Evaluation under Commercial Conditions of the Application of Continuous, Low Concentrations of Ozone during the Cold Storage of Table Grapes

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Abstract

***Botrytis cinerea* causes gray mold, a postharvest disease of table grapes. The ability of ozone (O_3) in air to inhibit gray mold in stored grapes was reported in chamber studies, but O_3 needed evaluation under commercial conditions. O_3 merits attention because it is pesticide-residue free and allowed as "organic" by the USDA National Organic Program. Tests were conducted at three commercial facilities using pallets of 'Flame Seedless', 'Thompson Seedless', and 'Princess Seedless' grapes in uncoated, corrugated fiberboard boxes containing grapes in ventilated cluster bags or hard plastic clamshell containers. Grape berries inoculated with *B. cinerea* were placed within grape clusters at the beginning of storage. After cold storage, the spread of infection, natural incidence of decay, and cluster appearance were evaluated. After initial pre-cooling in air, grapes were stored at -0.5 to 3°C with a day/night cycle of 100 ppb O_3 (day) and 300 ppb O_3 (night) for 5 to 8 weeks. At each exam, six 9-kg boxes with 9 cluster bags or 4 clamshell containers were examined. The mean number for all tested cultivars of berries adjacent to inoculated berries that became infected by the end of storage was 0.8 in the O_3 atmosphere compared to 3.1 among those in air. The mean percentage for all tested cultivars of naturally decayed berries at this time in O_3 was 2.4% compared to 5.8% among those in air. Cluster appearance was not harmed and storage life was extended by 2 to 3 weeks by O_3. The uncoated, paper fiber corrugated boxes used in these tests reduced O_3 diffusion into them more than coated corrugated fiberboard, expanded polystyrene, or corrugated plastic boxes. Selection of packaging that maximizes O_3 concentrations within packages should improve control of gray mold.**

INTRODUCTION

Botrytis cinerea causes gray mold, the most destructive postharvest disease of table grapes. In many controlled laboratory studies, ozone (O_3) gas inhibited the mycelial growth of this pathogen and its spread among stored grapes (Mlikota Gabler et al., 2010; Palou et al., 2002; Tzortzakis et al., 2007; Cayuela et al., 2009; Sharpe et al., 2009), but little has been published about its use under commercial conditions. O_3 for this application merits attention because it is a pesticide-residue free treatment, O_3 generation equipment is widely available, and it is allowed as 'organic' by the USDA National Organic Program. Meeting decay tolerances for table grapes is particularly challenging for grapes produced under 'organic' rules, since both vineyard fungicides to minimize gray mold during the production phase and sulfur dioxide fumigation to control it in storage are banned. For conventional growers, sulfur dioxide is a very effective and inexpensive treatment, but it causes bleaching injuries to the grapes after repeated fumigations (Luvisi et al., 1992). The tolerance for decayed berries is low. For example, assuming the average weight of one 'Flame Seedless' berry is 4 g, the number of berries per box would be about 2,400. Therefore, with the USDA shipping tolerance for decayed berries per box at 0.5%, only about 12 berries per box are allowed to be USDA Fancy. On receipt, the tolerance is 1%, or about 24 berries per box. Although preharvest vineyard fungicide regimes can significantly reduce postharvest gray mold, they are not effective enough to eliminate the need for postharvest fumigation (Smilanick et al., 2010).

MATERIALS AND METHODS

'Flame Seedless', 'Thompson Seedless' and 'Princess Seedless' table grapes in commercial boxes with internal packaging of clamshell boxes or cluster bags were evaluated in commercial facilities near Delano and Arvin, CA. Uncoated paper fiber corrugated boxes with a vented area of 5%, commonly used by the CA table grape industry, were employed. The final box weight was 10 kg of which approximately 0.5 kg was packaging. Internal packaging consisted of: 1) four clamshell containers each with clusters totaling 400 to 600 berries each; or 2) nine cluster bags, each ventilated with numerous round holes approximately 0.3 cm in diameter, with clusters totaling 150 to 250 berries each. After initial pre-cooling of grapes in air, they were stored at -0.5 to 3°C with a day/night cycle of 100 ppb O_3 in the day and 300 ppb O_3 at night, or in air for up to 8 weeks. O_3 was produced and controlled by a Purfresh-Cold Storage system (Purfresh, Inc. 47211 Bayside Parkway, Fremont, CA.).

