The Effect of Ethanol Dip and Modified Atmosphere on Prevention of Botrytis Rot of Table Grapes

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SUMMARY. Grape (Vitis vinifera) storage requires stringent control of gray mold caused by Botrytis cinerea. The commercial practice is dependent on sulfur dioxide (SO2) as a fumigant, which is applied by various means with well-known advantages and disadvantages. Many alternative technologies were developed over the years, most of them with limited efficacy or applicability. Modified atmosphere of table grapes suffers from a narrow threshold between control of gray mold and damage to the berries and stems due to high level of carbon dioxide (CO2) within the film-enclosed package. We demonstrated in the past that dipping table grapes in ethanol after harvest has a very pronounced effect on prevention of decay. However, ethanol does not leave a protective residue within the grapes, so it is not expected to prevent latent infections from developing decay nests during prolonged storage. However, if grapes of cultivar Superior were treated with ethanol and then subjected to...
a modified atmosphere using plastic films (Xtend), we achieved an additive effect and observed persistent control of gray mold without injury to the grapes. The advantage of this plastic film was mainly in its water conductance, which prevented accumulation of free water that is often the limiting factor in modified atmosphere packaging. This combination results in greater decay control, which is a prerequisite for commercial applicability. If undesired aftertaste did develop within the fruit due to the modified atmosphere, 1 day of exposure to ambient air was sufficient to dissipate it.

**Materials and methods**

'Superior' table grapes were harvested at commercial maturity from five vineyards over the course of two seasons (2002 and 2003). The bunches were sampled for maturity consisting of berry size, total soluble solids, and acidity (expressed as percent tartaric acid). The bunches were sorted and small and decaying berries removed. The bunches were placed in plastic boxes with a ventilated grid in the bottom and walls to an exact amount of 5 kg (11.0 lb). Ethanol dipping of sorted bunches was done by dipping the boxes in 25 L (6.6 gal) of 33% or 50% ethanol for 5 to 10 s. The boxes were dried in a wind tunnel of 3.5 m (11.48 ft) equipped with three vertical fans, each 50.8 cm (20 inches) in diameter, operating at 29 kW·h–1 and with a speed of 1.5 m·s−1 (4.92 ft/s). The box took 2 min to traverse the wind tunnel. If bunches remained wet after this process, they were further passively dried for up to 1 h at 20 °C (68.0 °F) and ambient relative humidity. Following this procedure, the bunches were packaged in liners by placing individual bunches in the modified atmosphere (MA) liner. The MA liners were sealed with a rubber band 24 h after the beginning of cold storage at 0 °C (32.0 °F).

Xtend bags (Stepac Ltd.) were compared to two commercial methods of storing grapes. The first was a polyethylene film of 40-µm [0.0016 inch (0.8 mil)] thickness with 6-mm (¼ inch) perforations covering 0.2% of the surface, which is the type of liner used in Chile (Zoffoli, 2002). The other storage method simulates the common practice in Israel in which the entire pallet is wrapped from the outside with stretch polyethylene of 20 µm [0.0008 inch (0.8 mil)] in thickness. In the experimental setup, three plastic boxes containing the untreated control or the SO2-generating pads (Osku, Chile) were wrapped with the polyethylene stretch film after cooling the grapes to 0 °C.

The Xtend films were of two types, low water conductance (designated L, with internal humidity of 93%) and high water conductance (designated H, with internal humidity of 90%). Variation among relative humidity (RH) of the bags has been tested previously and found to be ±1% (Aharoni and Richardson, 1997). Both films were tested with microperforations (designated MP) or without microperforations (designated NP).

Fruit examination was conducted by visual inspection after refrigerated storage [0 ± 0.5 °C (32.0 ± 0.9 °F), 90% RH] without touching the bunches, followed by a comprehensive examination after 3 d at 20 °C (70% to 80% RH). At removal from storage the bags were opened and the grapes were held in the open bags. Decay was scored by sorting and counting the decayed berries in the entire replicate (5 kg of fruit) according to causal organism, and data are presented as percent decayed berries. Bunch freshness was scored according to an index of 1 (highest grade, as at harvest) to 5 (wilting and aged bunches). The degree of stem and pedicel desiccation for each bunch was graded from 1 (no desiccation, green stems as at harvest) to 5 (dry, stems completely brown and desiccated). Firmness was measured subjectively on 20 berries per replicate by finger testing on a scale of 1 (firm) to 5 (very soft). Taste analysis of three samples was conducted by a panel of 16 individuals who evaluated taste according to a hedonic scale of 1 to 10 (Poste et al., 1991). The grapes were ranked using an index of 1 to 10 for sweetness, sourness, and aftertaste. When a large number of samples were analyzed repeatedly at different time points after opening the bags, a group of five to six experts trained panelists determined the taste.

