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Control of spoilage in table grapes

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Abstract
Purpose of the review: Diverse means to control decay and spoilage of table grapes during storage have been described in numerous research papers that were published recently. The purpose of this review is to describe some of the major publications in this field and to point out on their advantages and shortcoming.
Main findings: Studies pertaining to the control of postharvest decay of table grapes and prevention of quality losses were categorised according to the general approach used (dry, wet, physical). Of the "dry" treatments, active modified atmosphere packaging (MAP), which includes volatiles for continuous control of decay, seems to be an attractive approach. Of the methods that can be directly integrated into current commercial practices, biofumigation with the fungus *Muscodor albus* warrants further assessment. Wet treatments have some inherent disadvantages because they require additional handling of the grapes. However, postharvest treatments with disinfectants such as ethanol can effectively control decay during cold storage of medium duration. These wet treatments can be useful to remove visible deposits of soil and pesticides, to prolong the storage life of organic grapes, and to disinfect and retard the decay of "ready-to-eat" grape products. Advantages of ethanol are that its efficacy can be enhanced by heat, sorbates, or MAP, and that it dries faster than other wet treatments.
Directions for future research: There are specific issues to be addressed for every technology to be considered. However, many of the studies published on the control of spoilage of table grapes report results using specific cultivars, storage of limited duration, or small scale experiments. Any technology further to be considered must be confirmed in large-scale, semi-commercial experiments to reveal potential pitfalls and limitations and to evaluate all aspects of fruit quality. In addition, a cost analysis of each technology is essential.

Keywords: postharvest; table grapes; *Botrytis cienera*; cold storage

Abbreviations

CA	Controlled Atmosphere
GRAS	Generally Recognised as Safe
MAP	Modified Atmosphere Packaging
PP	Polypropylene
SO_2	Sulphur Dioxide
UV-C	Ultraviolet-C

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Introduction
Gray mould, caused by the fungus *Botrytis cinerea* Pers., is the most economically important postharvest disease of table grapes [1]. *B. cinerea* is especially troublesome because of its vigorous growth rate and ability to spread among berries even at low temperatures (–0.5°C). Infections that cause postharvest losses can originate from spores on the surface of the berries, latent infections that occurred before harvest during the growing season, or visibly infected berries that escaped removal during packaging [2]. If grapes are not treated after harvest or during storage, infection can affect the majority of the berries almost simultaneously. However, when external spore infection is eliminated, latent infection from single berries can lead to production of abundant white surface mycelia, which spread to adjacent berries creating, so called, nests of infection. The sporulating nest, which

may practically be single berries, can contaminate and infect an entire package of grapes. Other pathogens, such as *Rhizopus stolonifer* or *Aspergillus* spp., can also cause losses when temperature management is poor or during retail display of the fruit. Decay caused by *Penicillium expansum* or *Alternaria* spp. can become a problem, particularly during prolonged cold storage [1, 3, 4]. The purpose of the current review is to provide a wide and critical perspective of the technologies published in the recent years for prevention of spoilage of table grapes. Biological control was not addressed due to space limitations but it was recently reviewed elsewhere [5].

Current practices

When grapes are exported overseas, they are typically packaged with sulphur dioxide (SO_2) generator sheets followed by forced-air cooling immediately after harvest and cold storage at –0.5°C. The sheets, which contain sodium metabisulphite, are placed within grape packages. Later, when hydrated by water vapour, they continuously emit a low concentration of SO_2 during storage [5, 6]. Most sheets release SO_2 at a single, slow rate, while others, termed dual release, initially release a much higher concentration of gas. Generator sheets typically protect the grapes for up to 2 months from decay. Recently, to simplify packaging and to avoid disposal issues associated with generator sheets, SO_2 has also been incorporated into plastic cluster bags or box liners.

During the static cold storage of grapes practiced in California, table grapes are subjected to an initial fumigation with SO_2 during forced-air cooling [7, 8]. Initial fumigation is followed 1 week later by a 2 to 6 h-long fumigation, which is repeated weekly during cold storage. Under these conditions, the grapes can be protected from decay for up to 4 months. Fumigation can also be applied within mobile units at the time of shipment. Several variations of these technologies exist which are not described here.

