cryptococcus* sp. (LF) and *Candida pelliculosa* (R951) on table grapes (cvs. 'Barlinka', 'Dauphine', 'Red Globe', 'Sunred Seedless' and 'Thompson Seedless') from 2001 to 2003. Grapes were stored under regular (air), controlled atmosphere (CA, O_2 + CO_2) and SO_2 conditions at -0.5°C and subsequent storage at a 15°C to simulate shelf-life conditions. Results of this study showed that Botrytis decay levels did not develop rapidly due to low temperatures (-0.5°C vs 15°C), shorter storage periods (4 vs 8 weeks or 0 vs 7 vs 14 days), and CA treatment effects. The CA gas mixtures maintained commercially important low levels (less than 1%) of *B. cinerea* decay during the cold storage period at -0.5°C. However, during shelf-life storage at 15°C these low levels of decay could only be maintained by some of the SO_2 treatments. A necessary commercial requirement is to maintain low decay levels for longer at higher shelf-life temperatures, for which this study cannot conclusively recommend a CA and/or antagonistic yeast treatment as an alternative to SO_2. However, pre-harvest applications of the yeast and CA limited the general quality deterioration of the grapes at -0.5°C and 15°C compared to SO_2 treatments. Inclusion of macro- or micro-perforated polyethylene packaging liners in combination with CA and pre-harvest yeast treatments did not show obvious negative effects on quality parameters in this study. Discovery and selection of yeast strains that survive under low temperatures and CA conditions would make suitable candidates for continued control of decay development on the fruit surface during shelf-life storage periods.

INTRODUCTION

Botrytis cinerea Pers.:Fr., *Aspergillus niger* Tiegh. and *Rhizopus stolonifer* (Ehrenb.:Fr) Vuill. are common post-harvest fungal decay pathogens of table grapes in most regions of the world (Bulit & Dubos, 1988). Cold storage in combination with sulphur dioxide (SO_2) is the most common method used to control post-harvest fungal decay in table grapes (Artéz-Hdez *et al*., 2003). However, problems associated with the use of SO_2 include the following: (1) SO_2 residues that exceed the tolerance level of 10 mg/kg can occur if the application dosage is too high, (2) bleaching injuries on the skin of the berries and off-flavours when the fruit is eaten can occur during continuous exposure or after high dosages, (3) because of the susceptibility of some people to sulphite allergies , the dietary danger of SO_2 was recognised, and (4) there are concerns about the carcinogenic effect of long-term ingestion of the residues left on the fruit on consumption (Mlikota Gabler & Smilanick, 2001; Nelson, 1985; Yahia *et al*., 1983). Therefore, the development of alternative strategies to control post-harvest decay of table grapes that are safe, effective, and compatible with commercial practices is of interest to the South African table grape industry.

Controlled atmosphere (CA) has been successfully used to control post-harvest decay and for maintaining quality attributes in table grapes (Eris *et al*., 1993; Yahia *et al*., 1983). Yahia *et al*. (1983) and Berry and Aked (1997) reported the following potential benefits of CA storage: delayed senescence, decreased rachis and berry respiration, reduced rachis browning, maintained berry firmness, and retarded decay development. Studies by Lazlo

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(1985) suggest that CA storage of 'Waltham Cross' grapes at 5% O_2 and 10% CO_2 is insufficient to suppress pathogen development. Crisosto *et al.* (2003a) suggested a CA of 15% CO_2 combined with 3, 6 or 12% O_2 to limit Botrytis decay development without adversely affecting quality attributes of 'Thompson Seedless'.

Biological control agents that have been studied for the control of post-harvest decay of table grapes include yeasts, *Kloeckera apiculata* (Reess emend. Klöcker) Janke and *Candida guilliermondii* (Castellani) for control of Rhizopus rot (McLaughlin & Wilson, 1992), and *Candida oleophila* (Montrocher) for control of blue mould (Droby *et al*., 2002) and gray mould (El-Neshawy & El-Morsy, 2003). In field trials, biological control, or a combination of biological and chemical control, has resulted in control of gray mould (Elad, 1994; Harman *et al*., 1996). Harman *et al*. (1996) showed that as few as two late-season applications of *Trichoderma harzianum* were nearly as effective as up to five applications throughout bloom and fruit development for the post-harvest control of gray mould of grapes. The yeast *Metschnikowia fructicola* Kurtzman & Droby, when applied to table grapes 24 h before harvest, reduced the total number of decayed berries in storage at 1°C for 30 days followed by 2 days at 20°C (Karabulut *et al*., 2003; Kurtzman & Droby, 2001).

The main objective of this study was to evaluate treatments and storage conditions that could be used as alternatives to SO_2 treatment and simultaneously produce positive visual and quality effects in South African table grapes, without the need for additional harvest or post-harvest handling procedures.

MATERIALS AND METHODS

To meet the above objectives three experiments were conducted over three seasons, from 2001 to 2003, with conventionally grown Barlinka, Dauphine, Red Globe, Sunred Seedless and Thompson Seedless table grape cultivars within the Hex River table grape producing area in South Africa. All experiments were planned as complete randomised designs with four replications. The treatment design was a split-plot with pre-harvest antagonistic yeast and water (control) sprays as main plots and factorial treatment combinations as sub-plots. From each main-plot the harvested table grapes placed in cartons were pooled from which four were randomly selected as replications for each sub-plot combination. The sub-plot factors were CA-treatments consisting of various O_2 and CO_2 combinations plus regular atmosphere (RA) and SO_2 control treatments, as well as combinations with different types of perforated polyethylene packaging material. The other parameters were 4-, 6- or 8-week cold storage periods at -0.5°C and shelf-life storage periods of 0, 7 or 14 days at 15°C, as listed in Table 1.