Statistical analysis for decayed berries was performed by transformation of the ratio of decayed to healthy berries using the formula arcsin (p
Fig. 1. Levels of carbon dioxide (CO$_2$) and oxygen (O$_2$) are shown that accumulate inside three types of bags containing grapes [polyethylene (PE), non-perforated low water conductance (NP-L), and microperforated low water conductance (MP-L)] during storage at 0 °C (32.0 °F). Each type of bag had four replicates of bags containing 5 kg (11.0 lb) of grapes. Standard deviations are indicated.

Results

1. Xtend film compared to polyethylene film. During the first season, polyethylene film was compared to Xtend film. Polyethylene film with perforations on 0.2% of its surface (0.2PE) is the bag used to store grapes in Chile (Zoffoli, 2002). This bag was compared with an Xtend bag with microperforations, MP-L. In addition both films without perforation were examined (PE and NP-L). Bags of 0.2PE did not develop a modified atmosphere (data not shown), while PE without perforations had stable levels of CO$_2$ about 3% and O$_2$ levels were 12.8% after 10 d and 16.4% after 32 d (Fig. 1). MP-L bags had CO$_2$ levels of 6.7% after 10 d that increased to 10.2% after 30 d, while O$_2$ was about 10% after 10 d and decreased to 7.8% after 30 d. However, the NP-L showed a steady increase in CO$_2$, 6.6% after 11 d and reaching 18.4% after 32 d, and a decrease in O$_2$ from 8.1% after 11 d down to 4.3% after 32 d.

There was very little presence of either ethanol or acetaldehyde in the head space of the different bags (data not shown), except for NP-L. In this treatment, O$_2$ fell to 4% and CO$_2$ rose to 18%, ethanol and acetaldehyde were both 18 µL·L$^{-1}$ (ppm) after 40 d of 0 °C storage.

The two types of bags, with and without perforations, were stored either with a SO$_2$-releasing pad, or with grapes that had been dipped in 50% ethanol and then were dried before packing. With no additional protection against decay other than the modified atmosphere that developed inside the closed bags, there were high levels of decay after shelf life amongst the grapes from all treatments. The exact amount varied from experiment to experiment (Fig. 2A–B). In the first experiment, 0.2PE grapes had close to 100% decay,

Fig. 2. The amount of decay is shown that developed after storage for 40 d in Expt. 1 (A) or 47 d in Expt. 2 (B) at 0 °C (32.0 °F) plus 3 d at 20 °C (68.0 °F). Treatments were: polyethylene with 0.2% perforation (PE0.2), with sulfur-dioxide-releasing pad (SO$_2$) or with a dip in 50% ethanol (EtOH); polyethylene without perforation (PE), with a dip in 50% EtOH; non-perforated low water conducting Xtend bags (NP-L), with a dip in 50% EtOH. Each treatment had four replicates. Different letters above columns denote significant differences at a 5% level.
while in the second experiment grapes from this treat-
ment had 30% decay. MP-L (Fig. 2A) and NP-L (Fig. 2B) treatments had lower amounts of decay than PE; with 75% decay in Expt. 1 and 12% decay in Expt. 2. However, these levels of decay are unacceptable for commercial purposes. Grapes with a prestorage etha-
nol dip and sealed in bags had greatly reduced decay levels, ranging from 3 to 10 times less than the levels developing on grapes in bags with no protection. In the Xtend bags decay levels of the ethanol treatment were comparable to those on grapes in bags with an SO$_2$-generating pad in which SO$_2$ was expected to be extremely high. However, in the PE bags, with (PE0.2) or without (PE) perforations, ethanol did not have the impressive effect on preventing decay that SO$_2$ pads did. Ethanol reduced decay levels 3-fold in each case (from 100% to 34% in Expt. 1 and from 29% or 34% to 8% to 10% in Expt. 2), while SO$_2$ pads almost totally prevented decay.

