Alternatives to sulphites and another preservatives for table and dried grapes

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OIV COLLECTIVE EXPERTISE

ALTERNATIVES TO SULPHITES AND OTHER PRESERVATIVES FOR TABLE AND DRIED GRAPES

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SCOPE

The Sub-commission Table Grapes, Raisins and unfermented vine products (SCRAISIN) have been lately concentrated around the alternatives to sulphites and preservatives for table grape production, either in the vineyard, or at the post-harvest level.

Although some indications are mentioned in the adopted Resolution OIV-VITI 422-2011 (Specifications for the environmental aspects of sustainability for the table and dried grape sector), this document aims to gather more specific information on the alternatives for the use of SO_2 and other preservatives for the production of table grapes, mainly focusing on vegetal extracts and sustainable alternatives.

Finally, it should be remarked that all treatments have not been discussed within the OIV meetings and that authors should be informed on the treatments admitted in the relevant regulation. This review is based on the help of scientific literature and technical works founded and also, thanks to the inputs and subsequent revisions made by some OIV experts of the SCRAISIN, such the following research group:

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INTRODUCTION

Table grapes are subject to serious water loss and decay during postharvest handling. Gray mold, caused by the fungal agent *Botrytis cinerea*, and stem browning from desiccation are the two main factors that reduce the postharvest quality of table grapes (Candir *et al*., 2012; Lichter, 2016).

Gray mold is the most economically important postharvest disease for this crop. Occasional infections by *Penicillium* spp. (mainly P. *expansum*), *Aspergillus* spp. (mainly A. *niger*), *Alternaria alternata*, *Cladosporium* spp. and *Rhizopus stolonifer*, that cause blue mold, black mold, *Alternaria rot*, *Cladosporium* rot and *Rhizopus* rot, respectively, can also occur (Romanazzi *et al*., 2012). The symptoms of gray mold starts with small necrosis on the skin, which enlarge in brown spots. In those areas the fungus produces macerating enzymes below the skin, that separate the cuticle, being slippery, from the flesh, and this symptom is known as slip skin. Later, on those areas start growing white fungal mycelium, which originates gray conidia and giving the name to the disease. The fungus grows from infected berries to surrounding ones, leading to the production of “nests”, and this symptom is known as nesting (Romanazzi *et al*., 2012; Teles *et al*., 2014).

Gray mold, is responsible for severe losses of table grapes both in the field and after harvest, where it is a major threat to their long-distance transports and storage. Currently, gray mold is controlled by canopy management, preharvest fungicide applications, and postharvest sulfur dioxide fumigation (Ciccarese *et al*., 2013). Postharvest gray mold is usually controlled worldwide by an initial sulphur dioxide (SO_2) fumigation (Melgarejo-Flores *et al*., 2013), followed by weekly fumigations during cold storage at 0.5 ºC (Mlikota Gabler *et al*., 2010).

The use of SO_2 could prevent the decrease in sugar (fructose and glucose) and organic acid (malic and tartaric acid) contents without adversely affecting the anthocyanin content. Despite its efficacy, SO_2 treatments may compromise fruit taste, and can cause damage to the berry (cracks and bleaching) (Nelson and Richardson, 1967), resulting in excessive sulfite residues, and also, remaining on fruits at final market, which may represent a serious risk for human health and the environment (Ustun *et al*., 2012).

Ingestion of SO_2 residues can cause hypersensitive reactions in some people, which has resulted in the removal of SO_2 treatment from the United States Food and Drug Administration (USFDA) generally recognized as safe (GRAS) list and classified as a pesticide in USA (Anonymous, 1986). Some countries in Europe are proscribing its use for grapes imported into the country (Ustun *et al*., 2012; Candir *et al*., 2012).

On the other hand, the use of synthetic fungicides and of sulfur dioxide is not allowed on organic grapes (Romanazzi *et al*., 2012).