Pre-harvest application of yeast formulations

Pre-harvest applications of yeast formulations used in this study are listed in Table 1. Granular formulations of *Cryptococcus albidus* (Yieldplus®, strain YP, 10^8 cells/ml) originally isolated from apples, and *Cryptococcus* sp. (strain LF, 10^8 cells/ml) isolated from plums, were obtained from a yeast product manufacturer, Anchor Biotechnologies (Cape Town, South Africa). *Candida pelliculosa* (strain R951, 10^7 cells/ml) was isolated from grapevine material and formulated by Plant Health Products (Pietermaritzburg, South Africa). Yieldplus and LF were used at maximum label dosage of 1.5 g/L. The recommended dosage for R951 was 0.2 g/L.

For the first experiment in 2001, one pre-harvest YP spray was applied with a tractor-mounted mistblower on the day before harvest on 'Thompson Seedless', 'Dauphine' and 'Barlinka'. For experiment 2 in 2002, a single spray of YP and LF was applied on 'Thompson Seedless', 'Red Globe' and 'Sunred Seedless'. For experiment 3 in 2003, spray applications with YP and R951 were made three days before harvest, followed by a second application one day before harvest for 'Thompson Seedless', 'Red Globe' and 'Barlinka'. Grape bunches at optimum maturity were harvested into plastic picking lugs (27 x 35 x 53 cm) in the morning. Loose berries, small clusters, and damaged or rotten berries were removed. Eight packed cartons (7-8 clusters per carton, net weight approximately 4.5 kg) were randomly collected for each of the pre-harvest yeast treatment and post-harvest storage treatment combinations.

Post-harvest storage conditions and packaging

Post-harvest storage conditions and packaging material used in this study are listed in Table 1. For experiment 1 cartons were placed in a 1-m^3 static CA cabinet system (Icolcell Co., Italy) with the following CA mixtures, at -0.5°C for 4 weeks: 1.5% O_2 + 10% CO_2; 1.5% O_2 + 15% CO_2; 1.5% O_2 + 20% CO_2; 1.5% O_2 + 25% CO_2; 5% O_2 + 10% CO_2; 5% O_2 + 15% CO_2; 5% O_2 + 20% CO_2; 5% O_2 + 25% CO_2. Treatments with RA (air) and SO_2-generating sheets were included, to represent conventional storage conditions. For experiment 2 the storage conditions were 5% O_2 + 10% CO_2, 1.5% O_2 + 10% CO_2 and 1.5% O_2 + 15% CO_2 and RA in combination with macro- and micro-perforated polyethylene liners (MaPP, MiPP) as well as unspecified Xtend GR3 and Xtend GR4 liners (patent pending, StePac L. A. Ltd, Israel). Additional packaging material for RA treatments included SO_2-generating sheets: SO_2 Frescha, $Na_2S_2O_5$ = 97.0% (Quimetal Industrial, SA), and SO_2 Uvasys, $Na_2S_2O_5$ = 37.5% (Grapetek Pty. Ltd, SA). For experiment 3 the cold-storage treatments included 5% O_2 + 10% CO_2 and RA with combinations of MiPP as well as SO_2 Frescha, SO_2 Uvasys and SO_2 Uvaspec, $Na_2S_2O_5$ = 97.5% (Grapetek Pty. Ltd).

Evaluation of grapes

In experiment 1, at the end of the 6-weeks storage period at -0.5°C four cartons of each treatment were removed from the CA and RA cabinets for evaluation (0 days) and four moved to storage rooms at 15°C for a 7- and 14-day shelf-life storage simulation period. Experiment 2 had a 4- and 8-week storage period at -0.5°C followed by a shelf-life period of 0 and 7 days at 15°C. Experiment 3 had a 6-weeks storage period at -0.5°C and a 0- and 7-day shelf-life period. Quality evaluations included assessment of berry decay, loose berries, berry shatter, SO_2 damage, stem browning and berry firmness. Berry decay was identified as *B. cinerea* through signs of sporulation and the characteristic 'slip-skin' symptom (Bulit & Dubos, 1988). The percentage decay was calculated by the weight of decayed berries as a proportion of the total cluster weight per carton. The percentages of loose berries, berry shatter, as well as berries with SO_2 bleaching damage were calculated by determining the weight of the affected berries as a proportion of the total cluster weight per carton. Stem browning was scored on a 5-point scale, in which 1 = stems green and fresh,

TABLE 1

Summary of the experimental treatments used in the study of pre-harvest yeast applications and post-harvest use of controlled atmosphere or sulphur dioxide on the quality of table grapes for long term storage.

