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Review

Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes

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ABSTRACT

Gray mold, caused by *Botrytis cinerea*, is the main postharvest decay of table grapes. It can develop in the vineyard and spread rapidly among berries after harvest, during long distant transport, cold storage and shelf-life. In conventional agriculture, bunches are sprayed with fungicides after flowering, at pre-bunch closure, at veraison, and later, depending on the time of harvest. Harvested bunches are usually stored in the presence of sulfur dioxide. However, the use of synthetic fungicides and of sulfur dioxide is not allowed on organic grapes and the study of alternative methods to control postharvest decay has developed over several decades, along with the demand for safer storage methods. This review summarizes the results published in the field within the last 5 years (2006–2010). We can group these approaches as follows: (i) biocontrol agents; (ii) natural antimicrobials; (iii) GRAS type decontaminating agents; and (iv) physical means. Two biocontrol agents, *Muscodor albus* and *Hanseniaspora uvarum*, have shown equal or better effectiveness than conventional methods to control gray mold of table grapes in laboratory scale experiments. Currently, the bottleneck for the commercial use of biocontrol agents is that the registration process is comparable to that of fungicides, with similar costs but often with a narrower market. This delays their transition from experimental to practical use. Natural antimicrobials, such as salts, chitosan, and plant extracts, have demonstrated good results and often have been applied in various scales. Several GRAS-classified sanitizers have been tested to extend postharvest storage of table grapes, including acetic acid, electrolyzed oxidizing water, ozone, and ethanol. Physical technologies involving variations in temperature, UV-C irradiation, pressure or changing atmospheric composition, are all postharvest practices which require significant adaptation by an industry which is accustomed to minimal intervention during harvest. Overall, the use of ozone and of calcium chloride are two promising examples of treatments that are beginning to be adopted on a commercial scale. The requirements for the optimal treatment of grapes against gray mold before harvest or during storage are summarized.

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1. Introduction

Gray mold, caused by *Botrytis cinerea*, is the main postharvest decay of table grapes (*Vitis vinifera*) (Pearson and Goheen, 1988). It can develop in the vineyard and even more after harvest, during long-distance transport, cold storage, and shelf-life. Occasional infections by *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp., that cause blue mold, Aspergillus rot and Alternaria rot, respectively, can also occur. In conventional agriculture, bunches are sprayed with fungicides after flowering, at pre-bunch closure, at veraison, and later, depending on the time of harvest (Luvisi et al., 1992). These sprays can markedly reduce subsequent postharvest decay, but they do not eliminate the need for postharvest treatments (Smilanick et al., 2010a). Under commercial conditions, grapes may remain on the vines long after they are physiologically mature. Harvested bunches are usually stored in the presence of sulfur dioxide. This compound is registered as an adjuvant in most countries, while it has been removed from the GRAS list and classified as a pesticide in USA (Anon, 1986). However, the use of synthetic fungicides and of sulfur dioxide is not allowed on organic grapes (Mlikota Gabler and Smilanick, 2001), and there are increasing regulatory restrictions on the use of chemical fungicides. The study of alternative means to control postharvest decay has progressed over the past several decades, along with the expansion of organic agriculture and the concern of consumers about the possible presence of fungicide residues on fruit. The use of alternative means to control postharvest decay of table grapes was extensively reviewed most recently about 5 years ago (Droby and Lichter, 2004;

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Elmer and Reglinski, 2006; Lichter et al., 2006). However, interest in this subject remains high and significant research progress in this field has occurred in the meantime, although practical applications are still relatively few. The objectives of this review are to summarize the research published in the last 5 years on the use of alternatives to synthetic fungicides to control postharvest decay of table grapes and to describe requirements for the integration of alternative approaches in table grape vineyards and cold storage.

