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Evaluation of brief, high doses of gaseous ozone to control fruit decay fungi.

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Grapes are subject to infection by fungi that cause them to rot after harvest. The most important pathogen is *Botrytis cinerea*, which causes gray mold. It is particularly difficult to control because it grows at cold storage temperatures and spreads from infected to healthy berries. *B. cinerea* also develops rapidly in vineyards after rainfall, when it can cause numerous infections. Other fungi, such as *Penicillium* sp., *Aspergillus* sp., and *Rhizopus stolonifer*, and *Alternaria alternata*, can also cause decay, but typically earlier in the season during periods of hot, dry weather, and their growth stops or they do not spread rapidly among berries in cold storage.

Fumigation with up to 10,000 ppm ozone (O₃) for up to several hours was applied to table grapes in clusters bags, they were then placed in boxes, stored for one week at 15°C or one month at 1°C, and then examined to determine the amount of postharvest decay. O₃ more effectively inhibited *B. cinerea* conidia on grapes inoculated 24 h before treatment than those inoculated 1 h prior to fumigation. Gray mold incidence among Thompson Seedless grapes after fumigation for one hour with 0, 2500, or 5000 ppm O₃ followed by 7 days of storage at 15°C was 29.3, 21.2, or 11.5%, respectively, when grapes were inoculated 1 h prior to fumigation, and 15.6, 17.0, and 6.8%, respectively, when grapes were inoculated 24 h prior to fumigation. Gray mold incidence among

Redglobe grapes after fumigation with 0, 2500, 5000, or 10000 ppm O₃ followed by 28 days at 1°C was 57.7, 31.4, 27.6, or 17.4%, respectively, when grapes were inoculated 1 h prior to fumigation, and 34.2, 17.8, 13.4, or 10.3%, respectively, when grapes were inoculated 24 h prior to fumigation. Sporadic minor rachis injuries were observed after O₃ fumigation of some cultivars, but treated and untreated berries were identical in appearance.

Semi-commercial tests with several cultivars were conducted in the summers of 2005 with organic table grapes. The grapes were harvested, promptly treated with 5000 ppm O₃ for one hour, placed in cold storage, and examined after 4 to 5 weeks. This treatment reduced total natural gray mold decay about 45%. The natural decay of Redglobe grapes was reduced from 34.4 to 21.6%, of Black Seedless from 5.0 to 2.7%, and of Thompson Seedless grapes from 21.6 to 11.3%.

We also evaluated control of postharvest decay, mostly gray mold caused by *Botrytis cinerea*, using biofumigation with the volatile-releasing fungus *Muscodor albus*. The influences of time between inoculation and treatment, temperature, biofumigant dosage, and packaging type on decay control were evaluated. *M. albus* was more effective if present within 24 h after inoculation, under warmer storage than cold, at higher dosages, and biofumigation worked effectively in commercial packages, especially clam shell containers. Decay control varied among tests from about a 50% reduction in decay when *M. albus* was present beneath cluster bags, to more than 90% among grapes when *M. albus* was present inside clam shell containers. Effectiveness variations probably occurred because of differences in activation among *M. albus* formulations and packaging. In most tests, gray mold was controlled by *M. albus* only when it was present; if removed, gray mold growth resumed.

We combined O₃ and *M. albus* treatments and compared their effectiveness to sulfur dioxide (SO₂) fumigation in semi-commercial tests. We evaluated the effectiveness of integrated treatment with short-term O₃ fumigation and continuous biofumigation with in-package generators containing *M. albus*. *M. albus* survived fumigation with O₃. This is

important for practical reasons so it could be placed in grape boxes directly in vineyard, and then pre-cooled with O₃. Gray mold incidence among inoculated Autumn Seedless grapes was reduced from 91.7% (untreated) to 19.3% after a single, 5000 ppm O₃ fumigation, and further reduced to 10% when O₃ and *M. albus* were combined. O₃, *M. albus*, and SO₂ treatments were compared using four-box mini-pallets of Thompson Seedless grapes pre-cooled with air, SO₂, or O₃, and then wrapped with stretch-film. The polyethylene stretch-film pallet-wrap combined with SO₂ generator pads is used in Israel for storage of table grapes. Stretch-film minimizes rachis drying and maximizes the effectiveness of *M. albus* volatiles and SO₂ released from generator pads. Grapes were packaged either in vented cluster bags in EPS boxes, or in vented clamshells in RPC boxes. After one month in cold storage, O₃ and *M. albus* reduced decay but were inferior to SO₂ (Figure 1). Control of mycelial growth, decay spread from inoculated to adjacent berries, and natural decay was equally effective in treatments with SO₂ generator pads, where grapes were pre-cooled with air, as that in treatments with weekly SO₂ fumigation, where grapes were pre-cooled with SO₂. Less decay occurred within clamshell/RPC packaging compared to cluster bag/polystyrene packaging. Natural gray mold was more reduced when O₃ and *M. albus* were combined than by either used alone. Natural decay was reduced from 31.0% to 9.7% by O₃ fumigation and further reduced to 3.4% when *M. albus* was added to EPS boxes prior to O₃ fumigation. In this experiment, treatment with SO₂ generator pads or treatment with initial and weekly SO₂ fumigation reduced decay from 31.0% to very low levels of 1.1% and 0.2%, respectively. They also arrested mycelial growth on infected berries while the single initial O₃ fumigation and *M. albus* did not.