Pre-harvest main plot treatments	**Post-harvest sub-plot treatments**	
	Cold storage conditions [a] and packaging material [b]	**Shelf-life storage**
Experiment 1 / 2001 / 'Barlinka', 'Dauphine', 'Thompson Seedless'		
Storage period	6 weeks at -0.5 °C	0 days at 15 °C
	6 weeks at -0.5 °C	7 days at 15 °C
	6 weeks at -0.5 °C	14 days at 15 °C
Water (control)	CA 1.5% O_2 + 10% CO_2 + MiPP	
	CA 1.5% O_2 + 15% CO_2 + MiPP	
	CA 1.5% O_2 + 20% CO_2 + MiPP	
	CA 1.5% O_2 + 25% CO_2 + MiPP	
	CA 5% O_2 + 10% CO_2 + MiPP	
	CA 5% O_2 + 15% CO_2 + MiPP	
	CA 5% O_2 + 20% CO_2 + MiPP	
	CA 5% O_2+ 25% CO_2 + MiPP	
	RA (air) + MiPP	
	RA + SO_2 sheet + MiPP	
Cryptococcus albidus (Yieldplus or YP)	CA 1.5% O_2 + 10% CO_2 + MiPP	
	CA 1.5% O_2 + 15% CO_2 + MiPP	
	CA 1.5% O_2 + 20% CO_2 + MiPP	
	CA 1.5% O_2 + 25% CO_2 + MiPP	
	CA 5% O_2 + 10% CO_2 + MiPP	
	CA 5% O_2 + 15% CO_2 + MiPP	
	CA 5% O_2 + 20% CO_2 + MiPP	
	CA 5% O_2 + 25% CO_2 + MiPP	
	RA + MiPP	
Experiment 2 /2002 / 'Thompson Seedless', 'Red Globe ', 'Sunred Seedless'		
Storage period	4 weeks at -0.5 °C	0 days at 15 °C
	4 weeks at -0.5 °C	7 days at 15 °C
	8 weeks at -0.5 °C	0 days at 15 °C
	8 weeks at -0.5 °C	7 days at 15 °C
Water (control)	CA 5% O_2 + 10% CO_2 + MaPP	
	CA 1.5% O_2 + 10% CO_2 + MaPP	
	CA 1.5% O_2 + 15% CO_2 + MaPP	
	RA + MaPP	
	RA + Xtend GR3	
	RA + Xtend GR4	
	RA + MaPP+ SO_2 Frescha	
	RA + MiPP+ SO_2 Frescha	
	RA + Closed liner + SO_2 Uvasys	
	RA + MiPP + SO_2 sheet	
	RA + Xtend GR3 + SO_2 Uvasys	
	RA + Xtend GR4 + SO_2 Uvasys	
Cryptococcus sp. (LF)	CA 5% O_2 + 10% CO_2 + MaPP	
	CA 1.5% O_2 + 10% CO_2 + MaPP	
	CA 1.5% O_2 + 15% CO_2 + MaPP	
	RA + MaPP	
	RA + Xtend GR3	
	RA + Xtend GR4	
	RA + MiPP	
Cryptococcus albidus (YP)	CA 5% O_2 + 10% CO_2 + MaPP	
	CA 1.5% O_2 + 10% CO_2 + MaPP	
	CA 1.5% O_2 + 15% CO_2 + MaPP	
	RA + MaPP	
	RA + Xtend GR3	
	RA + Xtend GR4	
	RA + MiPP	
Experiment 3/ 2003 / 'Thompson Seedless', 'Red Globe', 'Barlinka'		
Storage period	6 weeks at -0.5 °C	0 days at 15 °C
	6 weeks at -0.5 °C	7 days at 15 °C
Water (control)	CA 5% O_2 + 10% CO_2 + MiPP	
	RA + MiPP	
	RA + MiPP+ SO_2 Frescha	
	RA + MiPP + SO_2 Uvasys	
	RA + MiPP + SO_2 Uvaspec	
Candida pelliculosa (R951)	CA 5% O_2 + 10% CO_2 + MiPP	
	RA + MiPP	
Cryptococcus albidus (YP)	CA 5% O_2 + 10% CO_2 + MiPP	
	RA + MiPP	
Candida pelliculosa (R951) – two sprays	CA 5% O_2 + 10% CO_2 + MiPP	
	RA + MiPP	
Cryptococcus albidus (YP) – two sprays	CA 5% O_2 + 10% CO_2 + MiPP	
	RA + MiPP	

[a] CA = Controlled atmosphere. RA = Regular atmosphere (air). [b] MaPP = Macro-perforated polyethylene liner. MiPP = Micro-perforated polyethylene liner. Xtend GR3 and GR4 = Unspecified liners. Other standard packaging material included carry bags and corrugated sheets for all treatments.

2 = stems green and wilted, 3 = stems more green than brown, 4 = stems more brown than green, and 5 = all stems brown and dry. Berry firmness was determined by gentle pressing of the berries by hand and classifying it as firm = 1, firm to soft = 2 or soft = 3.

Statistical analysis

Stem browning and berry firmness were recorded on an ordinal scale and the frequencies observed in the three and five classes were subjected to a general linear model (GLM) technique with a logistic link function. The maximum likelihood estimators (MLE = XBeta's) were calculated on an underlying scale (McCullagh & Nelder, 1989). These MLE's, which are on an interval scale, together with the other variables, were subjected to an appropriate analyses of variance, and were also separately performed for each shelf-life period. In order to compare the treatment means for significant effects Student's t-Least Significant Difference was calculated at a 5% significance level. The Shapiro-Wilk's test was performed to test for non-normality (Shapiro & Wilk, 1965). All analyses were carried out using SAS version 8.2 (SAS, 1999). Best performance treatment means were ranked according to the LSD letter groupings for each variable.