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 (Lichter and Romanazzi, 2017). 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 n°1 grade California grapes (Anonymous, 1999). Nowadays, the two notable exceptions of alternative treatments that can meet this standard are preharvest application of calcium chloride and postharvest fumigation with ozone (Romanazzi *et al*., 2012). Thus, innotative safe alternatives and technologies should aim to decrease the decay level, maintain berry quality, but without the use of synthetic pesticides.

POSTHARVEST TREATMENTS ALTERNATIVES TO SO_2FOR STORAGE OF TABLE AND DRIED GRAPES

Most of the treatments could be classified according to their nature, in four categories:

Biocontrol agents

Some biocontrol agents have shown an effectiveness comparable to that of conventional methods in the control of gray mold of table grapes in small scale experiments.

• *Muscodor albus*, a fungus that acts by producing volatile compounds. 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. (Romanazzi *et al*., 2012). However, this biocontrol agent was later retired from the market by the producing company.

• *Hansienaspora uvarum* a yeast that was reported to reduce natural decay from 55 to 15% after 50 d storage at 0 °C (Romanazzi *et al*., 2012).

• *Cryptococcus laurentii*. Uses combines with chitosan at postharvest or preharvest spraying to reduce natural decay (Meng *et al*., 2010).

Currently, the bottleneck for the commercial use of biocontrol agents is that the registration process is comparable to that of synthetic fungicides, with similar costs but often with a narrower market and inconsistent effectiveness. This delays their transition from experimental to practical use (Romanazzi *et al*., 2016).

Some other yeasts and yeast-like fungi have also been reported to counter the growth of mould by different natural mechanisms, such as nutrient competition, killer toxins character, iron depletion, ethyl-esters production or inducing host-plant resistance through the accumulation of phytoalexins and the synthesis of pathogenesis-related proteins. It should also be noted that for other fruit crops some yeast from wine products like *Saccharomyces cerevisiae* (strains N.826 & N.831) and *Zygosaccharomyces* (N. F30) are biocontrol agents, but their effectiveness need to be confirmed in large scale trials (Liu *et al*., 2013).

Natural antimicrobials

These alternatives include natural compounds from animal and plant origins (chitosan, essential oils, plant extracts), organic and inorganic salts, represent the approaches recently evaluated to ensure optimal fruit quality (Youssef and Roberto, 2014; Romanazzi *et al*., 2016).

Several **inorganic salts** have certain antimicrobial activity against several pathogenic fungi. Preharvest and post-harvest applications with salts like Boron, applied in the form of potassium tetra borate; potassium carbonate, sodium bicarbonate, and sodium carbonate, iron and ammonium based salts also, can significantly reduce the incidence of gray mold on table grapes using these salts, which are safe for consumers and the environment (Youssef and Roberto, 2014).

The pre-harvest applications of salts were generally more effective than individual post-harvest immersion and can be considered as a valid regime to enhance their activity since no impact of their application on quality profile was observed. But in large-scale tests, simulating practical commercial conditions, salt applications (30 and 90 d before harvest) of calcium chloride, sodium carbonate, or sodium bicarbonate, significantly reduced postharvest gray mold from 64% to 22%, respectively comparing with untreated controls, after 30 d storage at 0° C. Although, it seems that the best timing to apply salts is pre-harvest, because it is easily integrated into usual plant protection practices (Romanazzi *et al*., 2012).

Table grapes are not usually washed postharvest because wetting requires drying that can cause mechanical injuries to grape cluster beside the problem of poor appearance because of effect on the bloom (Youssef and Roberto, 2014).

Calcium (Ca), known for its ability to reduce or delay parasitic and/or physiological disorders in fruit and vegetables, gave promising results in controlling storage rots when applied both as organic and inorganic salts. Some studies have been carried out to verify the effect of calcium applications on controlling postharvest gray mold and demonstrated their efficacy of bunch treatments (Ciccarese *et al*., 2013).

