ACETIC ACID TREATMENTS TO KEEP POSTHARVEST QUALITY OF "REGINA" AND "TALOPPO" TABLE GRAPES

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SUMMARY

The most important postharvest pathogen for table grape is *Botrytis cinerea* (gray mold), which cause a rapid deterioration of fruit. An effective control of the disease during storage is difficult and remains an unsolved problem since no pesticide treatments are allowed by European legislation. GRAS compounds, employed with no restriction as preservatives in Europe and North America, are possible candidates to fulfil this gap.
The aim of this work is to study the efficacy of Acetic Acid (AAC), used as postharvest treatment to control *Botrytis cinerea* on "Regina" and "Taloppo" table grapes, by laboratory and storage tests.
The activity of this compound was first assessed with laboratory tests, treating at different concentrations (0, 5, 10, 20, 50, 75 and 100 µl/L) of AAC vapors, for 15 minutes, single berries inoculated with *B. cinerea*. After treatments fruit was incubated at 20°C for one week.
The in vivo experiment took place by using the most promising AAC concentrations (50, 75 and 100 µl/L) followed by eight weeks of storage at 5°C and 95% of relative humidity (RH) and four days at 20°C and 85% RH (simulated shelf-life conditions). At the end of the in vivo experiment decay, weight loss and visual assessment were evaluated.
Almost all treatments, after eight weeks of storage, reduced the incidence of gray mould. The best results were achieved by using 50 ppm of AAC, gaining a reduction of decay, compared to untreated "Taloppo" and "Regina" grapes of 61.0% and 41.4%, respectively. Following the simulated shelf-life period differences between treated and untreated (control) became no significant for "Taloppo" grape, while the lowest decay percentage was reached with 50 µl/L of AAC for "Regina" grape (52% of reduction if compared to control).
Regarding fruit weight loss all treatments did not affect significantly this parameter that ranged between 8.2% and 11.5% after eight weeks of storage and 13.5% and 18.2% after shelf-life.
At the end of storage the highest visual score was attributed to fruit treated with 50 µl/L of AAC evidencing a clear better keeping quality. During this period slight treatment damages were observed on berries following application of AAC at 75 and 100 µl/L.
The reported results obtained with these experiments showed that Acetic Acid could be a promising compound to be used as alternative to SO_2 in keeping grapes quality and controlling decay during storage.

Key words. Postharvest, GRAS, acetic acid, table grapes.

INTRODUCTION

The most important postharvest pathogen for table grapes (*Vitis vinifera* L.) is *Botrytis cinerea* Pers. (Droby and Lichter, 2004), which cause a rapid deterioration of fruit. Blue mould caused by *Penicillium* spp. is also considered a problem for table grapes because of its capability to develop even when fruit is stored at low temperatures, close to 0°C (Snowdon, 1990).

Currently, losses caused by these pathogens are controlled with a rapid refrigeration of the fruit, executed together with a fumigation of sulphur dioxide. Further fumigations with SO_2 are needed during storage, executed every 7 or 10 days, to avoid mould development (Luvisi *et al.*, 1992).
Sulphur dioxide 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 to high humidity values concurring to limit rachis browning.
However despite of such advantages, the use of SO_2 not always leads to satisfactory results, because of taste alterations of pulp and in some cultivars, more sensitive, damages to berries and browning of the skin or the rachis (Marois *et al.*, 1986). Moreover, the need of repeated fumigations during storage increases the residues of SO_2 on the berries, and the risk to exceed the maximum levels allowed by current laws. Furthermore the use of this gas is becoming more difficult to justify, because of the increasing concern for human health. Such motivations, as well as environmental considerations, have induced some European nations to proscribe the use of sulphur dioxide for table grapes.
For these reasons an effective control of storage rots is very difficult and remains an unsolved problem, since treatments with synthetic fungicides are not allowed by European legislation. GRAS compounds, employed with no restriction as preservatives in Europe and North America, are possible candidates to fulfil this gap.
In the search of new environment and consumer friendly technologies, that can reduce toxic residues, a GRAS compound such as acetic acid (AAC) has been tested as an alternative control mean.
AAC is a common food additive, classified by the U.S. Food and Drug Administration as generally recognized as safe (Barkai-Golan, 2001). This compound meets most of the environment and safety requirements and its use is allowed with no restriction in Europe and North America.
The aim of the present work was to evaluate the effectiveness of acetic acid, used as postharvest treatment, to control storage rots caused by Botrytis cinerea or other pathogens on "Regina" and "Taloppo" table grapes, by laboratory tests and storage trial.