Inoculated berries were placed among healthy berries initially in each test to quantify the O_3 effectiveness to stop the spread of decay among berries during subsequent cold storage. Berries were inoculated by injecting them at a depth of 5 mm with 0.05 ml volume of a dense suspension of conidia prepared from a 2-week-old culture of *B. cinerea* grown on potato dextrose agar at 22°C with a 12 h light/dark cycle. One berry was placed inside each clamshell box or cluster bag on the first day of the test just before the grapes were placed in O_3 or air storage. Boxes were arranged in pallets with all sides of the pallets exposed to the room atmosphere. Data recorded included: the number of decayed berries adjacent to the inoculated berry; the extent of mycelial growth on the inoculated berry on a 0 to 5 scale where 0 indicated no mycelium development while 5 indicated more than 60% of the berry surface was covered with mycelium; the number of grapes naturally infected with gray mold; the number of grapes naturally infected with other decay organisms; and the condition of the cluster rachis on a 0 to 5 scale, where 0 indicated the rachis was fresh and green while 5 indicated it was entirely brown.

In a second study, the diffusion of O_3 into 'Thompson Seedless' grapes packaged in one of five boxes with two types of internal packaging and O_3 concentrations was measured every 12 hours over four days at 1°C using two six-channel ozone monitors (Model 465L, API, Inc., San Diego, CA). O_3 was generated with the same system described previously inside a stainless steel environmental chamber 3 m wide, 2.7 m in length, and 3 m tall with two fans behind cooling coils. The air is mixed relatively uniformly in the chamber by these fans and air speed measurements taken on the exposed sides of the boxes with a hot wire air-speed meter (Model 9870, Alnor TSI Inc. Shoreview, MN) were 8.9±2.8 m s^{-1}. The boxes were arranged in a six-down pattern three boxes high, and only boxes in the middle layer were monitored. The O_3 concentration within the room was constantly 300 ppb. Sampling lines were placed inside the packages within grape clusters. Three replicates of each combination of box and internal packaging were done. The boxes were made of returnable plastic containers of hard, black plastic, expanded polystyrene, corrugated plastic, paper fiber corrugate with a water-resistant coating, or uncoated paper fiber corrugate (Table 1). The boxes contained either open poly zip bags or clear plastic clamshell boxes. The vent area of all of the boxes is shown (Table 1). The vent area of the clamshell boxes, with many slots and circular holes and a 3 mm wide open gap when closed, was approximately 10%, while that of the open zip bags, with vents composed of approximately 90 circular holes 4 mm in diameter, was 3.5% when closed, but these bags are always used in an open position so their vent area is variable.

RESULTS AND DISCUSSION

O_3 reduced the number of decayed berries in all three cultivars (Table 2). For

‘Thompson Seedless’ and ‘Flame Seedless’, O_3 was more efficient reducing the number of infected berries in clamshell compared to cluster bag packaging. O_3 did not significantly alter rachis or berry appearance, and caused no bleaching injuries to the berries which are commonly associated with sulfur dioxide use (data not shown).

Uncoated paper corrugated boxes impeded the diffusion of O_3 over four days more than any of the other boxes (Fig. 1) and open, poly zip bags consistently impeded O_3 diffusion more than hard plastic clamshell boxes. In general, package O_3 concentrations reached a constant concentration within one or two hours after the O_3 was introduced into the chamber and changed little over the subsequent four days (data not shown).

Like sulfur dioxide fumigation (Luvisi et al., 1992), it is likely packaging, air speed, vent size, pallet design and placement, and other factors influence O_3 diffusion under commercial conditions. For O_3 diffusion to be adequate and reliably effective under commercial conditions, quantitative evaluation of O_3 concentrations inside the various kinds of packaging now in commercial use is needed so measures can be taken to ensure the treatment is as effective as possible. Sulfur dioxide dosimeters are routinely used by table grape industry personnel now, where they are placed within packages to ensure sufficient sulfur dioxide diffusion to control decay (Luvisi et al., 1992). A similar dosimeter for O_3 applications would be of great value as well, since many combinations of box and internal packaging are now in use and changes in these products occur often. The results we report here are promising, particularly since the uncoated paper fiber corrugated boxes used in this study retarded O_3 diffusion more so than other grape packaging, such as coated paper fiber corrugated boxes, expanded polystyrene or corrugated plastic boxes. Prior work with citrus fruit packaging indicated uncoated paper fiber corrugate boxes offered greater resistance to O_3 diffusion compared to other kinds of packaging (Palou et al., 2003). Uncoated paper fiber corrugated boxes are popular for produce packaging due to their low price and ready acceptance for recycling, however, when storage is prolonged, they absorb moisture, which exacerbates product moisture loss and weakens the boxes. In our tests, the added storage life that resulted from the O_3 treatments may make the selection of other packaging worthwhile. Currently, we are continuing to evaluate the influence of box materials, internal packaging, box stacking patterns, and box and pallet locations within commercial cold storage rooms on O_3 diffusion into packages. We are evaluating some of the color change ozone dosimeters currently available and are establishing the minimum O_3 concentration needed to stop the spread of gray mold among stored grapes. Although further evaluation of O_3 use under commercial conditions is needed, it is likely O_3 can be an effective option now for growers who cannot use sulfur dioxide fumigation. Some commercial storage managers use O_3 in strategies to reduce, but not eliminate, sulfur dioxide use.