The **essential oil** of cinnamon leaves (CLO) is recognized for its aroma and medicinal properties and has been identified as a GRAS product by the USFDA (21CFR582.20), and its antifungal and antioxidant properties are due to volatile components such as eugenol and cinnamaldehyde. *Cinnamomum zeylanicum* leafs (CLO), their vapors and coating treatments on table grapes post-harvest can be an attractive, green, alternatives to decay control and improve the antioxidant berry compounds and quality (Melgarejo-Flores *et al*., 2013).

Others like **Aloe vera gel** coating, growth regulators (gibberellic acid) or grapefruit seed extracts and essential oils have been studied. **Grapefruit seed extract (GSE)** is a commercial product derived from the seeds and pulp of grapefruit (Citrus paradise Macf. Rutaceae); an effective broad-spectrum bactericide, fungicide and antiviral and antiparasitic natural extract (Xu *et al*., 2007). Essential oils of *Ocimum sanctum*, *Prunus persica* or *Zingiber officinale*, eugenol or thymol and sprayed with natural thyme (*Thymus vulgaris*) and summer savory (*Satureja hortensis*) oils could reduce the number of decayed berries (Romanazzi *et al*., 2012).

Chitosan (N-acetylated derivative of the polysaccharide chitin; a mostly deacetylated ß-1,4-linked d-glucosamine polymer), is a structural component of fungal cell walls and a natural biopolymer that has a dual mechanism of action: it inhibits the growth of decay causing fungi and induces defense response in host tissues. It was used both in pre-harvest and post-harvest applications: Pre-harvest chitosan treatment provided the highest decay reductions (over 80%) when applied 1 d before harvest, on three different cultivars (Romanazzi *et al*., 2012). At post-harvest, Chitosan must be dissolved in an acid solution in order to activate its antimicrobial and eliciting properties, and acetic acid is the best acid for this purpose and effective under low doses between 0.1- 1% (Romanazzi *et al*., 2017).

Finally, the use of all these compounds presents a minimal dietary residue and environmental issues. Their combination could show a promise future for the decay treatments.

GRAS type decontaminating agents

Several GRAS-classified sanitizers have been tested to extend post-harvest storage of table grapes, including acetic acid, electrolyzed oxidizing water, ozone, calcium chloride (CC) and ethanol; Hot water treatment, chlorinated wash, rachis removal and modified atmosphere packaging (MAP).