As expected, the SO$_2$-generating pad in the sealed liner, while preventing decay development, had detri-
mental effects on fruit appearance. The grapes from this treatment developed both SO$_2$ injury and appreciable levels of berry browning, even in bags with perfora-
tions (data not shown). The level of injury was higher in MP-L than in 0.2PE film, because the ventilation in the microperforated bags was minimal and higher levels of SO$_2$ developed. Therefore, although SO$_2$ was more effective in controlling decay in PE bags than ethanol, it had undesirable side effects on grape quality when sealed in the bag. In Xtend film the control of decay by ethanol was similar to that of SO$_2$ without any berry injury (data not shown).

2. COMPARISON OF XTEND BAGS. Two types of Xtend films, low and high water conductance, were compared, with microperforations (MP-L and MP-
H) or without microperforations (NP-L and NP-H). The low water conductance film is more transparent than the high water conductance film. Both films had similar impermeability to CO$_2$ and O$_2$ when they were without microperforations, and reached levels around 10% O$_2$ and CO$_2$ when microperforated (Fig. 3A–B). However, they had different permeabilities to water vapor (Aharoni and Richardson, 1997). Unperforated bags accumulated CO$_2$ steadily over the storage period, reaching levels above 20% after 40 d at 0 °C. The O$_2$ levels dropped rapidly to below 5% during the first 2 weeks of storage and then stabilized at between 3% to 5% O$_2$. Perforated bags increased in CO$_2$ levels steadily during storage, but reached maximum levels of only 10%, while the O$_2$ levels fluctuated around 10% also. Ethanol levels in the unperforated bags accumulated to much higher levels than in the experiments comparing PE to Xtend, with levels reaching 300 µL·L$^{-1}$ by 30 d of storage (Fig. 3C) when the CO$_2$ levels were above 15% (Fig. 3B). Acetaldehyde also accumulated in the NP-H and NP-L bags, but its rise was later than that of ethanol (Fig. 3D).

Decay after storage and 3 d at 20 °C was high in control grapes that were neither sealed, nor stored with a SO$_2$-generating pad, 28% in Expt. A (Fig. 4A) and 20% in Expt. B (Fig. 4B). There were no healthy bunches in Expt. A while there were 5% healthy bunches

![Fig. 3. Levels of (A) carbon dioxide (CO$_2$), (B) oxygen (O$_2$), (C) ethanol, and (D) acetaldehyde are shown that develop in Xtend bags containing grapes during 0 °C (32.0 °F) storage. Four different bags were tested: non-perforated low water conductance (NP-L), microperforated low water conductance (MP-L), non-perforated high water conductance (NP-H), microperforated high water conductance (MP-H). Each treatment was composed of three replicates. Standard deviations are indicated.](image-url)
in control fruit in Expt. B (Fig. 4C). Storing these grapes in Expt. A with an SO₂ pad, which is the normal method of storage, decreased decay 10-fold, as did a dip in ethanol before storage (Fig. 4A–B). Storing the grapes in microperforated bags (MP-L and MP-H) was as effective in preventing decay as SO₂ or ethanol, while grapes stored in unperforated bags (NP-L) had higher levels of decay. A dip in 33% ethanol followed by packaging in MP-H bags decreased these decay levels to below 2%. It appeared that in this experiment ethanol and the modified atmosphere in the Xtend films acted synergistically on decay. In Expt. B the combination of ethanol and modified atmosphere gave between 50% and 80% healthy bunches compared to 5% in control fruit and 20% in SO₂ treated fruit (Fig. 4C).

When the levels of CO₂ accumulating in MP-H and MP-L films were compared in two experiments, it was clear that various factors precluded the establishment of a very accurate level of CO₂. As can be observed in Fig. 5, in one experiment grapes in both bags were exposed to approximately 9% CO₂ after 40 d in storage, while in a second experiment the average CO₂ level inside the bags exceeded 11%. The reasons for these differences in CO₂ concentration are not known but may include the physiological age of the grapes as well as the head space left inside the package. In addition, the variability among bags of the same type increased after 30 d of storage, as can be seen by comparing the standard deviations at the early and later times of storage in Fig. 5.