2. Alternative means to control postharvest decay of table grapes

We searched relevant databases to find research papers dealing with the subject published since 2006 to 2010. The publications were clustered, according to the content, in four categories: (i) biocontrol agents; (ii) natural antimicrobials; (iii) GRAS type decontaminating agents; (iv) physical means. The combined treatments that use two or more applications, and data about investigated mechanisms of actions, were separately reported.

Few papers are related to the optimization of chemical control, such as the use of sulfur dioxide pads that release the appropriate dose of sulfur dioxide (Zoffoli et al., 2008; Zutahy et al., 2008). The large portion of the research in the control of postharvest decay of table grapes is devoted to alternative means to the use of synthetic fungicides, although most of them are still far from practical application.

2.1. Biocontrol agents

Over time, many biocontrol agents have been shown to approach the effectiveness of conventional means to control gray mold of table grapes in laboratory scale experiments. Most trials relate to decay control during storage but some biocontrol agents have proven to be effective when applied before harvest for postharvest control of gray mold. Database searches resulted in only a couple of reports dealing with the use of biocontrol agents to control gray mold of table grapes (Mlikota Gabler et al., 2006; Liu et al., 2010). Another three biocontrol agents were studied in a combined treatment approach and they will be discussed later (Ligorio et al., 2008; Meng and Tian, 2009; Mlikota Gabler et al., 2010b). *Muscodor albus*, a fungus that acts by producing volatile compounds, proved to be effective in the control of gray mold (Mlikota Gabler et al., 2006). In artificially inoculated grape bunches commercially packaged in ventilated polyethylene cluster bags incubated for 28 d at 0.5 °C, gray mold incidence was 43% among untreated fruit and 5 or 4% when the formulation at 5 or 10 g kg^{-1}, respectively, had been added. However, the process of registration of this biocontrol agent is now suspended because of the toxicity of one of the metabolites (J. Margolis, personal communication). A second report deals with the use of *Hansienaspora uvarum* that was reported to reduce natural decay from 55 to 15% after 50 d storage at 0 °C (Liu et al., 2010). This yeast is also reported to be associated with the "grape sour rot complex" (Guerzoni and Marchetti, 1987), so its use should be tested in field experiments to ensure that the selected isolate does not exacerbate sour rot. Currently, the bottleneck with the use of biocontrol agents is that their registration process can be as expensive and elaborate as that of fungicides, with similar costs but often with a narrower market, and this issue often affects the transition from the experimental to the practical phase.