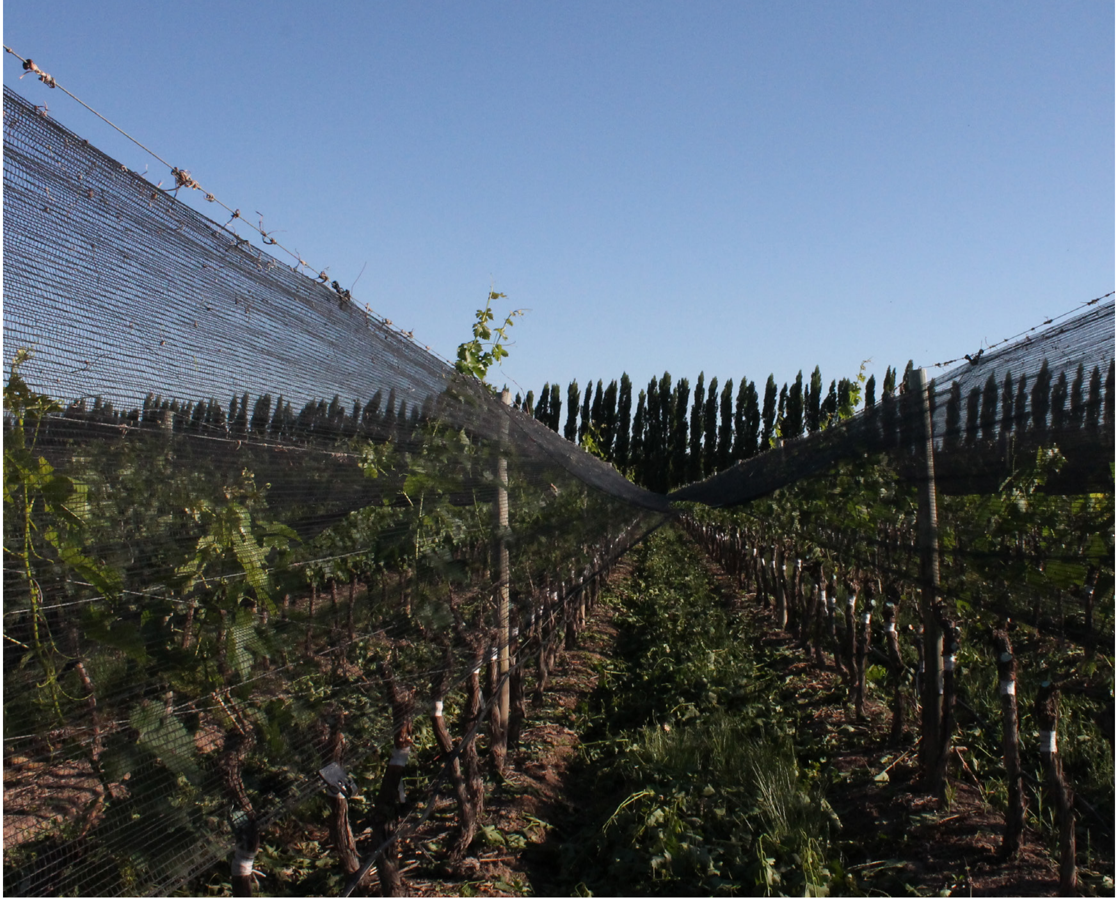
Some gas treatments may be a commercial alternative to the use of SO_2 generators for keeping quality of grapes, in cold storage for up to 2 months at 0 ºC followed by 1 week at 15 ºC in air. **MAP** of 5-15 kPa O_2 and CO_2 may be useful for commercial application and consumer satisfaction, due to being the cheapest and easiest technique. 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 ºC for 2–6 d, effectively reduced decay (Romanazzi *et al*., 2012).

Also, the near-neutral (pH 6.3–6.5) **electrolyzed oxidizing water** completely killed *B. cinerea* conidia at 10 g L^{-1} (Romanazzi *et al*., 2012).

Ethanol is a common food ingredient with antimicrobial activity, and is considered safe for use with food. Ethanol dips and vapors were reported to control postharvest diseases of table grapes, especially when heated. There is a gradual increase in the ethanol activity in killing fungal spores with the increase in its concentration. Rates higher than 30% rapidly killed conidia of *B. cinerea*, while those 20% and lower were sub-lethal (Romanazzi *et al*., 2007).

Ethanol was applied in 3 different ways to table grapes: by dipping (e.g. in a 50% solution for 10 s), or placing inside the package a container with a wick and ethanol (e.g. doses at 4–8 mL kg^{-1} grapes) or in a paper containing ethanol (Romanazzi *et al*., 2012).

Commercial ethanol (Antimold® sachets; Freund Industrial Co., Ltd., Tokyo, Japan) reduces decay of berries by 95–97% and is a good alternative to SO_2-generating pads to prevent



the decay of grapes during short term storage (Ustun *et al*., 2012) for one month. But if it is combined with SO_2, and packaged into MAP bags, the storage life of grapes could extend up to 3 months (Candir *et al*., 2012).

