Original article

In-package use of *Muscodor albus* volatile-generating sachets and modified atmosphere liners for decay control in organic table grapes under commercial conditions

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In-package use of *Muscodor albus* volatile-generating sachets and modified atmosphere liners for decay control in organic table grapes under commercial conditions.

Abstract — Introduction. In-package biofumigation with the volatile-producing fungus *Muscodor albus* was tested to control fungal decay in organic table grapes stored at a commercial packinghouse. **Materials and methods**. Sachets containing two different amounts of activated *M. albus* culture were inserted into shipping boxes containing approximately 4.5 kg of 'Thompson Seedless' or 'Red Seedless' table grapes. The volatiles were contained inside the boxes either by wrapping pallets of the boxes externally with plastic film after pre-cooling (pallet wrapping) or by using a modified atmosphere liner inside each box. Decay incidence was evaluated after 7 weeks of storage at 0 °C. **Results**. The *M. albus* sachets reduced decay incidence among 'Red Seedless' table grapes in both wrapped pallets and boxes with liners. In this cultivar, the modified atmosphere liner alone reduced decay incidence by about 70% and the *M. albus* treatment in the liner further reduced decay incidence, regardless of the amount of *M. albus* used. The combination of the *M. albus* sachet and the modified atmosphere liner proved to be the most effective decay control treatment. Decay incidence was lower among 'Thompson Seedless' table grapes and a significant decay control was only observed after the grapes had been allowed to warm up after storage with the 50-g rates applied inside the liner. No adverse effects were associated with the treatment or the liners. **Discussion**. Based on our results, biofumigation with *M. albus* sachets is compatible with the commercial handling of organic table grapes and could provide significant improvement in their shelf life.

USA / *Vitis vinifera* / dessert grapes / postharvest technology / *Muscodor albus* / controlled atmosphere storage / integrated control

Utilisation en conditionnement de sachets produisant du *Muscodor albus* volatil et du film à atmosphère modifiée pour le contrôle des maladies de conservation du raisin de table organique en conditions commerciales.

Résumé — Introduction. La biofumigation de la production volatile du champignon *Muscodor albus* a été étudiée pour le contrôle des maladies de conservation du raisin de table organique stocké en entrepôt commercial. **Matériel et méthodes**. Des sachets contenant deux quantités différentes de culture activée de *M. albus* ont été insérés dans des cartons d'expédition contenant approximativement 4,5 kg de raisins de table aspermes 'Thompson Seedless' ou 'Red Seedless'. Les composés volatils ont été maintenus à l'intérieur des boîtes soit en enveloppant extérieurement, après pré réfrigération, les palettes de cartons avec un film plastique (palettes enveloppées), soit en utilisant un film à atmosphère modifiée placé à l'intérieur de chaque carton. L'incidence sur le pourrissement a été évaluée après 7 semaines de stockage à 0 °C. **Résultats**. Les sachets de *M. albus* ont réduit l'incidence des maladies de conservation au sein des raisins de table 'Red Seedless' à la fois dans les palettes enveloppées extérieurement et dans les cartons munis de films intérieurs. Dans ce cultivar, le film à atmosphère modifiée seul a réduit l'incidence sur les moisissures d'environ 70 % et l'utilisation de *M. albus* à l'intérieur des cartons a réduit un peu plus cette incidence, indépendamment de la quantité de *M. albus* utilisée. La combinaison du sachet de *M. albus* et du film à atmosphère modifiée s'est avérée être le traitement de contrôle le plus efficace. L'incidence des maladies de conservation a été plus limitée au sein des raisins de table 'Thompson Seedless' et un contrôle significatif du pourrissement n'a été observé qu'après un réchauffement des raisins après stockage, en présence d'une dose de 50 g placée à l'intérieur du carton tapissé de film. Aucune conséquence négative n'a été associée au traitement ou à l'utilisation de films à atmosphère modifiée. **Discussion**. D'après nos résultats, la biofumigation avec des sachets de *M. albus* est compatible avec la manutention en conditions commerciales des raisins de table organiques ; cette technique pourrait permettre une amélioration significative de leur durée de conservation.