MATERIALS AND METHODS

Fruit

The experiment was carried out with table grapes (*Vitis vinifera* L.) of the cultivar "Regina" and the Sardinian cultivar "Taloppo", handpicked at commercial maturity from an experimental vineyard, belonging to the "Consorzio Provinciale per la Frutticoltura" of Sassari, located in the northwest part of Sardinia, Italy, managed using standard horticultural practices.
The same day of harvesting the fruit was immediately transported to the laboratory, where small and decaying berries were removed.
The bunches were sorted in three groups uniform in size, for the storage trial, weighed and placed in plastic boxes, while an additional group was used as control.
For the laboratory tests individual berries were cut with the pedicel, disinfected by immersion for 2 min in a sodium hypochlorite solution (0.2%), rinsed twice with distilled water and air-dried. Afterwards the pedicels were removed and the berries placed in multiwell plates for the inoculum.

Inoculation

The strain of *B. cinerea* used to infect the berries was isolated from naturally decayed grapes and cultured on potato dextrose agar (PDA, Fluka-BioChemika) for 10 days at 23°C in a thermo regulated cabinet. Spores were harvested from Petri dishes (9 cm diameter) by adding 10 ml of distilled water, containing 0.05% (w/v) of Tween 80 (Sigma) and gently scraping the spores from the surface with a sterile loop. The conidial suspension obtained was passed through two layers of cheesecloth and then adjusted to a concentration of 1×10^5 conidia/mL with a haemocytometer.
Infection of single berries was performed by injecting, with a micro syringe, 10 µl of the suspension of *Botrytis cinerea*, into the pedicel insertion point.

Treatments

Laboratory tests were first carried out to evaluate the effectiveness of AAC on the pathogen. The multiwell plates with the inoculated berries were treated at different concentrations (0, 5, 10, 20, 50, 75 and 100 µl/L) of vapours of the compound.
Fumigations were performed by placing the samples in six chambers equipped with a circulation fan attached to the top of the lid. Each lid was supplied with a rubber septum for AAC applications and once the chambers were closed, the lids provided an airtight system.
Each dose of glacial acetic acid (Carlo Erba) was injected with a micro syringe through the rubber septum into a heatproof glass vessel beneath the septum. The vessels of each chamber were placed on warm electric resistances connected with a power supply.
Once the required amount of AAC was injected, the circulation fans were turned on to evaporate and circulate the compound within the chambers. Samples were fumigated for 15 min, then incubated at 20°C for one week.
The *in vivo* experiment took place by using the most promising AAC concentrations used for the laboratory test (50, 75 and 100 µl/L). In this case the bunches were placed inside the chambers and then fumigated as described above.
The grapes were then stored for eight weeks at 5°C and 95% of relative humidity (RH) followed by four days at 20°C and 85% RH to simulate commercial shelf-life (SSL). At the end of the storage experiment, weight loss and decay percentage were determined.
Visual appearance of grapes was also assessed by using a scoring system proposed by Lurie *et al.* (2006). Bunch freshness and rachis desiccation were scored on a scale from 1 to 5, with 1 being the highest grade as at harvest, and 5 being wilted or desiccated bunches. Damages on berries such as cracking and browning were also assessed on a similar scale from 1 to 5.

Data Analysis

Analysis of variance (ANOVA) of all data was performed using the MSTAT-C software (Michigan State University, East Lansing, 1995), and when appropriate mean separations were performed according to the Duncan's multiple range test at $P< 0.05$. Angular transformation of decay percentage values was performed prior statistical analysis.