Ozone has been extensively tested for the control of table grape decay (Mlikota Gabler *et al*., 2010). It is fungistatic, effective to control decay, although it is dose dependent, and high concentrations (above 5000 ppm h^{-1}) can be phytotoxic. Even being classified as "organic" by the USDA, the risk of injury to table grapes from ozone has not been completely evaluated. So, the use of continuous low concentrations of ozone (rather than high concentrations) is preferred to minimize the risks of injury to people, the fruit, refrigeration equipment and to minimize the cost of the equipment (Feliziani *et al*., 2014). It was necessary to adjust ozone concentrations upward to ensure sufficient ozone diffused into the packages to control gray mold. Therefore, a constant concentration of 0.1-0.2 $\mu l \cdot l^{-1}$ would need to inhibit the development of aerial mycelial growth of *B. cinerea*. Ozone also, could be used in strategies with reducing sulfur dioxide doses (e.g. initial SO_2 fumigation or fumigation low frequency, from weekly to biweekly, with storage in ozone between fumigations; Feliziani *et al*., 2014). Postharvest ozone treatment has another benefit in that it enhances synthesis of resveratrol and other bioactive phenolics in grapes (Romanazzi *et al*., 2012).

Although in many controlled laboratory studies, ozone gas inhibited gray mold spread among stored grapes, little has been published about ozone use under commercial conditions (Feliziani *et al*., 2014). Therefore, the use of continuous 0.1 $\mu l \cdot l^{-1}$ O_3 was not sufficient for commercial purposes (Artés-Hernández *et al*., 2004).

In conclusion, fumigation with high doses of ozone gas during pre-cooling of grapes controlled postharvest decay and reduced residues of four commonly used fungicides.

Ozone is unlikely to replace SO_2 treatments in conventional grape production unless their efficacy is improved, but it could be an acceptable technology to use with grapes marketed under "organic" classification, where the use of SO_2 is prohibited, or if SO_2 use were to be discontinued. The reactive nature of ozone, like that of SO_2, requires these chambers to be designed to resist damage caused by these corrosive gases (Mlikota Gabler *et al*., 2010).

Physical means

Physical technologies involve variations in temperature, UV-C irradiation, microwaves, hypobaric treatments or changing atmospheric composition. They are all postharvest practices which require significant adaptation by an industry which is accustomed to minimal intervention during harvest.

Some combinations or sequential treatments technologies should help (Kou *et al*., 2009) in order to optimize a treatment for cluster grapes as an alternative to sulfur dioxide. These treatments are focused on reducing decay and microbial growth and maintaining grape quality during the storage. They are the following:

- Hot water treatment (e.g. at 45 ºC for 8 min).
- Sanitized or chlorine washing.
- Cutting the rachis 1 to 2 mm from the berries
- Modified atmosphere packaging (MAP).

Grapes treated under **controlled atmosphere** conditions (0.5 ºC with 95–98% relative humidity) with a continuous flow air pre-treatment (40% CO_2+ 60% N_2) applied from 24 h to 48 h (initial fumigation) and then, stored in air or controlled atmosphere (atmospheric air) could help to decrease the incidence of decay. It could be a commercially feasible alternative for postharvest handling of organic grapes (Teles *et al*., 2014). Recently, the use of microwaves for few minutes on table grape bunches kept in plastic boxes and covered with semi-permeable film was applied in large scale in packinghouse (Lichter and Romanazzi, 2017).