RESULTS

For experiment 1, a significant cultivar by shelf-life storage period interaction was observed for percentage decay, loose berries, berry shatter, stem browning and berry firmness (see Tables 2 and 3). Significantly higher levels of decay developed in 'Thompson Seedless' at 15°C than at -0.5°C, which increased with extended time at 15°C. Higher levels of loose berries and berry shatter also occurred for 'Thompson Seedless' grapes at -0.5°C, showing an increase at the 7-day storage period, and significantly less loose berries and berry shatter compared to 'Barlinka' and 'Dauphine' at the 14-day storage period at 15°C. Berry firmness of 'Dauphine" kept significantly well under all three shelf-life storage conditions compared to 'Barlinka' and 'Thompson Seedless'. SO_2 damage did not feature as a significant factor in the cultivar by shelf-life interaction of experiment 1, but showed a significant main effect for treatments (see Table 2). The effect was primarily due to SO_2 bleaching damage in the SO_2 treatments. No other treatment included SO_2, therefore none of the other treatments should show SO_2 damage.

For experiment 2, a significant treatment by cold storage (weeks) by shelf-life (days) was observed for percentage decay, berry shatter, stem browning and berry firmness (see Tables 2 and 4). A significant cold storage by shelf-life interaction was noted for decay, berry shatter, stem browning and berry firmness. A significant cultivar by treatment by shelf-life storage interaction was also observed for percentage decay, loose berries, berry shatter, SO_2 damage, and stem browning (see Table 2). Decay was significantly less where grapes had been exposed to the shorter (4 vs 8 weeks) and colder (-0.5°C vs 15°C) storage periods. It seems to be a matter of decay not developing as rapidly at the specific temperature, or a lower level occurring as a result of the shorter storage period. All the RA + SO_2 treatment combinations, except RA + MiPP + SO_2, showed decay at comparable levels to those observed for the -0.5°C cold-storage period. Controlled atmosphere + MaPP combinations including those with pre-harvest yeast applications (1.5% O_2 + 15% CO_2 + MaPP; 5% O_2 + 10% CO_2 + MaPP (LF); 1.5% O_2 + 10% CO_2 + MaPP) showed lower levels of decay in comparison to RA treatment combinations without SO_2. Decay levels of grapes at the 8-week cold storage period became very high during the 15°C storage period, which complicated recording of data for further analysis. Controlled atmosphere, SO_2 and yeast treatments in this experiment affected stem-browning levels during the -0.5°C and 15°C storage periods. At -0.5°C and 15°C the lower levels of stem browning were associated with CA + MaPP treatments alone or in combination with pre-harvest yeast, as well as the RA and yeast combinations. Berry firmness kept well at the -0.5°C storage period, but fruits were definitely softer after the 15°C storage period. CA1 (5% O_2 + 10% CO_2) and CA2 (1.5% O_2 + 10% CO_2) in combination with MaPP treatments seemed to influence the berry firmness positively at the 15°C storage period.

For experiment 3, a significant cultivar by treatment by shelf-life interaction was observed for percentage decay, berry shatter, stem browning and berry firmness (see Table 2). Significant treatment by shelf-life interaction was observed for percentage decay, berry shatter, stem browning and berry firmness. Cultivar by shelf-life, as well as cultivar by treatment interactions, were detected for percentage decay, loose berries, berry shatter, stem browning and berry firmness. Decay was lower where grapes had been exposed to the shorter (0 d vs 7 d) and colder (-0.5°C vs 15°C) storage periods. The best treatments that minimised stem browning at -0.5°C were CA (5% O_2 + 10% CO_2) and RA + MiPP treatments with pre-harvest yeast applications. The SO_2 treatments produced higher levels of stem browning during the shelf-life storage period compared to the CA treatments.

DISCUSSION

In this study, quality deterioration of grapes was mainly due to the occurrence of *B. cinerea*, which caused higher decay levels as a result of the shelf-life storage period at 15°C. The SO_2 generating material in all the experiments was more effective than all other treatments in reducing post-harvest decay. The results also showed that CA treatments affect the development of *B. cinerea* decay. Based on the results of this study, CA combinations of O_2 at a level of 1.5 and 5% and CO_2 levels at 10 to 20% could minimise decay development in cold storage at -0.5°C. Overall, this study shows that Botrytis decay levels did not develop rapidly due to the influence of a specific temperature (-0.5°C vs 15°C), or as a result of the shorter cold storage and shelf-life periods (4 vs 8 weeks or 0 vs 7 vs 14 days) as well as CA and SO_2 treatment effects. Studies by Lazlo (1985) suggest that CA storage treatment alone of 'Waltham Cross' at 5% O_2 and 10% CO_2 is not sufficient to suppress pathogen development. Pathogenic fungi are suppressed by low temperatures and CA with low O_2 and high CO_2 (Spotts *et al*., 1998, Tian *et al*., 2002), but studies have shown that *B. cinerea* can grow and cause decay even at -4°C (Tian & Bertolini, 1995). Statistical interactions between storage and treatment conditions that include CA treatments were detected in this study, which support suggestions made in other studies that CA treatments alone do not control decay development.