RESULTS

The laboratory trial was conducted to evaluate, preliminarily, the most effective dose of AAC in controlling *Botrytis cinerea* on artificially inoculated berries of the Sardinian cultivar "Taloppo". After 1 week of incubation at 20°C, the effect of fumigations on the pathogen was commensurate to the increase of the dose of AAC (data not shown). A complete inhibitory effect was achieved at concentrations of 50, 75 and 100 µl/L, whereas lower doses did not totally control the pathogen. Regarding visual assessment a slight browning was observed on the berries fumigated with 75 and 100 µl/L.

Storage trial

The storage experiment was conducted on the basis of the most promising AAC concentrations of the previous trial. Fumigations of table grapes were performed at 50, 75 and 100 µl/L and almost all treatments reduced the incidence of gray mould on bunches of both varieties, after a storage period of eight weeks. Among the three concentrations tested the best result was achieved by using 50 µl/L of AAC, that significantly ($P \leq 0.05$) reduced the percentage of decay, as compared to untreated control, with reduction ranging from 61.0% and 41.4%, respectively for "Taloppo" and "Regina" cultivars. Data reporting percentage of rots, weight loss and visual assessment for the two cultivars are shown in tables 1 and 2.
A significant reduction of rot incidence was also gained for "Taloppo" grapes, at the highest concentration (100 µl/L), whereas for "Regina" grape, fumigations with the doses of 75 and 100 µl/L did not improve the control of the pathogen.
During the following simulated shelf-life period of four days, at the relatively warm temperature of 20° C and 85% RH, differences between treated and untreated bunches of "Taloppo" grapes became no significant. "Regina" grapes showed a different behaviour, reaching the lowest decay percentage, when fumigated with 50 µl/L of AAC, with a reduction of 52% if compared to the control.
Regarding fruit weight loss, after eight weeks of cold storage, in "Regina" grapes all treatments did not affect significantly this parameter that ranged between 6.1 and 6.8%. In "Taloppo" grapes an increased weight loss was observed for bunches fumigated with the two highest concentrations of AAC (75 and 100 µl/L), while fumigation with 50 µl/L did not influence this parameter that was similar to the control. After the simulated shelf-life period, significant differences were not observed, for both cultivars, for any of the treatments performed.
Rachis drying was also evaluated at the end of storage and the same scores were attributed to the control and fruit treated with 50 µl/L, while increasing the AAC concentration, the rachis completely desiccated.
During this period treatment damages were also assessed for berries and the two varieties showed similar results. Slight browning was observed on berries following application of AAC at 75 µl/L, which became more evident for berries treated at 100 µl/L. The fruit fumigated with 50 µl/L of AAC gained the highest score, even better than the control.

Table 1. Rot incidence, weight loss, rachis drying and berry browning among "**Regina**" table grapes bunches fumigated with three AAC concentrations, after 8 weeks of storage and 4 days of SSL.

AAC (µl/L)	8 weeks				SSL			
	Rots (%)	Weight loss (%)	Rachis drying	Berry browning	Rots (%)	Weight loss (%)	Rachis drying	Berry browning
0	12.9 AB[x]	6.1 A	3	2	35.9 A	10.1 A	4	2
50	7.5 B	6.2 A	3	1.5	17.2 B	9.3 A	4	1.5
75	10.0 AB	6.7 A	5	2.5	44.4 A	10.6 A	5	2.5
100	14.9 A	6.8 A	5	3	39.8 A	12.3 A	5	3

[x] Values with the same letter are not significantly different

Table 2. Rot incidence, weight loss, rachis drying and berry browning among "**Taloppo**" table grape bunches fumigated with three AAC concentrations, after 8 weeks of storage and 4 days of SSL.