2.2. Natural antimicrobials

The research in the field of natural antimicrobials, such as salts, chitosan, and plant extracts, has been very active over the last 5 years (Table 1). Many salts were tested for their effectiveness to control gray mold, with both preharvest and postharvest applications. Boron, applied in the form of potassium tetraborate at 0.1–1%, was effective in the control of postharvest gray mold on table grapes stored 30 d at 0 °C. The best results were obtained with 1%, reducing decay on single berries artificially inoculated with the pathogen from 40 to 2–3% (Qin et al., 2010). In large-scale tests, simulating practical commercial conditions, two salt applications (30 and 90 d before harvest) of calcium chloride, sodium carbonate, or sodium bicarbonate, significantly reduced postharvest gray mold from 64% among untreated controls to 22, 31, and 29%, respectively, after 30 d storage at 0 °C. Calcium chloride controlled decay more effectively compared to a fungicide which was a mixture of cyprodinil and fludioxonil (Nigro et al., 2006). It seems that the best timing to apply salts is preharvest, because it is easily integrated into usual plant protection practices. Potassium tetraborate, potassium carbonate, sodium bicarbonate, and sodium carbonate also have a direct inhibitory effect on the pathogen (Nigro et al., 2006; Qin et al., 2010), while this was not the case for calcium chloride. One obvious requirement from application of salts is that residues should not be visible on the surface of the berries. Chitosan is a natural biopolymer that was used both in preharvest and postharvest applications. Several acids have been tested to dissolve chitosan; chitosan was most effective to control postharvest decay when it was dissolved in acetic acid, both on single berries stored at 15 °C and on small clusters stored 60 d at 0 °C (Romanazzi et al., 2009). Table grape bunches cv. Redglobe immersed in chitosan and stored 4 weeks at 0–1 °C had 10 infected berries per kg compared to 19 infected berries in the control (Xu et al., 2007). The same magnitude of decay reduction was observed on artificially inoculated grape bunches held under the same conditions. Bunches sprayed with chitosan before harvest, then coated after harvest with chitosan, had a decay index (McKinney index, that expresses the disease incidence as compared to the highest possible score, equal to 100) of 0.05, while the untreated control had a decay index of 0.15 after 42 d storage at 0 °C (Meng et al., 2008). Preharvest chitosan treatment provided the highest decay reductions (over 80%) when applied 1 d before harvest, on three different cultivars (Romanazzi et al., 2006). Several chitosan-based compounds have become available on the market (Elmer and Reglinski, 2006; Romanazzi, 2010), and recently Chito Plant (ChiPro GmbH, Bremen, Germany), and Armour-Zen (Botry-Zen Ltd, Dunedin, New Zealand) proved to effectively control decay of table and wine grapes, respectively (Reglinski et al., 2010; Romanazzi, 2010). Another approach is the use of *Aloe vera* gel coating, with a formulation under patent (Serrano et al., 2006), which was effective in preharvest and postharvest application for the control of postharvest gray mold of table grapes. Clusters sprayed 1 d before harvest with the *A. vera* gel solution, then stored 35 d at 2 °C had 1% decayed berries compared to 15% of the control, although additional preharvest sprays did not provide better efficacy (Castillo et al., 2010).

Growth regulators are usually applied to enlarge berry size. However, they may have an impact on the structural susceptibility of grapes to gray mold, especially when applied many times. For example, two applications of gibberellic acid on 'Thompson Seedless' grapes resulted in 1% gray mold compared to 12% after eight applications and increased doses, which was attributed to higher shatter, hairline cracks and splitting of berries (Zoffoli et al., 2009).

Grapefruit seed extracts and essential oils have been applied on harvested grapes with the aim of controlling decay. Table grape bunches immersed in grapefruit seed extracts and stored 4 weeks at 0 °C had 6 infected berries per kg compared to 19 of the control (Xu et al., 2007). When bunches were artificially challenged with *B. cinerea*, infected berries were 18 and 65 per kg when treated with grapefruit seed extracts or untreated, respectively (Xu et al., 2007). The time of appearance of initial gray mold symptoms was used

Table 1
Natural antimicrobials, decontaminating agents and physical methods used to contain gray mold of table grapes.

	Treatment	Application	Reference
Natural antimicrobial	Calcium chloride, potassium carbonate, sodium bicarbonate, sodium carbonate	Pre/post[a]	Nigro et al. (2006)
	Potassium tetraborate	Post	Qin et al. (2010)
	Carvacrol vapor	Post	Martínez-Romero et al. (2007)
	Chitosan	Pre/post	Romanazzi et al. (2006, 2009), Xu et al. (2007), Camili et al. (2007), Meng et al. (2008), Romanazzi (2010)
	Aloe vera gel	Pre/post	Serrano et al. (2006), Castillo et al. (2010)
	Essential oils	Post	Valero et al. (2006), Tripathi et al. (2008), Abdolani et al. (2010)
	Growth regulators	Pre	Zoffoli et al. (2009)
	Grapefruit seed extract	Post	Xu et al. (2007)
Decontaminating agent	Acetic acid	Post	Venditti et al. (2008), Camili et al. (2010)
	Electrolyzed oxidizing water	Post	Guentzel et al. (2010)
	Ethanol	Pre/Post	Yu et al. (2006), Romanazzi et al. (2007a), Lurie et al. (2006), Chervin et al. (2009)
	Ozone	Post	Cayuela et al. (2009), Mlikota Gabler et al. (2010a), Smilanick et al. (2010a)
Physical method	UV-C irradiation	Post	Romanazzi et al. (2006)
	Pressure (hyperbaric treatment)	Post	Romanazzi et al. (2008)
	Gas content (high CO_2)	Post	Sanchez-Ballesta et al. (2006)