Results over the three experiments could not conclusively demonstrate that pre-harvest applications of *Cryptococcus* and *Candida* yeast formulations resulted in control of decay under CA or RA storage conditions. However, some of the single and double pre-harvest applications of *Candida pelliculosa* (R951) and *Cryptococcus albidus* (YP) in combination with CA treatments

TABLE 2

Significance levels for analysis of variance of *Botrytis cinerea* decay, loose berries, berry shatter, SO_2 damage, stem browning and berry firmness of table grape cultivars stored for different storage periods after different treatment applications in three experiments, showing significant levels for main effects and all interactions.

		Variable (Pr > F)					
Source of variation	**df**	**Decay (%)**	**Loose berries (%)**	**Berry shatter (%)**	**SO_2 damage (%)**	**Stem browning (loc)[a]**	**Berry firmness (loc)**
Experiment 1							
Cultivar (Cult)	2	0.0001	<.0001	<.0001	0.2049	0.0046	<.0001
Treatment (Trt)	18	0.0513	0.1636	0.3117	<.0001	<.0001	0.9829
Shelf-life (Days)	2	<.0001	<.0001	<.0001	0.5257	<.0001	<.0001
Cult*Trt	36	0.9405	0.3248	0.8167	0.1543	0.0002	0.4030
Cult*Days	4	<.0001	<.0001	<.0001	0.3387	0.0005	0.0396
Trt*Days	36	0.0849	0.0702	0.4667	1.0000	0.0082	0.0711
Cult*Trt*Days	72	0.1175	0.0796	0.9972	0.3000	0.0013	0.9167
Experiment 2							
Cultivar (Cult)	2	<.0001	<.0001	0.0300	0.1558	<.0001	<.0001
Treatment (Trt)	25	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Cult*Trt	50	<.0001	<.0001	<.0001	0.0054	<.0001	<.0001
Cold storage(Weeks)	1	<.0001	0.4512	<.0001	0.3617	<.0001	0.0001
Trt*Weeks	25	<.0001	0.8853	<.0001	0.9999	<.0001	<.0001
Shelf-life (Days)	1	<.0001	<.0001	<.0001	0.9261	<.0001	<.0001
Cult*Days	2	<.0001	<.0001	<.0001	0.0021	<.0001	<.0001
Trt*Days	25	<.0001	0.0003	<.0001	0.9978	<.0001	<.0001
Cult*Trt*Days	50	<.0001	0.0284	<.0001	<.0001	<.0001	0.1603
Weeks*Days	1	<.0001	0.8831	0.0004	0.4674	<.0001	<.0001
Trt*Weeks*Days	25	<.0001	0.5876	<.0001	1.0000	0.0047	<.0001
Experiment 3							
Cultivar (Cult)	2	<.0001	<.0001	<.0001	0.0335	<.0001	<.0001
Treatment (Trt)	14	<.0001	0.0978	<.0001	0.5182	<.0001	<.0001
Shelf-life (Days)	24	<.0001	0.0372	<.0001	0.4937	<.0001	<.0001
Cult*Trt	1	<.0001	<.0001	<.0001	0.0636	<.0001	<.0001
Cult*Days	2	<.0001	0.0020	<.0001	0.0335	<.0001	<.0001
Trt*Days	14	<.0001	0.2437	<.0001	0.5182	0.0564	<.0001
Cult*Trt*Days	24	<.0001	0.0842	<.0001	0.4937	0.0307	0.0001

[a] Loc = Location values on the underlying scale.

TABLE 3

Shelf-life (days) X cultivar interactions in *Botrytis cinerea* decay, loose berries, berry shatter, stem browning and berry firmness for 'Barlinka' (BA), 'Dauphine' (DA) and 'Thompson Seedless' (TS) table grapes harvested for experiment 1.

Shelf-life[a]	**Cultivar**	**Decay (%)**	**Loose berries (%)**	**Berry shatter (%)**	**Stem browning (loc)[b]**	**Berry firmness (loc)**
0 d at 15°C	BA	0.13 b [c]	0.13 c	0.39 b	-1.4 a	1.5 a
	DA	0.06 b	0.89 b	0.17 c	-5.9 b	-7.7 b
	TS	0.87 a	2.74 a	0.64 a	-3.2 a	1.3 a
7 d at 15°C	BA	3.36 b	0.37 c	0.42 b	-0.4 a	3.6 a
	DA	1.09 c	0.78 b	0.23 b	0.7 a	-8.9 c
	TS	10.63 a	2.57 a	1.62 a	0.3 a	0.4 b
14 d at 15°C	BA	8.97 a	0.33 b	0.07 b	3.7 a	9.2 a
	DA	1.92 b	0.59 a	0.16 a	2.2 b	-3.1 c
	TS	10.07 a	0.00 c	0.00 c	4.0 a	3.8 b
0 d at 15°C	0.35 c	1.26 a	0.40 b	-3.52 c	-1.7 b	
7 d at 15°C	5.03 b	1.23 a	0.76 a	0.22 b	-1.6 b	
14 days at 15°C	6.98 a	0.31 b	0.08 c	3.29 a	3.3 a	

[a] Harvested grape bunches were stored at -0.5°C for 6 weeks in cartons at combinations of controlled atmosphere, regular atmosphere (air) and sulphur dioxide (SO_2) followed by self-life storage for 7 and 14 days at 15°C.