États-Unis / *Vitis vinifera* / raisin de table / technologie après récolte / *Muscodor albus* / stockage en atmosphère contrôlée / lutte intégrée

1. Introduction

Organic grapes are produced without synthetic pesticides, growth regulators or fertilizers. They usually have a more limited shelf life than conventional grapes, which benefit from field applications of synthetic fungicides and are usually fumigated after harvest with SO_2 during pre-cooling and periodically during storage [1, 2]. Because of the lack of products for suppressing mold development, producers of organic grapes have relied mostly on canopy management, manual sanitation and rapid cooling to protect their fruit from decay. Depending on the cultivar, conventional table grapes can usually be held in storage for over three months and shipped as needed by truck to distant markets. In the case of organic grapes, there is less flexibility in holding them because of their shorter shelf life and distant markets can be difficult to reach. Sales to markets in various parts of the United States and Canada can be infeasible since the North American production of organic table grapes is centered in California. Thus, better means of suppressing postharvest decay are needed to ensure retention of acceptable quality during the long-distance shipping required to supply these markets.

Much research has been done in recent years on the biological control of postharvest diseases, as evidenced by the large volume of publications on this topic and the registration of some microbial products for postharvest treatment [3]. Table grapes in California are not usually handled after harvest to preserve their appearance ("bloom"), and because visible residues of treatments are not accepted. The few biological or natural postharvest fungicides that are approved to treat organic fruits have not been developed for this market. For these reasons, biological control of postharvest decay is not being used on grapes.

The fungus *Muscodor albus* isolate CZ 620 produces a potent mixture of antimicrobial volatile compounds with broad-spectrum activity [4]. These volatiles, which are alcohol, acid, ester and terpenoid derivatives, were shown to be lethal to most postharvest decay pathogens [5, 6] and other fungi [4, 7]. Rye grain culture of *M. albus* was effective as a biofumigant in controlling postharvest diseases in inoculated apples, peaches, lemons and grapes [5, 6, 8]. Because of its excellent potential for killing or inhibiting plant pathogens, *M. albus* is being developed as a biopesticide for postharvest disease control and other agricultural applications [9]. *M. albus* has been registered as a biopesticide with the US Environmental Protection Agency [10] and is approved for organic agriculture in the USA according to the rules of the National Organic Program. Depending on the crop, storage conditions, types of storage containers and length of storage, *M. albus* can be more advantageously used to fumigate an entire storage room or individual shipping boxes. A sachet containing a desiccated culture of *M. albus* that is reactivated by hydration was designed for the fumigation of individual shipping boxes [11].

While biofumigation with *M. albus* can control fruit decay at ambient or cold storage temperatures, its use and delivery has to be optimized for the postharvest handling system used for each given commodity. In grapes, the sachet delivery system was first tested in conventional grapes placed in polystyrene containers without liners [12]. While significant reductions in decay occurred, a more optimal containment of volatiles in the shipping container might improve the efficacy of the treatment. The use of non-perforated liners or external pallet wraps could be used for this purpose. Also, the biofumigation treatment might be more appropriate for organically produced grapes, which are not fumigated with SO_2, and thus do not require storage in vented packages to facilitate SO_2 penetration into them during storage room fumigation. The purpose of our trial was to evaluate the efficacy of *M. albus* sachets for controlling naturally-occurring decay of organic grapes stored at a commercial facility. Two different volatile containment methods, pallets wrapped with plastic after pre-cooling and grapes placed in modified atmosphere liners before pre-cooling, were tested for decay control.

2. Materials and methods

2.1. Fruit

Organically produced 'Thompson Seedless' and 'Red Seedless' grapes grown commercially in Porterville, California, were used within hours of harvest. Approximately 500 g of fruit were placed as bunches in open plastic bags which were then put in open cardboard boxes, with nine bags per box. The grapes were not inoculated.