[a]Preharvest/postharvest.

to evaluate the efficacy of treatments on detached berries which were artificially wounded and inoculated with *B. cinerea*. Using this method, treatment with essential oils of *Ocimum sanctum*, *Prunus persica* or *Zingiber officinale* resulted in appearance of decay after 8, 9 and 10 d, respectively, while they appeared in the control after 4 d (Tripathi et al., 2008). Treatment of table grape clusters with the essential oils eugenol or thymol reduced the number of decayed berries after 56 d storage at 1 °C and 3 days shelf-life from 50% in the control to 10–22% (Valero et al., 2006). Gray mold from natural inoculum on clusters sprayed with natural thyme (*Thymus vulgaris*) and summer savory (*Satureja hortensis*) oils were able to reduce disease severity to 4.1 and 4.2, respectively, compared to 4.9 units (based on a 0–6 empirical scale) in the control, on table grapes after 60 d storage at 0 °C (Abdolani et al., 2010). These essential oils showed a different degree of inhibition of the in vitro growth *B. cinerea*. Carvacrol vapor at 0.05, 0.2, 0.5 and 1.0 mL L^{-1} completely suppressed *B. cinerea* growth on PDA and decreased decay of single table grape berries kept at 25 °C for 4 d from 93% in the control to 6% when exposed to the highest carvacrol concentration (Martínez-Romero et al., 2007).

2.3. GRAS type decontaminating agents

Several sanitizers classified as GRAS have been applied to extend postharvest storage of table grapes, including acetic acid (Venditti et al., 2008; Camili et al., 2010), electrolyzed oxidizing water (Guentzel et al., 2010) and ethanol (Lurie et al., 2006; Yu et al., 2006; Romanazzi et al., 2007a; Chervin et al., 2009) (Table 1). Acetic acid vapor concentrations ranging between 0.25 and 4 mL per 100 L room space reduced radial growth of *B. cinerea* after 4 d incubation at 22 °C from 8 cm in the control to less than 1 cm, with no differences among tested rates (Camili et al., 2010). In vivo trials on artificially inoculated bunches treated with vapor of 0.25 and 1 mL 100 L^{-1} of acetic acid, then stored at 22 °C for 2–6 d (Camili et al., 2010) effectively reduced decay. The treatment of table grape berries with 5 mL 100 L^{-1} acetic acid decreased postharvest gray mold on 'Regina' and 'Taloppo' table grapes by 61 and 41%, respectively, after 8 weeks storage at 5 °C (Venditti et al., 2008). The near-neutral (pH 6.3–6.5) electrolyzed oxidizing water completely killed *B. cinerea* conidia at 10 g L^{-1}, and decreased the gray mold incidence on artificially inoculated, single table grape berries, compared to a water dip (Guentzel et al., 2010). Ethanol was applied in 3 different ways to table grapes: by dipping in a 50% solution for 10 s, or placing inside the package a container with a wick and ethanol at 4–8 mL kg^{-1} grapes or a paper containing the same ethanol amount. Decay was reduced on grape bunches treated with ethanol, compared to the control; decay was equal or less compared to results from storage with SO_2 dual release generator pads with sodium metabisulfate (Lurie et al., 2006). A good decay control can also be obtained with reduced doses of ethanol. Immersion in a 20% ethanol solution of 'Autumn Seedless' grape bunches inoculated with *B. cinerea* conidia reduced gray mold from 95% among control to 7% among treated bunches (Romanazzi et al., 2007a). Preharvest application of 16% ethanol on 'Chasselas' grapes reduced decay at harvest from 16% in the control to 12%; after 6 weeks of cold storage this treatment increased commercially acceptable grape yield from 5% in the control to 38% in the treatment (Chervin et al., 2009). Ethanol is also applied after harvest on a small commercial scale for 'ready-to-eat' grapes.