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Tables

Table 1. Boxes used in O_3 penetration tests. Types of boxes, all in common commercial use, where O_3 diffusion was evaluated after four days of storage at 1°C in 300 ppb O_3. The vent area percentage is that of the entire box surface area. When O_3 diffusion was evaluated, they contained internal packaging of nine vented, open zip bags or eight clamshell containers filled with 'Princess Seedless' table grapes.

Package	Vent area (%)	Comment
Corrugate fiberboard	4.6	common, inexpensive, for brief storage
One- or two-side coated paper corrugate	7.6	for long storage, stronger, less hydration
Corrugate plastic		5.9 for UK, Australia, New Zealand export
Expanded polystyrene	5.2	EPS, tall for clamshell boxes, insulating
Expanded polystyrene	6.0	EPS, short for cluster bags, insulating
Returnable/reusable plastic container	6.3	RPC, hard plastic

Table 2. Number of gray mold infected berries adjacent to an initial *B. cinerea*-inoculated berry placed within clusters and the natural incidence of gray mold among grapes within commercial packages after cold storage (1 to 2°C) in commercial storage facilities. Each value is the mean of 6 boxes with 9 cluster bags or 4 clamshell boxes each.

Treatment	Flame Seedless (7)		Thompson Seedless (5)		Princess Seedless (8)
	Cluster bags	Clamshells	Cluster bags	Clamshells	Cluster bags
	Number of berries with gray mold around inoculated berry				
Air	4.6	3.5	3.8	3.0	0.4
Ozone	0.5	0.0	2.6	0.6	0.1
	Natural percentage of berries with gray mold per package				
Air	11.8	4.9	10.1	1.3	1.0
Ozone	3.0	1.8	6.6	0.3	0.2

Values in parenthesis indicate length (weeks) of cold storage.

Figures

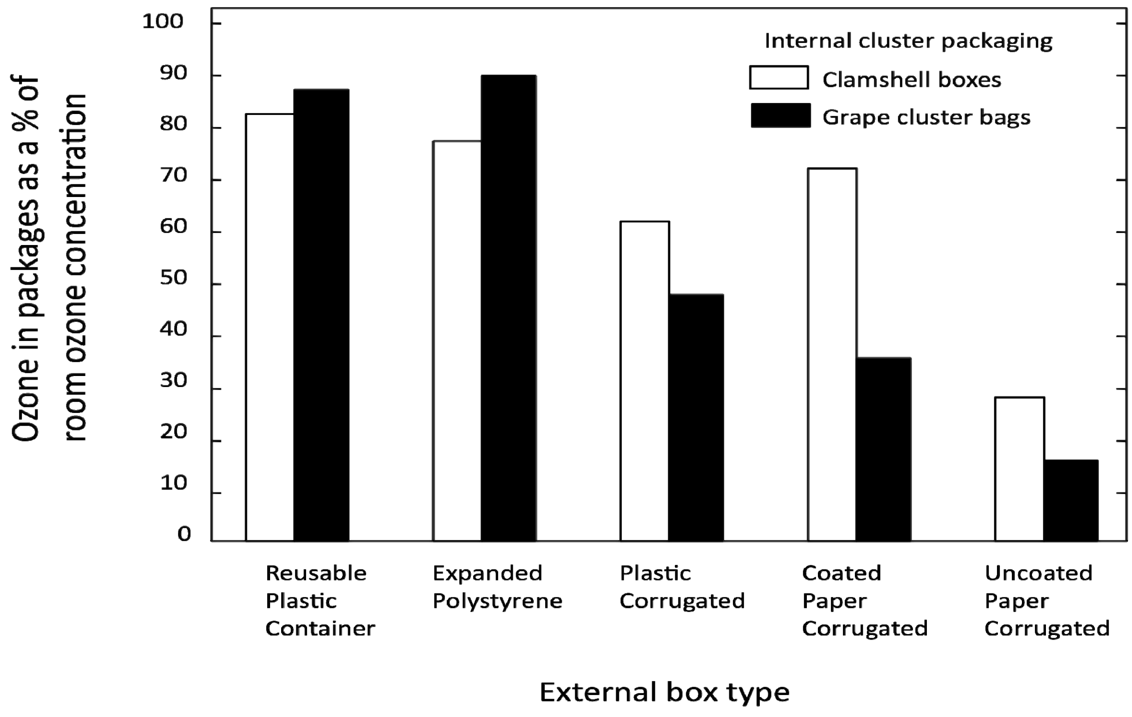

Fig. 1. O_3 concentration within 'Princess Seedless' table grape packages as a percentage of the room atmosphere concentration of 300 ppb ozone. These values were recorded after a 4-day period of exposure at 1°C. The boxes are described in Table 2.