Alternatives to SO_2

Alternatives to SO_2 are of interest for several reasons. Ingestion of SO_2 residues (sulphites) can cause hypersensitive reactions in some people, which caused it to be removed from the United States Food and Drug Administration "generally recognised as safe" (GRAS) list and to be reclassified as a pesticide with a 10 ppm residue tolerance by the United States Environmental Protection Agency [9]. While the tolerance is low and rarely exceeded in commercial practice [10], excessive residues of SO_2 can occur when it accumulates in wounded or detached berries [11]. Elevated levels of residues can also be expected if the cold-chain is broken and the SO_2 generator sheets become very wet. SO_2 can cause unacceptable bleaching injuries to berries [12] and berry taste may be compromised as well [13]. In the USA, its use is banned from certified "organic" grapes, some regulatory agencies do not allow the discharge of SO_2 to the air after fumigation, and workers cannot be exposed to the gas at a level above 2 ppm.

Dry treatments

Negative impacts on berry appearance and quality from handling are negligible when gaseous treatments are employed after harvest, because handling of fruit to apply gases is minimal. In contrast, the adverse impacts of additional handling and drying of the fruit cannot be avoided when wet treatments are applied.

Controlled atmosphere

Controlled atmosphere (CA) storage is a well-established technology that is used to preserve the quality of fruit by active manipulation of atmosphere composition in the storage rooms, transport containers or retail food packages. The advantage of CA is that any desired combination of O_2 and CO_2 can be used to protect the product from physiological or pathological disorders [14].

In the grape–*Botrytis* interaction, conventional CA is based on the inhibitory effect of CO_2 on germination of *B. cinerea* [15], where the inhibitory concentration of CO_2 was reported to be 15% or higher [16]. This threshold was corroborated by Retamales *et al.*, [17], but browning of the stems and pedicels (rachis) of 'Thompson Seedless' and 'Red-Globe' grapes was noticed. CO_2-induced rachis browning was previously described by Yahia *et al.* [18] who reported that 10% carbon monoxide and 2% O_2 were effective in preventing both decay and browning for 4 months of cold storage.

Flavour is another major issue when using CA. According to Artes-Hernandez [19], flavour and appearance of 'Autumn Seedless' grapes were compromised after 60 days of cold storage and 7 days at 20°C at 15% CO_2 and 5% O_2. The response of grapes to CO_2 can be influenced by their maturity, with late-season 'Red-Globe' or 'Thompson Seedless' grapes being less sensitive to CO_2 damage [20, 21]. It was therefore not recommended to store 'Thompson Seedless' grapes in CA due to "off-flavour", rachis and berry browning and to store late-harvested grapes at 15% CO_2. Late-harvested 'Red-Globe' grapes could be stored under 10% CO_2 for 12 weeks but for no more than 4 weeks for early harvested grapes. It should be noted that these experiments were carried out after initial SO_2 fumigation during precooling to eradicate the external *Botrytis* contamination on the berries. High O_2 is another approach to CA which was reported to preserve the quality of 'Kyoho' grapes [22]. However, in this study, high O_2 did not prevent decay and unpublished results suggest that *Botrytis* development *in vitr*o was not effected by 75% O_2. CA facilities have been available for many years, mainly in industrialised countries which store pome fruits for local consumption. However, currently grapes are not stored commercially in CA facilities.

Modified atmosphere packaging

In contrast to CA, modified atmosphere packaging (MAP) is a mobile and relatively simple technology of packaging in appropriate liners. In its simple form, MAP depends on CO_2 evolution by the grapes to prevent decay and some of the past

studies on this topic were summarised by Artes-Hernandez *et al.* [23]. In subsequent studies [19], they showed that 'Autumn Seedless' grapes could retain acceptable quality in MAP for 60 days, where a level of 10% CO_2 and 15% O_2 was established within 1 month of storage in solid polypropylene (PP) containers sealed with 35 µm PP. The level of decay was significantly reduced from 8.6% to 2.6%, but this method needs further evaluation with higher decay pressure. In an additional report, 'Superior Seedless' grapes were stored for a short duration of 7 days in cold storage, 4 days at 8°C, and 2 days at 20°C ("shelf-life") in oriented PP or micro-perforated PP. In the later packaging, CO_2 accumulated sharply to up to 21% during the shelf-life [24]. These treatments prevented weight loss of the clusters and preserved the visual, sensory, and chemical quality of the grapes. However, the short duration of storage employed was to brief for the development of *B. cinerea* and did not permit evaluation of gray mould control by these treatments. One of the major issues with MAP is accumulation of condensation and respiratory water within the liner during storage, which in turn can increase decay caused by *B. cinerea*. This problem can partially be alleviated by the use of anti-fog coating, paper pads to adsorb humidity or by liners with high permeability to water. The latter approach was attempted by Lichter *et al.* [25] who found that MAP in itself reduced decay significantly but not sufficiently under high decay pressure during the cold storage of 'Superior' grapes for 45 days followed by 3 days at 20°C. However, when the grapes were additionally dipped in ethanol after harvest (elaborated on later in this review), the control of decay was much more effective than by either technology alone. Quality was sufficiently preserved during these experiments when the level of CO_2 did not exceed 10%, and the "off-flavour" that developed under these conditions dissipated within 24 h after opening the liners. The rationale behind this combined technology is that the ethanol dip treatment prevents development of *B. cinerea* in the first phase of storage before sufficient CO_2 accumulation occurs. The conclusion from this and other studies is that MAP may be a viable alternative to SO_2 only if it is to be combined with another method of active protection against decay before or during storage.