2.2. Biofumigant

A formulation consisting of desiccated rye grain culture of *Muscodor albus* isolate CZ 620, as described previously [9], was used for this study. The culture was stored at 4 °C prior to use. Volatile-generating sachets containing 50 g or 90 g of *M. albus* culture were made with heat-sealable grade 126/3 tea bag paper (Schoeller and Hoesch N.A. Inc., Pisgah Forest, N.C.). The *M. albus* sachets were activated by dipping in water for 15 s, and then held in a plastic tub at ambient room temperature for 2–6 h to ensure reactivation of the culture before use at low temperature [13].

2.3. Storage treatments

A single reactivated *M. albus* sachet was placed over the grapes in the middle of each box. Control boxes received no sachet. Two methods to contain the volatiles from *M. albus* with the fruit were attempted.

One method ("wrapped pallet") was to wrap the pallet of treated boxes with an outside plastic cover after each treated box had received a sachet and was pre-cooled for 12 h. The control grapes were wrapped separately.

The other volatile containment method consisted of a gas-selective liner (high-density polyethylene liner, Xtend®, StePac USA, Encinitas, CA, USA) that is used to retard water loss, and generate a modest increase in carbon dioxide and decrease oxygen content within the package to extend the storage of the grapes. The liner was placed inside each box, with the grapes inside. One *M. albus* sachet was placed inside each treated box and the liners were sealed. Then, the treated and control boxes were pre-cooled for 12 h to about 2 °C. The liners were closed before pre-cooling because otherwise it would have required handling each box again, something that would not be done in a commercial facility. After pre-cooling, the grapes were stored at 0 °C in a commercial cold room. Because of the distant location of the storage facility, changes in atmosphere composition were not measured.

There were two sets of four to five replicate boxes for each treatment and volatile containment method combination.

2.4. Fruit evaluation

After seven weeks of storage, the boxes were taken out and the incidence of decay was evaluated by counting the number of rotten and total fruit in four to five replicate boxes for each treatment. In addition, the liner treatments were also evaluated after a 24-h warming period in a second set of boxes because of concerns that the low decay incidence with the liners, especially in the 'Thompson Seedless', would not allow one to detect differences among treatments. In order to achieve this, the additional set of boxes with liners was taken out of storage and the *M. albus* sachets were removed, after which the boxes were allowed to warm up to ambient room temperature and decay evaluation was done the next day. Percent decay data were analyzed statistically using SuperAnova from Abacus Concepts (Berkeley CA) with analysis of variance and mean separation performed with Fisher's Least Significant Difference ($P = 0.05$).

3. Results

There was a very high incidence of decay among 'Red Seedless' grapes after seven weeks, with approximately 50% decay in the control stored in the wrapped pallet, while the incidence of decay was only 15% among the 'Thompson Seedless' stored under the

Table I.
Effect of postharvest storage treatments on percentage decay in 'Red Seedless' grapes after seven weeks of storage. Means with different letters are significantly different according to Fisher's protected LSD.

Treatments	% decay immediately after storage	% decay after storage + 24 h warming
Boxes in wrapped pallets		
Control	50.6 a	–
50 g biofumigant	33.5 b	–
90 g biofumigant	24.0 bc	–
Boxes with liner		
Control (liner only)	14.6 c	40.3 a
50 g biofumigant	1.4 d	5.2 b
90 g biofumigant	2.6 d	3.9 b
P > *F*	0.0001	0.0084

Table II.
Effect of postharvest storage treatments on percentage decay in 'Thompson Seedless' grapes after seven weeks of storage. Means with different letters are significantly different according to Fisher's protected LSD.

Treatments	% decay immediately after storage	% decay after storage + 24 h warming
Boxes in wrapped pallets		
Control	15.3	–
50 g biofumigant	13.9	–
90 g biofumigant	7.4	–
Boxes with liner		
Control (liner only)	5.5	30.3 a
50 g biofumigant	2.0	4.4 b
90 g biofumigant	2.9	11.4 a
P > *F*	0.0580	0.0157

same conditions (*tables I, II*). The main cause of decay was *Botrytis cinerea*. The use of the modified atmosphere liners significantly reduced decay incidence in 'Red Seedless'. The same tendency could be seen in 'Thompson Seedless', although the statistical analysis had a *P* value slightly above 0.05. The effect of the liner on decay appears to be mostly suppressive, as there was a large increase in decay incidence after the liner was removed and the fruit was left to warm up to room temperature (*tables I, II*).