The quality of grapes was evaluated immediately after refrigeration and examined in detail 3 d later (Table 1). Control and SO₂ treated grapes were softer than the fruit stored in Xtend bags. In the first evaluation after refrigeration, there was no difference in the freshness between the treatments (data not shown). After 3 d at 20 °C there were no consistent and significant differences in the freshness parameter among the different treatments. However, grapes stored in NP-H and NP-L liners had reduced freshness and greater rachis desiccation than grapes stored in MP-H or MP-L liners, although these differences were not always significant statistically. Rachis desiccation scores reflect the difference between perforated and unperforated films being significantly worse in the latter case.

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**Figure 4.** Percentage of decay is shown from two experiments (A and B) and percentage of healthy bunches (C) in Expt. B. In Expt. A the control had 28.3% decay (the number is written above the bar graph). Four different bags were tested: non-perforated low water conductance (NP-L), microperforated low water conductance (MP-L), non-perforated high water conductance (NP-H), microperforated high water conductance (MP-H). In addition, the grapes were either dipped in 33% ethanol (EtOH) or stored in the presence of a sulfur-dioxide-releasing pad (SO₂). Each treatment was composed of three replicates. Different letters above columns denote significant differences at a 5% level.
Table 1. Quality of grapes after 7 weeks of 0 °C (32.0 °F) storage plus 3 d at 20 °C (68.0 °F) is shown. Quality parameters are rated as an index of 1 to 5, where 1 is quality at harvest and 5 is very poor quality. Generally values up to 3 are considered marketable when considering grapes after shelf life. The values are the average of three experiments with each treatment represented in four replicates. Treatments are: untreated (control), with a sulfur dioxide–generating pad (SO2), with a dip in 33% ethanol (Ethanol), in non-perforated low or high humidity conducting bags (NP-L and NP-H, respectively), or in microperforated low or high humidity conducting bags (MP-L and MP-H, respectively).

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<th>SNK</th>
<th>Mean ± SE</th>
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These results may reflect, in part, CO2 induced browning of the rachis.

3. Organoleptic quality of grapes stored in XTEND films. During the second season taste tests were made on grapes after storage. Taste panels sampled treatments 3, 24, and 72 h after cold storage, respectively (Table 2). Aftertaste was the parameter that changed significantly in correlation to final CO2 levels. Three h after opening the bags control and ethanol treated grapes had a value of 1 (no aftertaste) and SO2 or grapes in final concentration of 7.3–7.8% CO2 obtained a value of 1.8–2. A final concentration of 10.6% CO2 gave an aftertaste score of 4 and 21.7% CO2, a value of 6. After 24 h at 20 °C, the score of SO2 treated grapes was 2.8 whereas grapes exposed to 7% to 9% CO2 obtained a score of 1–1.5. Grapes exposed to 10.6% CO2 had reduced aftertaste score of 2.8, down from a score of 4 reported initially, and those exposed to 21% CO2 remained with a score of 6. The aftertaste scores after 24 and 72 h at 20 °C are also described in Fig. 6; according to these results a final concentration of 13% but not 16% CO2 reached a reasonable low aftertaste score pending 24 h ventilation.

In the second experiment 16 individuals ranked the grapes (Table 3). Sweetness and sourness did not differ substantially among the treatments and were in normal values. General taste was significantly higher for the untreated grapes at the day of opening of the liners but 24 h later there was no difference in this parameter. Aftertaste was 2.5, which is a relatively high score for untreated control in the first analysis and it was around 4 for grapes held in liners. However, 24 h later it was around 1.6 for both the control and the treatments which were exposed to higher CO2 levels. Because more than half of the panelists did not detect aftertaste in this treatment, it is probably insignificant and results from another factor than the CO2 level (e.g., being the first sample in the taste analysis).

