Postharvest Biology and Technology 125 (2017) 91–98

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Repeated treatments with acetic acid vapors during storage preserve table grapes fruit quality

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ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 8 November 2016
Accepted 10 November 2016
Available online xxx

Keywords:
Postharvest
Botrytis cinerea
GRAS
Simulated marketing period

ABSTRACT

Postharvest losses on table grapes caused by *Botrytis cinerea*, are controlled with SO_2 fumigations carried out every 7 or 10 d. The use of this gas is becoming more difficult to justify because of undesirable effects on the fruit and the increasing concern for human health. Objectives of the paper were to evaluate if repeated treatments with acetic acid (AC) during storage, were effective in preserving table grapes quality, comparing in addition the effects of AC and SO_2 treatments. Experiments carried out *in vitro* on *B. cinerea* proved that the effect of AC on mycelia growth and conidia germination was related not only to the dose and exposure period, but also to the elapsed time between fungal inoculation and treatment. The reinoculum test demonstrated that a treatment with 20 $\mu L L^{-1}$ of AC for 15 min had a fungicidal effect. A laboratory test was performed, to evaluate *in vivo* the effectiveness of AC on *B. cinerea*. Results suggested that higher doses were needed to control the pathogen. On naturally infected table grapes two storage experiments were carried out: in the first trial a single AC concentration of 50 $\mu L L^{-1}$ was used to perform one or two fumigations after 4 or 8 weeks (w), while three different AC concentrations (30, 50 and 75 $\mu L L^{-1}$) were used in the second trial and fumigations were repeated 5, 3 and 2 times respectively. Treatments lasted 15 min and fruit was stored for 8 w at 5 °C and 90% RH, followed by 3 d of a simulated marketing period. All treatments reduced gray mold incidence, with respect to untreated fruit, after 8 w of storage, but repeated treatments resulted the most effective. Two fumigations at 50 $\mu L L^{-1}$ or 5 fumigations at 30 $\mu L L^{-1}$ reduced gray mold incidence by 63.6 or 57.1% respectively.

Fruit weight loss was significantly reduced by all treatments, while quality parameters resulted not to be affected by any of the treatments.

1. Introduction

The most important postharvest disease for table grapes is gray mold caused by *B. cinerea* Pers.:Fr. (Droby and Lichter, 2004), which lead to a rapid deterioration of the fruit. The pathogen causes severe economic losses, because of its latent behaviour only causes symptoms after ripening of the grapes. Control of gray mold is particularly important during storage because it also develops at cold temperatures and spreads rapidly throughout berries. Currently, losses caused by this pathogen are controlled with a rapid refrigeration of the fruit, executed together with a postharvest fumigation with SO_2. Further fumigations with SO_2 are needed during storage, carried out every 7 or 10 days, to avoid mold development (Luvisi et al., 1992; Droby and Lichter, 2004). SO_2 is an additive employed in the food manufacturing and its use turns out to be an effective method in the control of the pathogens and allows storing the grapes at high humidity values. However, despite of such advantages, the use of SO_2 not always leads to satisfactory results, because it causes off-flavour, bleaching of the berries and browning of the rachis (Nelson and Richardson, 1967; Marois et al., 1986; Chervin et al., 2005). Moreover, the need of repeated fumigations during storage increases the risk of SO_2 residues on the berries (Karabulut et al., 2003), that can be harmful to people allergic to sulphites, although in a previous paper, Austin et al. (1997) reported that "current industry practices, leave sulfite residues well below the official legal tolerance". An additional reason to find alternatives is that the use of synthetic fungicides and SO_2 is not allowed in organically certified grapes (Mlikota Gabler and Smilanick, 2001) and there is an increasing demand of products with low chemicals levels. Mounting concerns of

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http://dx.doi.org/10.1016/j.postharvbio.2016.11.010
0925-5214/

consumers and health authorities about risks associated with chemical residues in food have led to imposing strict regulations and even banning the use of certain chemical groups. These developments have driven the search for alternative strategies that are effective and not reliant on conventional fungicide applications (Romanazzi et al., 2016).