Active MAP

Active MAP can be regarded as any modification of atmospheric gas composition by packaging supplemented by compounds that may enhance its effect. According to this definition, SO_2 cannot be included in this category because SO_2 is normally added to ventilated packages. One of the first studies towards this approach was the use of (E)-2-hexenal to fumigate 'Crimson Seedless' grapes [26], which showed reduced decay under experimental conditions. Further improvements in active MAP for grapes may be achieved by using natural essential oils, as was convincingly shown recently by Valverde *et al.* [27]. Adding 0.5 mL of thymol, menthol, or especially eugenol into oriented PP liners containing 160 g cluster fragments of 'Crimson Seedless' grapes retained quality and controlled decay during cold storage. The characteristic aroma of the oils dissipated shortly after opening the sealed liners but an off-flavour in the berries was detected. Further studies by this group on 'Autumn Royal' grapes with reduced doses of the oils showed preservation of many important quality and nutritive parameters of grapes (eg, thymol prevented decrease tartaric acid during storage), as well as very effective control of decay during 1 month of storage under high decay pressure [28]. Off-flavour was not perceived as a problem although sweetness was higher in the control.

Ethanol vapour

The feasibility of using ethanol vapour for the storage of grapes was demonstrated on 'Chasselas' grapes stored in PP liners with ethanol delivered from pre-soaked paper sheets. The optimal level of ethanol was 2 mL/kg; at higher doses, rachis browning occurred. The level of CO_2 within the packages was not reported but SO_2-treated grapes were significantly less acceptable by the consumers than the controls when assessors tasted the grape samples [13]. The viability of this approach was confirmed on ethanol-dipped grapes stored in water permeable liners using two ethanol delivery methods [29]. However, rachis and berry browning occurred on 'Thompson Seedless' grapes using the ethanol-impregnated papers but not when ethanol was applied from a paper wick dipped in ethanol. The efficacy of ethanol as a volatile may be associated with its conversion by the berry alcohol dehydrogenase to acetaldehyde, which is very toxic to microorganisms. Fumigation with acetaldehyde at 0.3 or 0.5% for 24 to 40 h at 20 or 0°C reduced decay on grapes caused by *Botrytis cinerea, Rhizopus stolonifer, Aspergillus niger* and *Alternaria alternata* [30]. Due to its cross-linking capacity, higher concentrations of the volatile may promote polymerisation of phenolic compounds and browning.

One of the major problems with MAP for grapes, which are usually ignored in research papers, is the greater expense and time of pre-cooling required for non-ventilated packages. In addition, respiration and condensation water that may accumulate in the liners require the usage of films with high permeability to water, or anti-fog coating and adsorbent paper pads, or closing the liners after pre-cooling.

Chlorine fumigation

Chlorine gas produced by a salt mixture and combined with 25 days of storage at 0°C significantly reduced gray mould among artificially inoculated table grapes cvs. 'Flame Seedless', 'Thompson Seedless', and 'Ribier' [31]. Infections by conidia or mycelium of *B. cinerea* were suppressed for up to 45 days in cold storage, providing a similar degree of protection to that of one SO_2-generator pad per box. No deleterious effects due to chlorine gas generation were reported. However, there is some concern, unrelated to grapes in particular, from generation of mutagenic trihalomethanes by interaction of chlorine with humic and fulvic acids [32]. As a result, the use of chlorination for produce disinfection has been banned by some countries other than the United States, and this may restrict the export of chlorinated produce.

Acetic acid
Decay of table grapes caused by *Botrytis cinerea* and *Penicillium* spp. was reduced when grapes were periodically fumigated for 30 min with 0.27% (vol/vol) acetic acid during 6 week's storage at 2 or 5°C [33]. No significant difference was detected between SO_2 and acetic acid fumigation in cluster or berry weight and fruit soluble solids, titratable acidity, pH and colour, as well as and the degree of rachis drying. Brief fumigation with acetic acid at 8 mg/L followed by MAP for 74 days at 0°C reduced the percentage of decayed grapes from 94% to 2% [34].