Ozone, classified as a GRAS substance by the US Food and Drug Administration since 2001, has been extensively tested for the control of table grape decay (Cayuela et al., 2009; Sharpe et al., 2009; Mlikota Gabler et al., 2010a; Smilanick et al., 2010b). It is fungistatic, effective to control decay, although it is dose dependent, and high concentrations (above 5000 ppm h^{-1}) can be phytotoxic. Treatment with 5000 ppm h^{-1} ozone in a commercial chamber of organically grown 'Autumn Seedless' and 'Black Seedless' table grape bunches reduced gray mold incidence from natural inoculum by about 50% after 6 weeks storage at 0 °C and on 'Redglobe' grapes decay reduction was 65% (Mlikota Gabler et al., 2010a). Many cold storage facilities in California have installed equipment that generates a constant low dose of ozone (100 ppb day and 300 ppb night cycle) and it reduces the spread of gray mold and prolongs the storage of grapes for several weeks (Smilanick et al., 2010b). Control and delivery of the optimal dose during cold storage is essential for its effectiveness; this could be challenging because some commercial packages can impede ozone penetration into the grapes. The risk of injury to table grapes from ozone has not been completely evaluated. There are no reports indicating that ozone harms grape berries themselves; when injuries have been reported, the rachis was harmed. Constant low concentrations of ozone (0.3 ppm) caused no harm to the rachis of 'Flame Seedless' grapes after 4 weeks or 'Thompson Seedless' grapes after 7 weeks (Palou et al., 2002; Smilanick et al., 2010b), while rachis injuries developed in

Table 2
Physiological changes in host tissues after treatment with alternative methods.

Treatment	Parameter	Effect[a]	Reference
Chitosan	Superoxide dismutase	–	Meng et al. (2008)
	Respiration	–	Romanazzi et al. (2007a, 2009)
	Hydrogen peroxide	–	Romanazzi et al. (2007b)
High CO_2	PAL[b]	+	Sanchez-Ballesta et al. (2007)
	Chalcone synthase	+	
	Stilbene synthase	–	
	trans-Resveratrol	–	
	Anthocyanins	–	
Aloe vera gel	Phenolics	+	Serrano et al. (2006)
	Ascorbic acid	+	
	Antioxidant activity	+	
	Anthocyanins	–	
Carvacrol vapor and *UV*-C	Ethylene	–	Martínez-Romero et al. (2007)
	Respiration rate	–	
	trans-Resveratrol	+	Romanazzi et al. (2006)
	Catechin	+	
Grapefruit seed extract and chitosan	Weight loss	–	Xu et al. (2007)
	Color	–	
	Ripening	–	
	Sensory quality	+	
Ozone	*trans*-Resveratrol	+	Cayuela et al. (2009)
	Sensory quality	+	

[a] +: increase/improve; –: decrease.
[b] PAL: phenylalanine ammonia-lyase.

some tests after treatments of 30 min with very high concentrations (5000 ppm) of ozone (Mlikota Gabler et al., 2010a). Postharvest ozone treatment has another benefit in that it enhances synthesis of resveratrol and other bioactive phenolics in grapes (González-Barrio et al., 2006; Artés-Hernández et al., 2007; Cayuela et al., 2009), confirming earlier work on this subject (Sarig et al., 1996) (Table 2).

2.4. Physical means

Physical methods of controlling gray mold include UV-C irradiation, and various atmospheric pressures or atmosphere compositions (Table 1). UV-C treatment (254 nm) effectively controlled gray mold, which was reduced from 22 and 52% in the control to 14 and 38% in 'Autumn Black' and selection B36–55 grapes, respectively. The same treatment reduced blue mold of table grapes from 13% in the control to 8% in selection B36–55 and induced in the berries the production of *trans*-resveratrol and catechin, phytoalexins linked to increased resistance of host tissues to the pathogens (Romanazzi et al., 2006) (Table 2).