AGRONOMIC SOLUTIONS

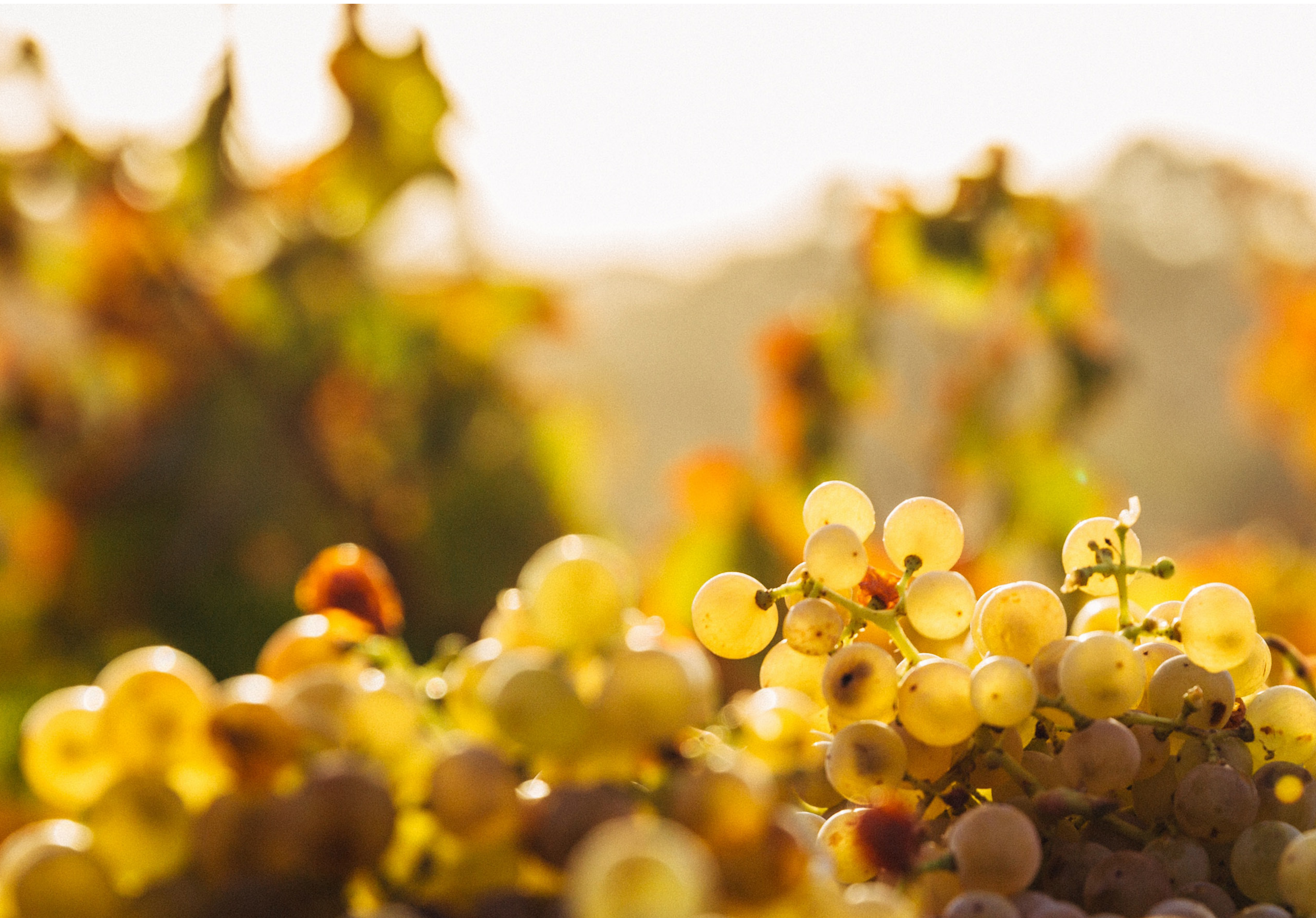
The above mentioned alternatives can be accompanied by a series of agronomic solutions in order to assist the alternatives of use of both SO_2 and hormones. Irrigation, light, summer and winter pruning, girdling, fertilizing, planting distances, etc. could be mentioned.