Ozone gas
Ozone is a natural substance in the atmosphere and one of the most potent sanitisers known against a wide spectrum of micro-organisms at relatively low concentration [35]. It can be applied both in the gaseous or aqueous states and ozone or its decomposition products (eg, hydroxyl radical) react with various biological molecules. For commercial use, ozone must be produced on site and it is declared to be GRAS for food contact applications in the USA [36, 37]).

Shimizu *et al.* [38] reported that overnight fumigation with 500 ppm ozone controlled *B. cinerea,* did not control *Alternaria* spp. decay and caused some injuries to 'Kyoho' grapes. A single application of 0.1 mg per g of grapes for 20 to 80 min prevented decay by *Rhizopus stolonifer* and reduced berry microflora [39]. Continuous fumigation with a low dose of ozone (0.1–0.3 ppm) during storage prevented gray mould nesting in 'Thompson Seedless' grapes stored for 7 weeks at 5°C. but did not decrease the incidence of decay after spray-inoculation with *B. cinerea* [40]. Application of ozone at 0.1 µL/L in MAP was not effective in prevention of decay [19]. Evaluation of high-dose, one-time fumigations of grapes with 5,000 or more ppm/h ozone, applied during initial pre-cooling, is in progress in California for the control of postharvest gray mould of table grapes with irregular results (Mlikota Gabler and Smilanick, unpublished). Grey mould was reduced by 30 to 80%, however, decay caused by *Alternaria* and *Penicillium* spp. was poorly controlled and minor rachis injury occurred. The treatments did not injure berries, but in some tests rachis browning was noticed. Ozone also has a significant physiological effect on grapes as it was shown to increase berry stilbene content [39, 41].

Although there is no doubt about the potential of ozone as a fumigant, it seems that there is a relatively narrow window between the lethal threshold to the pathogens and its detrimental effect on the berry. In addition, the corrosive nature of ozone requires suitable technological adaptation in the structure of the storage facilities.

***Muscodor albus* volatiles**
A novel alternative for controlling postharvest decay is biological fumigation, or biofumigation, with the fungus *Muscodor albus*. The volatiles produced by *M. albus* are a mixture of low molecular weight compounds that are biocidal or biostatic to a broad variety of micro-organisms including *Botrytis cinerea* [42]. The *M. albus* formulation consists of rye grain colonised by the fungus, which is activated by water. The fungus creates a "musky" odour that declines rapidly after its removal from the package. *M. albus* was effective in controlling gray mould in many types of grape packages and storage conditions [43]. For example, gray mould incidence was reduced from 20.2% among untreated grapes to less than 1%, when ≥5 g of the formulation per kilogram of grapes was added to inoculated clusters inside clamshell boxes and incubated for 7 days at 15ºC. In another experiment, *M. albus* was applied to ventilated polyethylene cluster bags stored for 28 days at 0.5ºC. Gray mould incidence was 42.8% among untreated fruit and 4.8% in fruit treated with 5 g/kg [43].

An advantage of biofumigation is that the biocontrol agent is never in direct contact with the commodity avoiding microbe residue on fruit. The treatment could be applied passively by simply placing active *M. albus* formulations within packages of grapes as is now done with SO_2 generator pads. The level of biofumigation is directly affected by the storage temperature and hence smaller doses may be required at higher storage temperatures. Biofumigation with *M. albus* should be further evaluated with cultivars other than 'Thompson Seedless' and for various aspects of fruit quality. An issue with *M. albus* use is that it is alive and its metabolism and effectiveness depend on the rye grain substrate on which it is grown. It was observed that different rye grain batches can affect its effectiveness.

Physical treatments

UV-C irradiation
Ultraviolet (UV)-C light doses from 0.125 to 0.5 kJ/m modestly reduced both the number of infected berries and the diameter of decay lesions on cv. 'Italia' grapes [44]. The UV-C treatment also enhanced berry resistance because the level of disease was lower among berries inoculated 1 day after treatment compared with fruit inoculated immediately after treatment. These results were similar for 'Autumn Black' and grapes of selection B36-55, artificially inoculated with *B. cinerea*. UV-C was also shown to induce synthesis of catechin and trans-resveratrol [45]. Although it doesn't seem that UV-C alone is sufficiently effective in prevention of decay, it is possible that it may enhance the effectiveness of other postharvest treatments.

Hypobaric treatments
Hypobaric or sub-atmospheric pressure treatment of grapes in 0.25 atm for 24 h reduced the infection index of gray mould by as much as 53% [46]. Shorter treatment duration was not effective, making this approach less feasible.