Hyperbaric treatments have been applied to control postharvest gray mold of table grapes. Laboratory scale applications of 1140 mmHg (1.5 atm) for 24 h decreased the percentage of infected berries and lesion diameter of gray mold on artificially inoculated berries (Romanazzi et al., 2008). Although widely applied in the sterilization of foods, the use of pressures higher than atmospheric could pose safety issues and these treatments need to be further studied in large scale tests. Postharvest treatments can reduce decay of table grapes and affect putative virulence-associated pathways. The pretreatment of 'Cardinal' table grapes with 20% O_2 + 20% CO_2 + 60% N_2 for 3 d reduced decay to 5%, compared to 25.5% in the control, after 33 d cold storage at 0 °C (Sanchez-Ballesta et al., 2006). Exposure of grapes to high concentrations of CO_2 for 3 days at 0 °C decreased activity of phenylalanine ammonia-lyase, chalcone synthase, and stilbene synthase, and the contents of *trans*-resveratrol and total anthocyanin (Sanchez-Ballesta et al., 2007) (Table 2).

2.5. Combined treatments

A single alternative approach might not effectively reduce decay, compared to chemical fungicides, so the integration of two or more alternative means, in a multifaceted approach, can be worthwhile (Wilson, 1997). This approach can reduce the decay following the "multiple hurdle concept" that consists of the reduction of decay by applying to the pathogen several consecutive hurdles, with each one contributing a portion of the reduction (Ippolito, 2010). The combination of several means, some of which may not be effective on their own (priming effect, see Conrath et al., 2006), allow their use at lower concentrations and may result in additive or synergistic effects (Romanazzi et al., 2007a). For example, the biopolymer chitosan was more effective when combined with UV-C, ethanol or grapefruit seed extracts (Romanazzi et al., 2006, 2007a; Xu et al., 2007) (Table 2). Preharvest chitosan treatment and postharvest UV-C irradiation had a synergistic interaction in reducing gray (from 22% in the control to 3%) and blue (from 13% in the control to 1%) molds on single berries, while it increased the amount of *trans*-resveratrol and catechin compared to each treatment applied alone (Romanazzi et al., 2006) (Table 2). Similarly, reduced doses of 0.1 and 0.5% chitosan and ethanol at 10 and 20% provided additive, and at times synergistic effects in the control of gray mold. In experiments conducted on 'Autumn Seedless' single berries, decay was 92% in the control and 2% in the combination of 0.5% chitosan and 20% ethanol. Similar experiments carried out on small bunches showed a reduction of decay from 9% in the control to 0.5% when treated with the combination of chitosan and ethanol (Romanazzi et al., 2007a) (Table 3). The combination of 1% chitosan and 0.5% grapefruit seed extract decreased *B. cinerea* conidia germination and mycelium radial growth on agar plates from 95% and 85 mm in the control to 5% and 35 mm, respectively. The same combination decreased the number of decayed berries kg^{-1} on grapes stored 4 weeks at 0–1 °C to 10%, compared to 65% recorded in the control. Chitosan and grapefruit seed extract, alone and in combination, produced changes in weight loss, color change, ripening, and improved sensory quality of grapes (Xu et al., 2007) (Table 2). It should be noted that the influence of treatments on

Table 3
Combined treatments to contain gray mold of table grapes.