The *M. albus* sachets reduced decay incidence in both wrapped pallets and boxes with liners (*tables I, II*). For 'Red Seedless', decay control was of particularly large magnitude because of the high decay incidence in the control treatment (*table I*). In this situation, the combination of the modified atmosphere liner with the biofumigant sachets provided the best decay control and both biofumigant rates were equally effective (*table I*). This effect persisted after grapes stored with liners were allowed to warm up to room temperature, suggesting a fungicidal effect (*table I*). The lower decay incidence among the 'Thompson Seedless' grapes makes the assessment of the treatments more difficult, as the effect of the treatments was not statistically significant when the grapes were evaluated immediately after cold storage (*table II*). In boxes with liners that were allowed to warm up after storage, the most effective decay control was obtained with the lower (50 g) rate of *M. albus* (*table II*). For both grape cultivars, there was no effect of biofumigation or modified atmosphere liners on the appearance of the fruit or the rachis. Although no formal taste evaluation was made, no off-flavor or odors were noticed by the evaluators.

4. Discussion

The *M. albus* sachets provided significant decay control in organic grapes, whether the volatiles were contained by an external plastic film wrapping or a modified atmosphere liner. In 'Red Seedless' grapes, where decay incidence was the highest and the effect of the treatments was highly significant, the combination of the *M. albus* sachet and the modified atmosphere liner proved to be the most effective treatment, regardless of the rate of *M. albus* used (*table I*). The evaluation of the 'Thompson Seedless' was more difficult because of the variability associated with a lower decay incidence. In this case, the effect of the 50-g sachet in the liner was highly significant when the grapes had been allowed to warm up to room temperature (*table II*). The modified atmosphere liners and the resulting modified atmosphere did

not prevent *M. albus* from having an antifungal volatile activity, although the effect the liner had on the quantity and profile of volatiles produced is not known. Similarly, *in vitro* experiments showed that conditions of high CO_2 or low O_2 did not affect the ability of *M. albus* to inhibit other fungi through the production of volatiles [14].

While the volatile mixture produced by *M. albus* is effective in killing exposed conidia and hyphae of *B. cinerea* on grapes and controlling decay at various temperatures [9, 15], there are several options for optimizing the use of this biofumigant for commercial use. Containing the volatiles externally by wrapping pallets of boxes with plastic film would probably be the easiest application method, as it allows the use of different types of boxes and does not require modification of packaging protocol, except for adding the reactivated *M. albus* pads during packaging. Wrapping of pallets of grape boxes to contain SO_2 emitted from generator sheets is done commercially in Israel [16].

The gas-selective liner used in our study was effective on its own in suppressing decay and would be worth considering for the storage and shipping of organic grapes, whether or not *M. albus* is used. Modified atmosphere packaging has been found to be an alternative to sulfur dioxide fumigation for several cultivars such as 'Italia' [17], 'Napoleón' [18] and 'Autumn Seedless' [19]. However, modified or controlled atmosphere storage of grapes is rarely employed commercially [20]. The atmospheres are generated slowly with modified atmosphere packaging, because of the relatively low respiration rate of grapes, and elevated carbon dioxide levels can cause browning of the rachis or berries [21–23]. In our study, we did not notice any adverse effect of these liners, although it is likely that they could have slowed down the cooling process. Enclosing warm grapes in the liners before pre-cooling could have accelerated the generation of a modified atmosphere. The combination of modified atmosphere packaging with natural fungicides has also been investigated by others. Inclusion of the natural volatile compound (*E*)-2-hexenal [24] with packaged strawberries or of ethanol or acetic acid with several grape cultivars [25–28] reduced postharvest decay.