[b] Loc = Location values on the underlying scale.

[c] Means within columns followed by unlike letters differ significantly according to Student's-t-least significant difference (P = 0.05).

TABLE 4

Treatment X cold storage (weeks) X shelf-life (days) interactions in *Botrytis cinerea* decay, loose berries, berry shatter, stem browning and berry firmness for 'Red Globe' (RG), 'Sunred Seedless' (SR) and 'Thompson Seedless' (TS) table grapes harvested for experiment 2.

Cold storage and shelf -life Treatments [a]	Decay (%)	Berry shatter (%)	Stem browning (loc) [b]	Berry firmness (loc)
Treatment X cold storage X shelf-life				
4 weeks at -0.5°C and 0 d at 15°C				
CA1 + MaPP	0.10 b [c]	0.47 bcdefg	1.6 a	-3.8 abcd
CA2 + MaPP	0.08 b	0.22 efg	-14.2 j	-3.9 abcdef
CA3 + MaPP	0.10 b	0.14 fg	-2.8 abcd	-3.9 abcd
CA1 + MaPP (LF)	0.05 b	0.08 g	0.8 ab	-4.1 abcdef
CA2 + MaPP (LF)	0.14 b	0.10 g	-9.0 ghi	-3.9 abcdef
CA3 + MaPP (LF)	0.15 b	0.35 bcdefg	-6.3 defg	-3.9 abcde
CA1 + MaPP (YP)	0.13 b	0.58 bcdefg	-13.1 ij	-4.2 abcdef
CA2 + MaPP (YP)	0.07 b	0.30 defg	-5.8 defg	-5.4 abcdef
CA3 + MaPP (YP)	0.11 b	0.39 bcdefg	-8.8 ghi	-3.8 ab
RA + MaPP (LF)	0.14 b	0.69 bcdef	-3.8 cdef	-5.5 abcdef
RA + MaPP (YP)	0.42 b	1.92 a	-0.6 abc	-7.1 def
RA + MaPP	0.52 ab	0.22 efg	1.1 a	-3.7 a
RA + Xtend GR3 (LF)	0.05 b	0.15 fg	-7.7 fgh	-3.8 abc
RA + Xtend GR4 (LF)	0.31 b	0.13 fg	-10.1 ghij	-4.1 abcdef
RA + Xtend GR3 (YP)	0.13 b	0.34 cdefg	-10 8 hij	-4.4 abcdef
RA + Xtend GR4 (YP)	1.27 a	0.31 defg	-8.6 gh	-7.0 cdef
RA + Xtend GR3	0.52 ab	0.40 bcdefg	-7.4 efgh	-5.4 abcdef
RA + Xtend GR4	0.27 b	0.92 b	-0.9 abc	-3.3 a
RA + MiPP (LF)	0.73 ab	0.91 bc	-8.9 ghi	-5.8 abcdef
RA + MiPP (YP)	0.31 b	0.73 bcde	-6.0 defg	-7.1 cdef
RA + MaPP + SO_2 Frescha	0.14 b	0.19 efg	-0.1 abc	-5.5 abcdef
RA + MiPP + SO_2 Frescha	0.28 b	0.47 bcdefg	0.9 ab	-5.7 abcdef
RA + Closed liner + SO_2 Uvasys	0.76 ab	0.43 bcdefg	-2.0 abcd	-5.7 abcdef
RA + MiPP + SO_2 sheet	1.28 a	0.43 bcdefg	-3.3 bcde	-4.2 abcdef
RA + Xtend GR3 + SO_2 Uvasys	0.70 ab	0.42 bcdefg	-2.9 abcd	-7.2 ef
RA + Xtend GR4 + SO_2 Uvasys	0.78 ab	0.81 bcd	-1.9 abcd	-7.2 f
4 weeks at -0.5 °C X 7 d at 15°C				
CA1 + MaPP	14.85 cde	4.97 bcdef	2.3 efgh	0.6 def
CA2 + MaPP	9.00 defgh	3.92 bcdefg	-0.1 j	-2.6 ef
CA3 + MaPP	8.34 efgh	10.18 a	2.3 efgh	0.6 def
CA1 + MaPP (LF)	9.76 defgh	6.50 abcd	6.3 ab	-4.3 f
CA2 + MaPP (LF)	15.22 cde	7.77 ab	2.2 efghi	-4.2 f
CA3 + MaPP (LF)	11.93 cdefg	3.51 cdefg	2.0 fghi	0.6 def
CA1 + MaPP (YP)	20.43 bc	4.08 bcdefg	0.8 hij	0.6 def
CA2 + MaPP (YP)	13.3 cdef	7.26 abc	1.9 fghi	2.2 bcde
CA3 + MaPP (YP)	16.99 bcde	5.17 bcde	0.9 hij	0.8 def
RA + MaPP (LF)	12.93 cdef	7.51 abc	3.2 defg	8.7 a
RA + MaPP (YP)	16.62 cde	1.34 efg	3.3 cdefg	7.0 abc
RA + MaPP	18.72 bcd	1.04 g	3.9 cde	7.3 ab
RA + Xtend GR3 (LF)	16.94 bcde	3.55 cdefg	2.1 efghi	0.7 def
RA + Xtend GR4 (LF)	20.61 bc	0.29 g	1.7 ghij	0.7 def
RA + Xtend GR3 (YP)	22.01 bc	2.57 defg	0.4 ij	7.0 abc
RA + Xtend GR4 (YP)	35.63 a	1.98 efg	0.4 hij	4.1 abcd
RA + Xtend GR3	21.57 bc	2.45 defg	2.2 efghi	5.5 abcd
RA + Xtend GR4	27.03 ab	1.50 efg	2.2 efghi	1.7 cde
RA + MiPP (LF)	12.7 cdef	1.06 efg	3.2 defg	4.2 abcd
RA + MiPP (YP)	10.18 defgh	3.41 cdefg	2.8 defg	5.6 abcd
RA + MaPP + SO_2 Frescha	3.35 fgh	1.82 efg	3.4 cdefg	2.3 bcde
RA + MiPP + SO_2 Frescha	1.71 h	1.48 efg	4.4 bcd	7.0 abc
RA + Closed liner + SO_2 Uvasys	3.57 fgh	1.80 efg	5.1 bc	0.5 def
RA + MiPP + SO_2 sheet	15.38 cde	1.61 efg	3.6 cdef	3.4 abcd
RA + Xtend GR3 + SO_2 Uvasys	1.90 gh	1.41 efg	7.6 a	2.1 bcde
RA + Xtend GR4 + SO_2 Uvasys	0.76 h	3.04 defg	7.3 a	3.6 abcd
Cold storage X shelf-life				
4 weeks at -0.5°C and 0 d at 15°C	7.13 b	1.99 b	-1.1 b	-1.2 b
8 weeks at -0.5°C and 0 d at 15°C	26.7 a	3.65 a	3.24 a	3.7 a