Thermal treatments
Heat is always an attractive tool for controlling postharvest diseases. The heat treatment required to kill conidia of *B.*

cinerea is approximately 30 s at 49°C [47]. However, effective control of decay of table grape berries with hot water requires temperatures higher than 50°C [48]. Under these conditions hot water treatment can injure the berries and prompt drying is crucial for preventing berry cracking.

Water vapour heat treatments can circumvent some of the problems associated with immersion in water. Vapour treatments for 12 to 32 min at 50 to 55°C, applied after inoculation, reduced *B. cinerea* infections compared with the controls [49]. Conversely, berries inoculated after treatments were more susceptible to gray mould than the controls. Treatments of up to 55°C for 27 min did not harm berry and bunch quality and reduced rachis browning, although their effect on berry bloom appearance was not reported [50]. The water vapour heat treatment is relatively easy to implement but drying, additional cooling costs, the prevention of recontamination of the grapes, and the risk of berry cracking in the wet environment remain issues that have to be addressed with this approach.

Wet treatments

The current practice of packing dry grapes with minimal handling makes implementation of wet treatments commercially less attractive. However, a high microbial load, the presence of dust and insect frass on the berries, and the demand for an alternative to SO_2, may make a wet process more acceptable in the future. Wet postharvest treatments may be implemented immediately for products such as "ready-to-eat" berries which are sold in transparent boxes that can be conveniently sized to suit a single consumer purchase. The handling to remove berries from clusters increases distribution of the inoculum of *B. cinerea* and other microbes, and the detachment of berries from pedicels creates large wounds. At the same time this additional handling offers an opportunity to apply wet postharvest treatments. SO_2 fumigation would be a less suitable treatment for packages containing single berries with their pedicels detached, because the wound created by pedicel removal provides an entry point for the accumulation of excessive SO_2 residues, probably above the tolerance of 10 mg/kg, and unsightly bleaching of the berries would occur [11].

Chlorine

Chlorine is a potent disinfectant with powerful oxidising properties that is commonly used to sanitise fresh fruits and vegetables. It is soluble in water, either by injection of chlorine gas or by addition of hypochlorite salts. Chlorine in aqueous solutions consists of a mixture of chlorine gas, hypochlorous acid, and hypochlorite ions in ratios controlled by pH. The concentrations generally used in postharvest applications are 50 to 200 µg/mL, expressed as free chlorine [51]. Concentration of free chlorine is reduced by presence of organic material in solution, so additional chlorine needs to be added to compensate for this loss. A brief spray application of 200 µg/mL chlorine reduced gray mould on grapes about 40–60% [52].

Ozone

Disinfectant properties of ozone in a gas phase were addressed earlier in this review. In water, ozone has limited solubility (29.9 µg/mL at 20°C) and in practice, it is difficult to exceed 10 µg/mL [53]. Ozone rapidly decomposes to oxygen leaving no traces and, in water, is a better disinfectant than chlorine against a wide variety of micro-organisms [54].

Shimizu and coworkers [38] reported that immersion of table grapes in ozonated water reduced postharvest decay, although long contact periods were needed and it was incapable of stopping infections in wounds. Milkota Gabler and Smilanick [52] reported that control of gray mould on inoculated grape berries by immersion in ozonated water was less effective, even after treatment for periods much longer and at concentrations much higher than those that controlled spore germination. Its efficacy was irregular and very dependent on grape condition. In addition, minor rachis injuries were observed after ozone treatment and consisted of thin longitudinal, parallel light brown lines. It was also recognised that micro-organisms embedded in the plant tissue survive the treatment better then than those readily exposed to ozone, allegedly due its low concentration within the tissue [55].

The disadvantage of using ozone in water is that it is extremely unstable and it is challenging to predict how it will react in the presence of organic matter. Therefore it can be difficult to maintain a concentration of ozone that would be reliably effective.