Treatment	Application[a]	Reference
Chitosan + UV-C	Pre/post	Romanazzi et al. (2006)
Chitosan + ethanol	Post	Romanazzi et al. (2007a)
Chitosan + grapefruit seed extract	Post	Xu et al. (2007)
Chitosan + *Cryptococcus laurentii*	Pre/post	Meng and Tian (2009) and Meng et al. (2010)
MAP + essential oils	Post	Guillén et al. (2007)
Ethanol + calcium chloride	Pre	Chervin et al. (2009)
Ethanol + MAP	Post	Lurie et al. (2006)
Muscodor albus + ozone	Post	Mlikota Gabler et al. (2010b)
Pichia anomala + *Cryptococcus humicolus* + bentonite + potassium caseinate + calcium chloride	Post	Ligorio et al. (2008)

[a] Preharvest/postharvest.

flavor is often ignored because laboratory-scale experiments tend to focus on the effectiveness of a treatment to control decay and do not sufficiently take into consideration the final quality of the produce, which is essential for a potential commercial application. Table grape bunches sprayed 10 d before harvest with the combination of 0.1% chitosan and *Cryptococcus laurentii* stored 17 and 42 d at 0 °C, then exposed to 3 d shelf-life had a decay index of gray mold of 0.15 (based on a 0–1 empirical scale) compared to 0.30 recorded in the control (Meng and Tian, 2009). Bunches treated with the same antagonist preharvest and dipped in 1% chitosan solution after harvest, then stored in the same conditions as above, had a decay index of 0.35 in the control to 0.15 in treated bunches (Meng et al., 2010). Modified atmosphere packaging (MAP), obtained by wrapping table grape bunches in films with different permeabilities, combined with the use of eugenol–thymol–carvacrol essential oils, reduced decay incidence from 37% in the control to 7% in treated bunches after 56 d cold storage at 1 °C, independent of the film used (Guillén et al., 2007) (Table 3). The combination of 16% ethanol and 1% calcium chloride applied on bunches 4 times prior to harvest reduced gray mold at harvest from 16% in the control to 5% (Chervin et al., 2009). The same treatment on bunches stored 6 weeks at 0–1 °C and 24 h at ambient temperature produced an amount of commercial grapes of 53%, compared to 6% in the untreated control. The initial fumigation with high concentrations of ozone (5000 ppm for 1 h), followed by biofumigation with *M. albus* reduced gray mold incidence among inoculated 'Autumn Seedless' grapes stored 30 d at 0.5 °C from 92% in the control to 10% among treated grapes. The same combined treatment applied to organically grown 'Thompson Seedless' grapes reduced gray mold incidence from natural inoculum from 31% in the control to 3.4% among treated grapes. However, the combined treatment was less effective than the standard sulfur dioxide treatments (Mlikota Gabler et al., 2010b). Among grapes stored at 2 °C for 15 d, the decayed berries were reduced from 22% in the control to 10% by using a treatment that combined two yeasts (*Pichia anomala* and *Cryptococcus humicolus*), bentonite, potassium caseinate and calcium chloride (Ligorio et al., 2008). The 'multiple hurdle' or the combined treatments approaches are more difficult to integrate into commercial practice compared to a single alternative treatment, and this complexity comprises another barrier to their implementation.

3. Commercial aspects, examples of success and future outlooks

Most of table grapes (around 90% in Italy and in California) are packed directly in the field. This practice is common due to the fragility of the produce and due to other logistic aspects including the higher cost of indoor or shed packing. For this reason, preharvest applications are the desired method of treating grapes against gray mold because they are easier to integrate into current conventional practices. Moreover, postharvest applications are not always more effective than preharvest applications of alternative methods (Ippolito, 2010). Table grapes are not usually washed postharvest, because wetting requires drying that can cause mechanical injuries to the cluster and in some cases can alter the bloom on the berry surface, which is an important part of the appearance of the berries for marketing purposes. Wetting the berries may also cause them to crack.

One practice, to increase the value of clusters with inferior quality, is to clean and dissect them into small clusters and to pack the better part of the clusters in containers of 0.5 or 1 kg. In order to reduce the logistic costs, some packinghouses in Italy are setting up an intermediate system to pack the grapes in small boxes directly in the field.