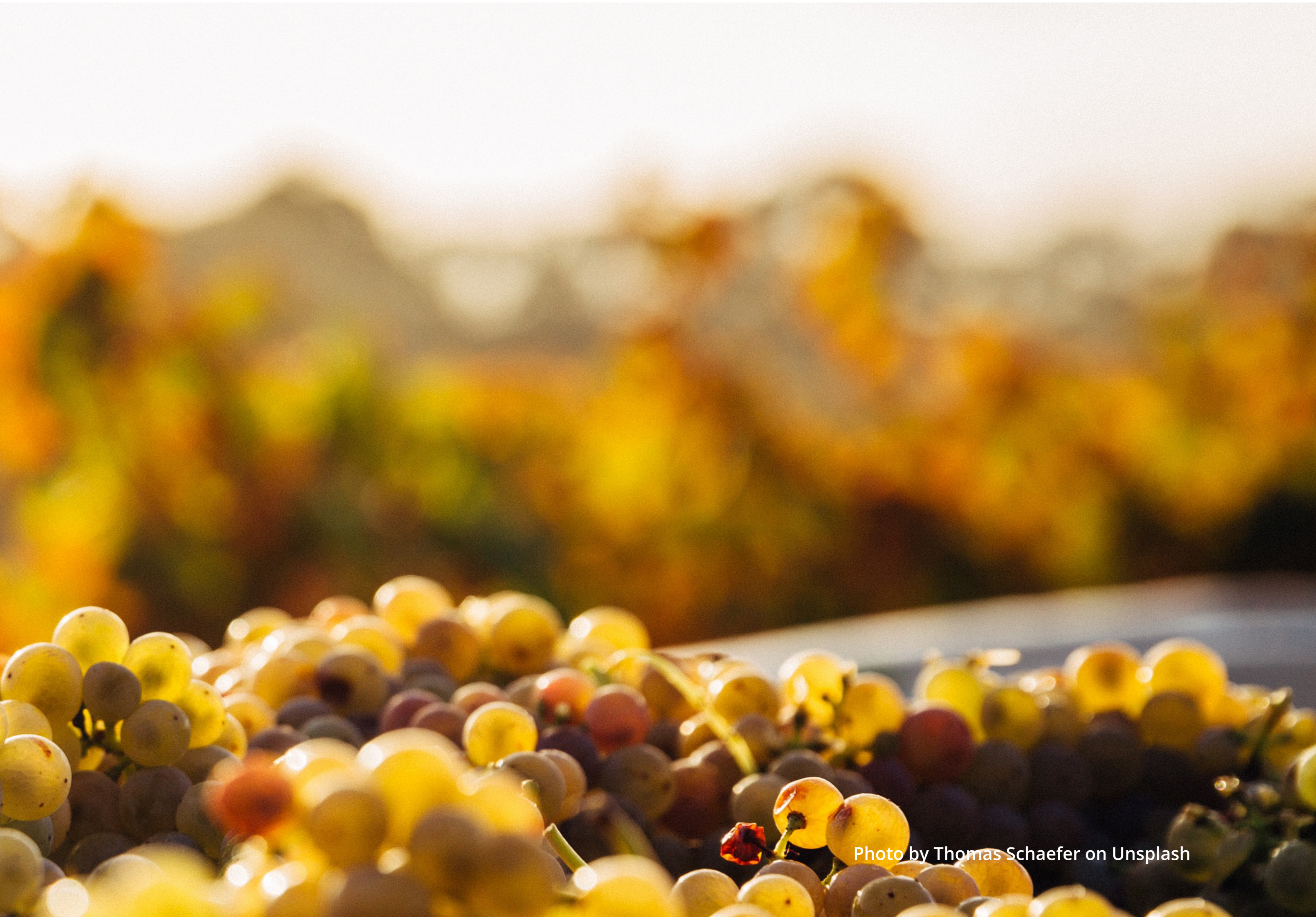
Usually, the integration of canopy management and fungicide treatments before harvest with the use of SO_2 and cold storage after harvest, are the commercial strategies implemented to control this disease (Youssef and Roberto, 2014).

FUTURE OBJECTIVES

The ideal alternative (Romanazzi *et al*., 2012) of controlling gray mold of table grapes, should meet the following criteria:

- Efficacy equivalent or better than the current practice.
- Will not injure or cause phytotoxic effects.
- Will not compromise the organoleptic quality of the grapes.
- Will not be a threat to human health and the environment.
- Compatible with standard practices, affordable and easy to implement.
- Compatible with the principles of organic agriculture.
- Offer substantial benefits to the technology manufacturer which often plays a pivotal role in commercialisation of novel treatments.

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