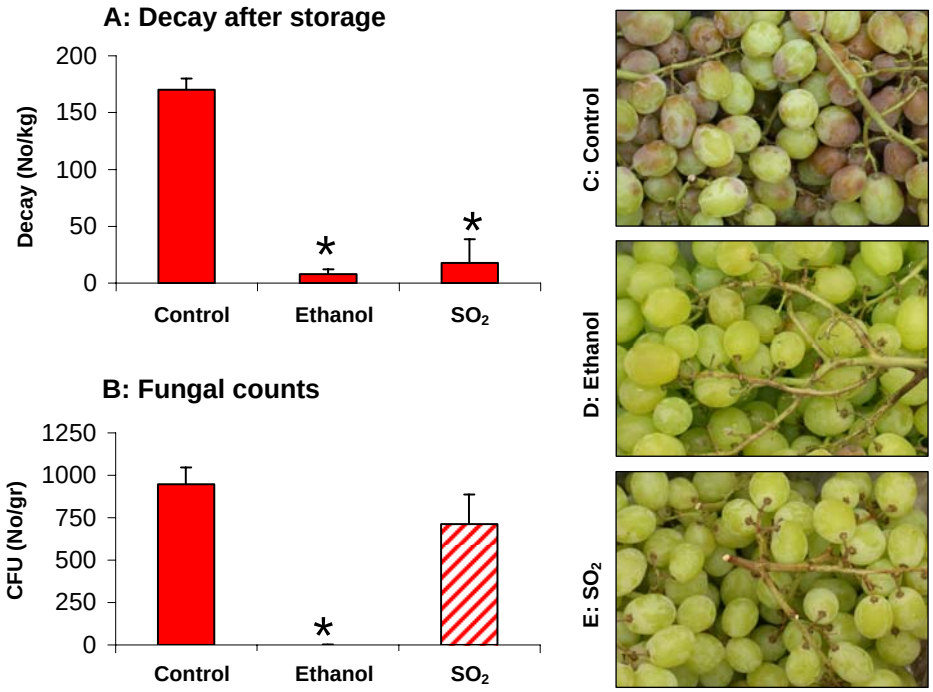
Bicarbonate and carbonate salts

Bicarbonate and carbonates are simple salts that are often used as food additives and were shown to effectively prevent the germination of *B. cinerea* [52]. Among solutions of bicarbonate salts, each applied at 500 mM, ammonium bicarbonate was significantly more effective in controlling gray mould on table grapes. Ammonium bicarbonate was also superior to potassium carbonate (100 mM) and chlorine (200 µg/mL) and equal in effectiveness to sodium carbonate (100 mM) and ethanol (70%, vol/vol). Carbonates, but not bicarbonates, were phytotoxic, probably due to their high pH, and caused immediate darkening of pedicels and dark brown spots on berries. Bicarbonates were unable to protect wounded and inoculated grape berries from decay. Such wounds on detached berries are prone to subsequent inoculation during handling. Bicarbonates have minimal environmental or worker safety issues associated with their use and they pose a minimal ingestion hazard because of their low toxicity to animals. They are inexpensive, do not injure berries, and their effectiveness against gray mould was reproducible.

Chitosan

Chitosan, is a natural polymer that produces a film on the surface of treated fruit. It inhibits the growth of decay-causing fungi [56, 57] and induces defence responses in several plant systems, mostly in dicotyledon plants [58]. Post-

Fig.1: Decay after storage and fungal counts after treatment of table grapes with ethanol or SO_2. Table grapes cv. 'Early Sweet' were treated after harvest by dipping in 50% ethanol or during storage with an SO_2 generator pad. **A**. Decay was monitored after 35 days at cold storage and 3 days at 20°C as the number of decaying berries per kg fruit. **B**. Fungal counts in the control and after ethanol dip. Sampling was performed before storage. Fungal counts in the SO_2 treatment (diagonal pattern) represent the initial inoculum for this treatment which is activated only during storage. In a subsequent experiment when sampling was performed after storage, SO_2 effectively eliminated fungal counts. Asterisks indicate significant differences compared to the control. **C-E:** Pictures of control, ethanol, and SO_2-treated grapes after storage.



harvest immersion of grape clusters and individual berries in 1% chitosan, followed by inoculation with *B. cinerea*, reduced the percentage of infected berries and severity of infection, as well as decay nests during cold storage [59]. The disadvantage of postharvest chitosan application on table grapes is that it requires a relatively prolonged drying period before the grapes can be placed in storage. The acceptability of chitosan-film on grapes by consumers has not yet been determined.

Aloe vera gel

Coating of 'Crimson Seedless' grapes with *Aloe vera* gel was reported to enable cold storage for 35 days [60]. This edible coating reduced fruit dehydration, rachis browning and respiration rate while it decreased the deterioration in flesh firmness, acidity and colour. The coating had a positive effect on all sensorial aspects including appearance and taste. In addition to these physiological benefits, *Aloe vera* gel reduced microbial counts after 21 days at 1°C and 4 days at 20°C. However, shelf-life was not reported for later cold storage periods and there are no indications whether decay was a problem. In another report by the same group, further benefits of the treatment were reported on functional food aspects such as phenolics, ascorbic acid and anthocyanin content, as well as total antioxidant activity [61].