Discussion

The results of the trials conducted in this study show that storing grapes in sealed bags combined with a prestorage dip in either 33% or 50% ethanol, maintained grape quality for up to 7 weeks with 3-d shelf life at 20 °C as well or better than the commercial practice of storing them with SO2-releasing pads. However, the type of film used is crucial, since modified atmosphere close to 10% CO2 will have fungicidal
Regarding the role of CO₂ in delaying decay development on grapes in storage, little has been published on modified atmosphere, but some work has been done on controlled atmosphere. The influence of controlled atmosphere conditions with an emphasis on decay by *B. cinerea* has been evaluated for ‘Emperor’ grapes (Uota, 1957), ‘Alphonse Lavallee’, and ‘Rakazi’ grapes (Eris et al., 1993), ‘Thompson Seedless’ and ‘Red Globe’ grapes (Retamales et al., 2003). The controlled atmosphere of above 10% helped to prevent decay, but formation of off-flavors and berry browning was reported (Uota, 1957; Nelson, 1969; Retamales et al. 2003). In early harvested ‘Thompson Seedless’ grapes, Nelson (1969) found that berry internal browning overshadowed the potential benefits of controlled atmosphere in reducing decay. A more recent study of ‘Thompson Seedless’ found that the damage level for the grapes was above 15%, and that this concentration of CO₂ with reduced oxygen could control decay for up to 12 weeks (Berry and Aked, 1997). In ‘Red Globe’ grapes atmospheres of 10% CO₂ or higher controlled decay incidence and spread among berries (nesting) independent of O₂ concentrations during storage at 1 °C (Crisosto et al., 2002). Rachis browning and off flavor were found in grapes stored above 10% CO₂. Artes-Hernandez et al. (2004) also found that 10% CO₂ and 15% O₂ were the best atmospheres for maintaining grape fruit quality in ‘Autumn Seedless’ grapes. This atmosphere was able to avoid browning of the rachis and increased softness of berries, and maintain visual quality, flavor and eating texture.

The variability in CO₂ level between the packaging units can become a significant problem after 1 month of storage (Fig. 5). This may result from minute holes in the film, from non-even scaling of the film or from biotic factors. These can be the developmental stage of the grapes, the effect of preharvest conditions and treatments, or from slow development of decay leading to increased rate of CO₂ evolution and O₂ consumption. Technological improvements in the level of the film can probably solve part of the problem and further studies are required in the biotic level.

The efficacy of ethanol in preventing postharvest decay of table grapes was demonstrated in a number of cultivars (Lichter et al., 2002). The lowest concentration of ethanol which was found effective as a dip treatment after harvest was 30% and it maintained the fruit free of decay during storage for 6 to 8 weeks. This concentration reflects the upper threshold required to kill *B. cinerea* spores with the lower threshold around 25% (Lichter et al., 2003). Interestingly, attempts to apply 25% ethanol dips followed by MP packaging failed to yield a favorable result in one experiment on ‘Thompson Seedless’ (not shown). The loss of ethanol effectiveness after prolonged storage was probably because the decay that developed was the result of latent or secondary infections, rather than of surface wound infection. Also ethanol may remove microorganisms antagonistic to fungal pathogens, leading to a biological vacuum and possible decay development.
Physiological effects of ethanol in the postharvest area are varied. Exogenous application of ethanol can enhance acetaldehyde evolution which can be injurious to plant tissue (Perata and Alpi, 1991) but which can also act directly against decay agents (Avissar and Pesis, 1991). Exogenous ethanol application has also been found to improve taste of various fruits including grapes (Pesis and Marinansky, 1992, Lichter et al., 2002).

In conclusion, Xtend films were able to partially prevent decay from developing in ‘Superior’ grapes during storage for 7 weeks with shelf life of 3 d at 20 ºC. Adding a prestorage dip of 33 or 50% ethanol improved the effect of each treatment alone, and gave decay control levels as good as or better than storage of grapes with SO2-generating pads. The generation of CO2 inside the bags of up to 10% by SO2-generating pads. The generation of CO2, inside the bags of up to 10% by using Xtend bags with microperforation only minimally affected taste, and any off-flavors disappeared after 24 h at 20 ºC. Non-perforated bags had too high levels of CO2, and although decay was prevented, there were detrimental effects to grape quality and taste. Xtend film was found to be superior to PE bags when combined with an ethanol dip, perhaps due to the impermeability to water vapor of the PE bags. The Xtend high water conductance film gave higher quality fruit than the low water conductance film. Obviously, these results require optimization in order to reduce variability for as long as possible. The technology, however, seems to be a promising alternative to the current practice for storage of table grapes.

Literature cited