[a] Harvested grape bunches were stored at -0.5°C for 4 or 8 weeks in cartons followed by self-life storage for 7 days at 15°C. CA = Controlled atmosphere: CA1 = 5% O_2+10% CO_2. CA2 = 1.5% O_2+10% CO_2. CA3 = 1.5% O_2+15% CO_2, RA = Regular atmosphere or air, MaPP = Macro-perforated polyethylene liner, MiPP =Micro-perforated polyethylene liner, Xtend GR3 and GR4 = unspecified liners and SO_2 = sulphur dioxide. LF = *Cryptococcus* sp. and YP =*Cryptococcus albidus* = yeast spray applications one day before harvest.

[b] Loc = Location values on the underlying scale.

[c] Means within columns followed by unlike letters differ significantly according to Student's t-least significant difference (P = 0.05).

resulted in improved decay control during shelf-life storage. Previous studies have shown that yeasts can function over a wide range of environmental conditions to control decay. Chand-Goyal & Spotts (1997) showed that the application of *Cryptococcus laurentii* and *Rhodotorula glutinis* effectively controlled blue mould of pear when stored at -1°C under CA (O_2, 1.08%; CO_2 0,01%; N_2 98.91%). Most yeast requires CO_2 as a carbon source for growth, and about 10% CO_2 enhances sporulation (Spotts *et al*., 1998). Populations of *C. laurentii* in apple wounds reached a maximum after 4 days at 1-2°C in both air and CA (1.5% O_2, 2% CO_2, 96.5% N_2) (Shefelbine & Roberts, 1990). The effectiveness of *Cryptococcus oleophila* in preventing *P. expansum* infection of nectarine fruits was not reduced by CA (3% O_2, 10% CO_2, 87% N_2) (Lurie *et al*., 1995). Three applications of the yeast *C. oleophila*, made at 10-day intervals, 15 days before harvest provided post-harvest control of the gray mould pathogen in grapes (El-Neshawy & El-Morsy, 2003). The tolerance of the yeast strains to the storage temperatures and CA treatments is unknown. Both *Cryptococcus* strains (YP, LF), in comparison with the *Candida* strain R951, were derived from environments different from those which they effectively control *B. cinerea*. Better biocontrol is likely to occur if organisms are obtained from a niche similar to that where biocontrol is desired (Harman *et al*., 1996). Screening and selection of yeast strains that survive and multiply under more extreme environmental conditions should be investigated.

TABLE 5. Shelf-life (days) X cultivar X treatment interactions in *Botrytis cinerea* decay, berry shatter and stem browning for 'Barlinka' (BA), 'Red Globe' (RG), 'Thompson Seedless' (TS) table grapes harvested for experiment 3.