Ethanol

Ethanol is an approved substance for use as a disinfectant or sanitiser in organic crop production by the United States Department of Agriculture. Evidence from independent studies performed on several cultivars suggests that immersion of grape clusters in 50, 40 or 33% (but not in 20%) ethanol, prior to packaging, inhibited berry decay and was equivalent to, or better than, the effectiveness of SO_2, released from generator pads. Decay was acceptably controlled for a cold storage period of 4–5 weeks and sometimes longer and the quality of the grapes was not affected [48, 62, 63]. *In vitro* studies showed that 30% ethanol was lethal to conidia of *B. cinerea*, which was confirmed by the postharvest treatments of grapes. Grapes artificially contaminated with *Escherichia coli* were

exposed to increasing concentrations of ethanol by dipping bunches of grapes for 1–10 min. *E. coli* populations were typically reduced 1–3 log 10 cfu/g on grapes by treatment with 50% ethanol or more, although the results were highly variable [64]. An example of the potential control of decay by ethanol dip of 'Prime' seedless grapes by the elimination of fungal inoculum on the surface of the berries is given in Figure 1.

Heated ethanol

The flammability limit of ethanol in air is 33,000 μL/L and the air in manned workplaces cannot contain ethanol at more than 1,000 μL/L [65]. Therefore, reducing the concentration used in applications is important. The combined application of ethanol and heat to un-germinated spores reduced the lethal threshold of ethanol [47]. In addition, sub-inhibitory concentrations of ethanol became inhibitory to spores of *Rhizopus stolonifer, Aspergillus niger, Botrytis cinerea* and *Alternaria alternata* (all common postharvest pathogens of table grapes), when heated to temperatures lower than those that would cause thermal destruction of the spores in water alone [66]. When grape berries were immersed in 35% ethanol at 50°C, gray mould incidence was significantly lower then when they were immersed in the same concentration of ethanol at 25°C [48, 63]. Infections were controlled by both cool and warm ethanol on berries inoculated with the pedicel intact. Only heated ethanol controlled gray mould on berries that were wounded by detachment of the pedicel before inoculation. Heated ethanol treatments could be applied up to 24 h after inoculation and remain effective [63]. The only adverse effect observed for heated ethanol was a slight change in the red colour of 'Crimson Seedless' grapes [48]. The concentration of ethanol residues within the berries was low [63].

Ethanol and sorbate

Sorbates have been used as common food preservatives for many years [67]. A postharvest treatment of grapes with a combination of potassium sorbate and ethanol has been evaluated by Karabulut *et al.* [68]. While 20% ethanol or 0.5% potassium sorbate modestly reduced gray mould decay incidence on 'Thompson Seedless' grapes, their combination was comparable in efficacy to a commercial SO_2 generator pad. One advantage of this combined treatment is that, like heat, it allows a lower concentration of ethanol to be used. Although the ethanol-sorbate treatment did not harm the appearance of the fruit, further studies are needed on berry quality, taste and reproducibility of these results with other cultivars. Sorbate residues should be quantified; if persistent and of sufficient concentration, they might control subsequent infections.

Perspective on the use of ethanol

Sanitising grapes with ethanol could be particularly useful for the postharvest treatment of grapes marketed under "organic" classifications, where SO_2 treatments are prohibited. An additional benefit of ethanol treatment would be the cleaning of the grape berries, which is especially important for the late ripening cultivars that may have visible deposits of dust and insect frass. Ethanol also has a significant advantage over other wet treatments because fruit dry faster after immersion in ethanol compared with other aqueous solutions. Ethanol usage may be most feasible and practical for reducing decay and the microbial load on detached berries marketed as "ready-to-eat".

Integrated strategies

One of the conclusions from this review is that prevention of spoilage of table grapes during storage can benefit from combining different approaches, thereby complementing the deficiency of each method. For example, the use of MAP alone to inhibit decay may require high levels of CO_2 in the packages that would lead to undesired off-flavour and rachis browning. However, the combination of MAP and volatiles from essential oils allows keeping of the volatiles around the grapes and reduces the dependency of the MAP on CO_2 [27, 28]. Likewise, the use of ethanol vapour and MAP offers the advantage of a mild treatment with continuous efficacy [13, 29]. The combination of ethanol dip and MAP relies on the initial elimination of surface-borne pathogens until the level of CO_2 accumulates and delays development of decay from secondary or latent sources [25]. The use of physical treatments can also enhance the activity of chemical treatments as demonstrated for heated ethanol [48, 63]. The role of heat in this case is probably to make the membranes of the pathogens more vulnerable to the effect of ethanol. Another integrated approach is to use two different chemicals that act synergistically, such as that of ethanol and sorbates [68]. Similarly, the effectiveness of the bicarbonate solutions for controlling gray mould was significantly improved when combined with 200 μg/mL of chlorine [52]. Another successful combination was a preharvest spray application of chitosan followed by postharvest UV-C irradiation, which caused a synergistic reduction in gray mould incidence and severity and blue mould incidence [45]. Theoretically, such dual treatment can lead to complementary enhancement of host defence in combination to surface protection.