The extensive research efforts of the last 20 years to find alternatives to conventional chemical fungicides for table grapes have resulted in treatments that provide significant levels of postharvest decay control. In spite of these accomplishments, however, most are not regarded in the conventional table grape industry as effective enough to be acceptable. The accepted maximum decay level on commercial stored table grapes is 0.5% at the point of shipping for US no. 1 grade California grapes (Anon, 1999). The two notable exceptions of alternative treatments that meet this standard are calcium chloride for preharvest storage on the vine and ozone for postharvest storage. Calcium chloride is now applied in Italy, both in conventional and in organic vineyards to assist in preharvest storage on the vine and it is reported to effectively reduce decay, to leave no residues on berry surface, and to delay ripening. This is beneficial for growers that cover the grapes with plastic late in the season for rain protection, in the trellising system called "tendone", to prolong the harvest until the price increases. These grapes are left on the vine sometimes until the end of December, utilizing natural plant defense systems. After harvest, ozone can successfully control the spread of gray mold during storage and extend the storage life of the grapes by several weeks. It leaves no residues and it is allowed on certified organic grapes in the USA by the USDA National Organic Program (Anon, 2010). This technology is becoming popular in cold storage facilities in California and it is estimated that ozone is used on about 75% of organic table grapes after harvest. In Italy the technology is currently under evaluation in several packinghouses, while in Israel it is not used on table grapes but in other commodities, e.g. tomatoes. Research is in progress now to define the optimal material and ventilation needed to optimize table grapes packaging for use with ozone gas. The use of ozone can be beneficial for conventional grapes because it can reduce residues of certain pesticides on grape berries, thus allowing them to comply with the requirements of some commercial chains of buyers that often require levels of fungicide residues considerably lower than legislative thresholds. Residues of fenhexamid, cyprodinil, pyrimethanil, and pyraclostrobin were reduced by 68, 75, 84, and 100%, respectively, after a single fumigation of table grapes with 10,000 ppm ozone for 1 h, while, residues of iprodione and boscalid were not significantly reduced (Mlikota Gabler et al., 2010a). Currently, it seems that ozone is unlikely to replace sulfur

dioxide technologies in conventional grape production unless its efficacy is further improved and its cost of production becomes economically viable in global terms. This situation can change if the use of SO_2 becomes restricted or prohibited, as in the case of organic grapes. Hence, ozone is likely to become a leading technology for grapes marketed under the organic classification (Mlikota Gabler et al., 2010a).

Finally, if we try to portray a picture of the ideal alternative means of controlling gray mold of table grapes, they should meet the following criteria:

1. efficacy equivalent or better than the current practice.
2. will not injure or cause phytotoxic effects.
3. will not compromise the organoleptic quality of the grapes.
4. will not be a threat to human health and the environment.
5. compatible with standard practices, affordable and easy to implement.
6. compatible with the principles of organic agriculture.
7. offer substantial benefits to the technology manufacturer which often play a pivotal role in commercialization of novel treatments.

To date it looks as though those requirements are met in large part by the application of ozone. Relatively basic ozone systems are capable of ozonating a large storage room with 1 or 2 ppm of ozone. However, systems with sophisticated remote operation of similar ozone generation capacity are available. The more expensive systems may include controlled ducting to several rooms at the same time. Other alternative treatments to the use of synthetic fungicides can potentially meet those requirements and can be easily moved toward a practical application in coming years.

There are several prerequisites that may influence the outcome of faster implementation of safe alternatives over conventional methods to control postharvest gray mold of table grapes: (i) researchers should take more consideration into a possible practical application when they design experiments, (ii) there is a tendency for consumer and retail chain companies to demand even more pesticide residue free food; (iii) the willingness of companies to invest in innovative means; (iv) the willingness of producers to take the risk to introduce new changes that can be beneficial for the industry, consumers, and the environment.

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