AAC (µl/L)	8 weeks				SSL			
	Rots (%)	Weight loss (%)	Rachis drying	Berry browning	Rots (%)	Weight loss (%)	Rachis drying	Berry browning
0	17.8 A[x]	8.2 C	3	2	44.6 A	13.5 A	4	2
50	6.9 B	8.7 C	3	1.5	46.6 A	14.0 A	4	1.5
75	11.3 AB	10.0 B	5	2.5	53.2 A	15.5 A	5	2.5
100	9.6 B	11.5 A	5	3	52.0 A	18.2 A	5	3

[x] Values with the same letter are not significantly different

DISCUSSION

The aim of the present work was to evaluate if acetic acid, performed as a fumigant, can be used as a postharvest treatment to reduce storage decay on table grapes.

Currently, losses are controlled with an initial sulfur dioxide treatment, followed by subsequent fumigations during storage, executed every 7 or 10 days. However sulfite residues on the fruit and phytotoxicity are the main problems associated with this type of treatment.

In the last years new strategies have been exploited to overcome these difficulties. Hydrogen peroxide performed as vapour phase was evaluated by Forney *et al.* (1991), but H_2O_2 applications even if reduced germination of *Botrytis* conidia, caused drying of pedicels and rachis.

Acetaldehyde vapours have also been considered as a possible alternative to SO_2, but this compound negatively affected grapes composition and sensory quality. Pesis and Frankel (1989) found that treatment with acetaldehyde vapours damaged berries of "Thompson seedless" grapes and left some off-flavour.

Most recently Crisosto *et al.* (2002) evaluated a range of CO_2 and O_2 concentrations for controlling decay development during storage of table grapes. They found that concentrations of CO_2 above 10 kPa limited *Botrytis* decay even if accelerated stem browning and off-flavor.

Lurie *et al.* (2006) tested new methods of applying ethanol to prevent storage decay of table grapes. The ethanol vapor was successful in controlling rots as well as or better than SO_2, but in some cases caused high levels of berry browning, perhaps because of elevated concentrations of acetaldehyde inside the packages.

Pre-harvest applications of salts were also assessed by Nigro *et al.* (2006), for the control of storage rots. Some salts showed an activity higher or similar to that of conventional chemical treatments.
In the search for new products and new methodologies as alternative control means to SO_2, we tested fumigation with acetic acid on two cultivars of table grapes.
The effectiveness of the treatments with AAC vapors in preventing postharvest decay, caused by various pathogens such as *Botrytis cinerea* and *Penicillium expansum*, is reported in previous trials conducted on many fresh produces (Sholberg and Gaunce, 1996; Sholberg *et al.*, 2001; Liu *et al.*, 2002; Sholberg *et al.*, 2004).
Fumigation with AAC was also tested on table grapes by Sholberg *et al.* in 1996. They concluded that fumigation of table grapes with AAC is a realistic alternative to SO_2, even if the method needs to be developed to avoid fruit injury.
In this trial we tested a different technique of application of the AAC, with respect to those utilized in previous studies, since we used higher concentrations of the GRAS compound, but shorter time of fumigation.
Laboratory tests were conducted as a preliminary screening, to evaluate if AAC fumigation performed with a different technique could result in an inhibitory activity against *Botrytis cinerea*, without damages to the berries. This GRAS completely prevented the pathogen development starting from a concentration of 50 µl/L and berry browning was observed at the two highest values used.
The storage trial confirmed that 50 µl/L was the most effective fumigation. Vapor of AAC performed at this concentration significantly reduced decay, with respect to the untreated grapes of both varieties, after eight weeks of cold storage at 5°C. This was also the concentration that reached the highest visual score, evidencing a better keeping quality.
Regarding the simulated shelf-life period it is likely that will be necessary to repeat the treatments during storage to improve the control of decay, as well as it happens for fumigations with sulfur dioxide.
In our experiment we did not contemplate a sensory evaluation, but informal tasting of the fruit did not indicate any difference between treated grapes and the control. These, preliminary results indicate that AAC fumigations do not affect the internal quality of the berries.
In conclusion fumigations with AAC are promising and represent a realistic alternative to SO_2 that could maintain table grapes quality in cold storage.

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