This is only a partial list of the possible combinations that were described for grapes during storage. One must take into consideration that a combined approach may be more costly or may become too complicated to implement.

Conclusions and future prospects

The control of decay and preservation of the quality of table grapes during storage are complex issues because of the interplay among effectiveness, phytotoxicity, physiological compatibility, acceptability, simplicity of use and cost. This presents a significant challenge to alternative technologies to replace SO_2 which in some aspects, such as its low cost and the ease and simplicity of repeated application, can be considered a "silver bullet". None of the alternatives to SO_2 have been shown to be more effective or more practical than SO_2 on a commercial scale. The challenges of commercial implementation of alternative treatments are how efficient, repro-

ducible and compatible the technology, is while accommodating those practical aspects previously mentioned. Another issue is that alternatives requiring additional handling or packinghouse processing would be implemented with some reluctance by California table grape growers, who normally pack their fruit into commercial packages in vineyards [12]. However, this situation may change in the future due to food safety issues.

Some of the studies presented in this review suggest original ideas which prove to be effective but does not cover all the range of commercially relevant aspects. Thus, it may be difficult to predict from these studies whether a specific treatment can be proposed as an alternative to SO_2. Confirmatory tests with naturally inoculated table grapes are needed to establish accurately the effectiveness of a treatment, and an SO_2 treatment is needed in these tests for comparison purposes. In addition, it should be emphasised that incidence of decay above 0.5 to 1% is not acceptable under commercial conditions, but in many reports this level is exceeded. One commonly misinterpreted aspect of decay control treatment in grapes is that if a compound can kill, inhibit, or wash away the surface inoculum from the berries, it would then necessarily control a substantial part of the decay on fruit. In reality, some treatments that can kill spores effectively, such as ozone or chlorine, are not particularly effective for the control of postharvest decay. Conversely, some substances that do not kill the spores, such as bicarbonate salts, can control decay significantly.

Among the physical treatments, an acceptable technology to apply a heat vapour treatment for a period of 20 min, that would be followed by a post-treatment drying period and cooling, has not been developed in a commercial scale.

Among the wet treatments, it seems that ethanol treatment is an alternative which may be attractive for cold storage duration of 4 weeks due to its acceptable effectiveness, rapid drying rate and few regulatory issues. If higher efficacy is required, or due to regulatory issues, or if storage has to be prolonged for marketing purposes, heat, sorbates or MAP can be used as complementary technologies with ethanol. While in the near future most conventional growers would prefer to retain the current practice of dry packaging of table grapes, the use of a wet ethanol treatment might be feasible enough to be adopted by producers of "organic" or "ready-to-eat" grapes, to alleviate concerns about microbial contamination, or to reduce visual deposits such as dust and insect frass.

The dry treatments are probably the most attractive for immediate integration into current practice. Among the dry treatments, CA is probably the least feasible because of the high initial investment and the narrow margin between efficacy and the phytotoxicity of CO_2. MAP exhibits similar problems and it is unlikely that it can be used as a stand-alone technology under high decay pressure. However, active MAP is a promising approach, with either ethanol, essential oils or other specific volatiles. Physiological and sensory evaluation of these approaches is incomplete and the technologies have to be tested on a wide variety of cultivars and situations to reveal potential pitfalls. One shortcoming of MAP is the need to avoid the accumulation of condensation and respiration water within the packages by various means as outlined in the MAP section. Another problem is the lower rate of cooling of the fruit which requires very efficient forced-air cooling systems or sealing the liners after cooling. The difficulties associated with MAP may be avoided if volatiles can be used in ventilated packages, as shown for volatiles produced by the fungus *M. albus*. The approach of biofumigation can probably be integrated into current practice with fewer alterations in current industry practices.

Other technologies, some of which are mentioned above, may also enter into commercial practice. However, this will happen only if the technology proves to be as cost-effective, reliable and effective as SO_2 is now, or if regulatory actions demand that the industry switches to another technology.

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