Cold storage and shelf -life treatments [a]	Cultivar	Decay (%)	Berry shatter (%)	Stem browning (loc) [b]
Shelf-life X cultivar X treatment				
0 d at 15°C	BA			
CA + MiPP	0.00	0.00	-4.9	
CA + MiPP (R951)	0.27	0.00	-13.4	
CA + MiPP (YP)	0.00	0.81	-9.1	
RA + MiPP (R951)	0.00	0.45	-13.4	
RA + MiPP (YP)	0.41	1.56	-8.8	
RA + MiPP	0.00	2.85	-13.4	
CA + MiPP (2x R951)	0.00	0.45	-13.4	
CA + MiPP (2x YP)	0.00	0.00	-9.1	
RA + MiPP (2x R951)	0.00	2.13	-9.2	
RA + MiPP (2x YP)	0.28	2.92	-9.1	
RA + MiPP + SO_2 Frescha	0.00	0.99	-4.7	
RA + MiPP + SO_2 Uvasys	0.00	1.06	1.6	
RA + MiPP + SO_2 Uvaspec	0.00	0.00	5.8	
RA + Xtend GRx + SO_2 Uvasys	0.00	1.19	-5.2	
RA + Xtend GR + SO_2 Uvasys	1.20	0.31	3.3	
7 d at 15 °C	BA			
CA + MiPP	0.56	1.44	-5.2	
CA + MiPP (R951)	0.00	1.22	3.4	
CA + MiPP (YP)	1.80	2.09	4.4	
RA + MiPP (R951)	6.14	2.03	-1.1	
RA + MiPP (YP)	11.97	0.00	4.2	
RA + MiPP	4.19	1.66	-0.6	
CA + MiPP (2x R951)	0.00	2.51	-5.2	
CA + MiPP (2x YP)	0.45	1.67	-1.1	
RA + MiPP (2x R951)	8.95	0.87	-9.1	
RA + MiPP (2x YP)	24.45	0.00	-5.0	
RA + MiPP + SO_2 Frescha	0.38	0.79	12.2	
RA + MiPP + SO_2 Uvasys	0.29	1.87	16.3	
RA + MiPP + SO_2 Uvaspec	0.28	0.42	15.1	
RA + Xtend GRx + SO_2 Uvasys	0.00	1.67	12.8	
RA + Xtend GR + SO_2 Uvasys	0.00	3.04	12.7	
Shelf-life X cultivar				
0 d at 15°C	BA	0.11 b	0.98 b	-6.9 c
	RG	4.07 a	7.41 a	-0.9 a
	TS	3.12 a	2.41 b	3.7 b
7 d at 15°C	BA	3.96 c	1.39 b	3.6 b
	RG	18.17 a	0.00 c	10.8 a
	TS	9.8 b	4.19 a	-2.3 c

[a] Harvested grape bunches were stored at -0.5°C for 6 weeks in cartons, followed by self-life storage for 7 days at 15°C. CA = Controlled atmosphere = 5% O_2 + 10% CO_2, RA = Regular atmosphere or air, MiPP = Micro-perforated polyethylene liner, Xtend GR3 and GR4 = unspecified liners and SO_2 = sulphur dioxide. R951 = *Candida pelliculosa* and YP = *Cryptococcus albidus* = yeast spray applications one day before harvest. 2x R951 and 2 x YP = applications three days before harvest followed by a second application one day before.

[b] Means within columns followed by unlike letters differ significantly according to Student's t-least significant difference (P = 0.05).

[b] Loc = Location values on the underlying scale.

In this study CA and SO_2 treatments influenced the visual quality of grapes, especially in terms of stem browning. Reduced stem browning at -0.5°C and 15°C was associated with combinations of CA and single as well as double pre-harvest yeast applications. However, the significance of these interactions to reduce stem browning or confining grape bunch deterioration requires further studies.

Controlled atmosphere has increasingly been used in the commercial storage of fruits and vegetables to reduce decay, delaying senescence and maintaining quality (Kader, 1997; Tian *et al*., 2002). Altering the atmospheric composition around fruit, in conjunction with temperature and humidity control, can slow the process of deterioration and hence maintain the quality of the commodity and its resistance to pathogens for longer periods (Sommer, 1992). Crisosto *et al*. (2003a & b) suggested a CA combination of 3, 6 or 12% O_2 with 10% CO_2 for 'Red Globe', and 3, 6 or 12% O_2 and 15% CO_2 for 'Thompson Seedless' for up to 12 weeks storage to limit decay, but maintaining quality attributes.

Inclusion of MaPP and MiPP packaging liners in combination with CA and pre-harvest yeast treatments did not show obvious negative effects on quality parameters in this study. Decay of pears by *P. expansum* was suppressed in modified atmosphere packaging (0.003-cm liners) compared to standard air storage, and decay suppression was increased by the application of biocontol agents (yeast and bacteria) (Miller & Sugar, 1997). Closed packaging systems (barrier and semi-barrier film) extended the shelf-life storage period and inhibited mould development in strawberries and raspberries compared to the nowadays macro-perforated packages (Jacxsens *et al*., 2003). The same study also showed that the closed packaging systems had a profound inhibitory effect on the development of yeasts compared to the macro-perforated packaging. Since improved packaging material for quality control of table grapes is a science on its own, the latest developments in this regard should be considered in studies related to post-harvest pathogen control. Understanding increases or decreases of antagonistic yeast populations in new packaging material is important.

Results of this study show that the action of CA mixtures of O_2 and CO_2 maintained commercially important low levels (less than 1%) of *B. cinerea* decay during cold storage at -0.5°C. However, during the shelf-life storage period at 15°C these low levels of decay could only be maintained by additional SO_2 treatments. A necessary commercial requirement is to maintain low decay levels for longer at higher temperatures, for which this study cannot conclusively recommend CA and/or the application of antagonistic yeast as an alternative to SO_2. In this study, pre-harvest applications of yeasts and CA did however have an impact on confining the general deterioration of the grapes at -0.5°C and 15°C compared to SO_2. Selection of yeasts that survive and multiply under CA and low-temperature storage conditions would make them suitable candidates for the continued inhibition of pathogens on fruit surfaces. Control strategies against *B. cinerea* infections that occur between the flowering and bunch-closing growth stages (Holz, 2001) should also be taken into consideration for an effective post-harvest control strategy.

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