Both volatile containment methods are likely to be an improvement over early studies where *M. albus* sachets were used in polystyrene boxes without wrapping or the use of a liner ([12]; also J. Mercier, unpublished data). Although it was observed that vents in some polystyrene boxes could become obstructed by the fruit, providing some containment for the volatiles, this effect was not consistent among boxes and some boxes had mostly open vents, which would result in poor efficacy of the biofumigation. Reducing the number and/or size of the vents to retain the volatiles, although it may delay cooling, has the benefit of retarding the loss of water by grapes. Minimizing water loss is a crucial component of table grape quality, since a loss of only 2% is associated with declining market quality [29]. As in previous experiments with grapes [8, 15], we did not see any adverse effect of the *M. albus* volatiles on the appearance of the fruit or rachis. A sensory evaluation conducted previously by an independent food science laboratory in California did not detect any effect of the biofumigation treatment in grapes, while the use of sulfur dioxide fumigation was detected by the sensory panel (unpublished results).

While the biological control of postharvest diseases is relatively easy to demonstrate in small-scale studies using artificial inoculation, it is considerably more difficult to achieve similar results under the constraints of commercial postharvest handling. Factors contributing to making biocontrol more difficult under such realistic situations are the presence of several species of decay pathogens, various infection times, and the need for compatibility with practices required for optimal crop quality and appearance such as waxing, degreening, controlled atmospheres, pre-cooling, use of specific liners and container types, and other pesticides. For these reasons, biocontrol agents eventually need to be tested under commercial postharvest settings [30–33]. This research provides an example of a biocontrol agent being integrated into the commercial handling of table grapes. Because the *M. albus* culture reactivates

readily and produces volatiles with broad-spectrum fungicidal activity [7, 9, 13], even at cold storage temperature, it can be made compatible with most postharvest systems as long as the volatiles can be contained without affecting packaging requirements or fruit quality.

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Empleo en acondicionamiento tanto de bolsas productoras de *Muscodor albus* volátil como del film en atmósfera modificada para el control de enfermedades de conservación de la uva de mesa orgánica, en condiciones comerciales.

Resumen — Introducción. Se estudió la biofumigación de la producción volátil del hongo *Muscodor albus* se estudió para controlar de las enfermedades de conservación de la uva de mesa orgánica guardada en almacén comercial. **Material y métodos**. Se introdujeron bolsas que contenían dos cantidades diferentes de cultivo activado de *M. albus* en cajas de cartón de expedición, cuyo contenido aproximado era de 4.5 kg de uvas de mesa sin semillas 'Thompson Seedless' o 'Red Seedless'. Los compuestos volátiles se mantuvieron en el interior de las cajas de cartón o bien mediante envoltura exterior, tras pre-refrigeración, de las paletas de las cajas de cartón con un film plástico (paletas envueltas), o bien mediante el empleo de un film en atmósfera modificada situado en el interior de cada caja de cartón. Se evaluó la incidencia de pudrimiento tras 7 semanas de almacenaje a 0 ºC. **Resultados**. Las bolsas de *M. albus* redujeron la incidencia de enfermedades de conservación de las uvas de mesa 'Red Seedless' tanto en las paletas envueltas exteriormente como en las cajas de cartón provistas de filmes interiores. En este cultivar, el film de atmósfera modificada sólo redujo la incidencia de moho en cerca de un 70 %; y, el empleo de *M. albus* en el interior de las cajas de cartón redujo un poco más dicha incidencia independientemente de la cantidad empleada de *M. albus*. La combinación de la bolsa de *M. albus* y del film de atmósfera modificada resultó ser el tratamiento de control más eficaz. La incidencia de enfermedades de conservación fue más limitada en las uvas de mesa 'Thompson Seedless'. Un control de putrefacción no se observó más que después de un precalentamiento de las uvas, tras su almacenaje, en presencia de una dosis de 50 g insertada en el interior de la caja de cartón tapizada de film. No se asoció ninguna consecuencia negativa con el tratamiento ni con el empleo de filmes de atmósfera modificada. **Discusión**. Según nuestros resultados, la biofumigación con bolsas de *M. albus* es compatible con la manutención en condiciones comerciales de las uvas de mesa orgánicas; esta técnica en cuestión podría permitir una mejora significativa de su duración de conservación.

EUA / *Vitis vinifera* / uvas de mesa / tecnología postcosecha / *Muscodor albus* / almacenamiento atmósfera controlada / lucha integrada