Many researches have been carried out as alternatives for the control of postharvest decay of table grapes. Romanazzi et al. (2012) have grouped the alternative means in four categories: biocontrol agents, natural antimicrobials, Generally Recognized As Safe (GRAS) type decontaminating agents and physical means. On table grapes, reduction of the most economically important disease has been accomplished with preharvest and/or postharvest treatments with GRAS salts such as sodium bicarbonate, sodium carbonate, potassium sorbate, potassium bicarbonate, potassium carbonate or calcium chloride (Palou et al., 2016). Furthermore, the use of compounds such as ethanol (Lichter et al., 2002; Lurie et al., 2006), chitosan (Xu et al., 2007), ozone (Mlikota Gabler et al., 2010), boron (Qin et al., 2010) and combination of chitosan and ethanol (Romanazzi et al., 2007) have been investigated with some success. In the search of new environment and consumer friendly technologies that can reduce toxic residues, a GRAS compound such as AC is a valid candidate that meets most of the safety and environment requirements. The efficacy of this compound was extensively investigated on a variety of fruits such as apples and pears (Sholberg et al., 2001, 2004), stonefruit (Sholberg and Gaunce, 1996), mandarins (Venditti et al., 2009) and kiwifruit (Lagopodi et al., 2009), resulting effective in preventing many decay-causing pathogens including *B. cinerea*, *Penicillium expansum*, *P. digitatum* and others. AC was also employed on table grapes for the control of gray mold (Sholberg et al., 1996), where four fumigation levels were tested. In this experiment in addition to the control and the pre-storage treatment, further fumigations were performed during cold storage: treatments on fruit were repeated biweekly (4 fumigations) and weekly (7 fumigations). The outcome of this research was that among treated fruit, fumigation frequency resulted to be irrelevant in decay control, differences were only found between control and treated fruit, independently of the number of fumigations. Since results of this paper suggest that only one fumigation with AC can be effective in controlling decay on table grapes for 6 w, the present work was performed to evaluate: (I) the possibility to store table grapes for 8 w at 5 °C; (ii) the effectiveness of repeated fumigations during storage, establishing the most appropriate timing, also considering a subsequent simulated marketing period (SMP); and (iii) to evaluate and compare the effect of AC and SO_2 treatments on table grapes, considering the external appearance and sensory quality.

2. Materials and methods

The effectiveness of treatments with AC in controlling the pathogen development was investigated *in vitro*, by a laboratory test with detached berries and with storage trials.

2.1. Fruit

The experiment was carried out with local table grapes (*Vitis vinifera* L. cv. 'Taloppo'), a late ripening cultivar, with seeded green berries medium in size. Fruit was handpicked from a commercial vineyard, located in northwest Sardinia, Italy (40° 43′ N), managed using standard horticultural practices and delivered on the same day to the laboratory where small, loose and decaying berries were removed. Some clusters were used to cut the berries needed for the laboratory test, while the other bunches were sorted in groups uniform in size for the storage trials, weighed and placed in plastic boxes.

2.2. Pathogen

The strain of *B. cinerea* was isolated from naturally decayed berries and cultured on potato dextrose agar (PDA, Fluka-Sigma-Aldrich Buchs, Schweiz) for 10 d at 23 °C in a thermo regulated cabinet. Spores were harvested from Petri dishes by adding 10 mL of distilled water, containing 0.05% (w/v) of Tween 80 (Sigma-Aldrich Buchs, Schweiz) and gently scraping the spores from the surface with a sterile loop. The conidial suspension obtained was passed through four layers of sterile cheesecloth and then adjusted to a concentration of 1×10^5 conidia mL^{-1} with a haemocytometer.

2.3. Treatments

All treatments were performed by using airtight chambers specifically created for AC fumigations as described in Venditti et al. (2009). Briefly, each dose of glacial AC (Carlo Erba, Milan, Italy), according to the different treatments, was injected in a heated glass vessel and the samples were treated for 15 min by turning on the circulation fan.

2.4. Effect of AC on B. cinerea growth on PDA

The effect of AC on the radial growth of the pathogen mycelia was evaluated *in vitro* on PDA, by performing treatments at different concentrations (0, 5, 10 and 20 $\mu L\,L^{-1}$) for 15 min. PDA was prepared as recommended by the manufacturer and added to Petri dishes, left overnight in a hood under ultraviolet light and laminar air flow. The conidial suspension was introduced in the center of each dish by injecting an aliquot of 20 μL. Treatments were applied after 0, 24 and 48 h (T0, T24 and T48) the inoculum and dishes, before or after fumigations, were stored at 20 °C with 12 h light/dark cycles. The increase in radial growth of the pathogen was daily recorded up to 8 d using a digital ruler. Results are reported as the diameter of the fungal colony. Each treatment consisted of nine replicates and the entire experiment was repeated twice.

In order to investigate if the effect of AC was fungistatic or fungicide a reinoculum test was also carried out. The experiment was performed by transferring and placing upside-down a disk of agar (2 cm Ø), containing the mycelia obtained from dishes treated 48 h after the inoculum, in the center of a Petri dish containing fresh PDA.

2.5. Treatment of single berries

A laboratory test was carried out to evaluate *in vivo* the effectiveness of AC on the pathogen. Healthy berries cut from the clusters with the pedicel were disinfected by immersion for 2 min in a NaOCl solution (0.2%), rinsed twice with distilled water and air-dried. Afterwards the pedicels were removed and the berries randomized and placed in multiwell plates to avoid the contact among them. Inoculum was performed by injecting 10 μL of the spore suspension, prepared as above, into the pedicel insertion point. The multiwell plates were treated 0, 24 and 48 h after the infection at different concentrations (0, 5, 10, 20, 50, 75 and 100 $\mu L\,L^{-1}$) of AC. The first fumigation was performed after the inoculums had dried in air for 60 min. The plates were subsequently aerated for 30 min, placed in plastic boxes with a wet filter paper on the bottom, to ensure a relative humidity close to saturation and held at 20 °C for 7 d. The treatments were applied at three replicates of 24 berries each and the experiment was repeated twice.