In conclusion, O₃ gas fumigation during pre-cooling of grapes as well as continuous fumigation of grapes with *M. albus* during storage controlled postharvest decay, but these were inferior in effectiveness to SO₂, and they are unlikely to replace SO₂ treatments in conventional grape production unless their efficacy is improved. Berry injury was never observed, but some rachis browning was observed after O₃ fumigation. They might be good technologies to use with grapes marketed under “organic” classification, where the use of SO₂ is prohibited, or if SO₂ use is discontinued for some reason.

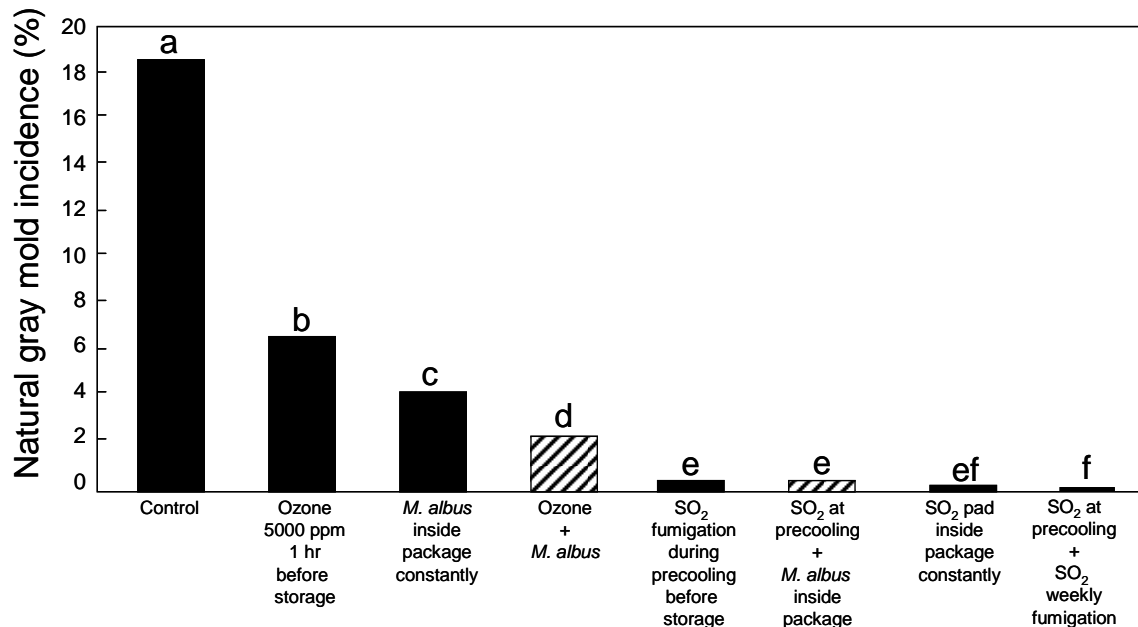


Figure 1. The natural incidence of decayed berries of Thompson Seedless grapes after treatment and storage approximately one month at 32°F. The grapes were; 1) placed in storage without any treatment (control) other than pre-cooling; 2) treated for one hour with 5000 ppm ozone during pre-cooling before storage; 3) a package containing *Muscodor albus* was placed inside the package before pre-cooling and storage; 4) a package containing *Muscodor albus* was placed inside the package before pre-cooling that included 5000 ppm ozone for one hour followed by storage; 5) pre-cooled with sulfur dioxide fumigation alone before storage; 6) a package containing *Muscodor albus* was placed inside the package before pre-cooling with sulfur dioxide fumigation followed by storage; 7) a sulfur dioxide generator pad was placed inside packages before the package was pre-cooled in air and stored; and 8) pre-cooled with sulfur dioxide fumigation initially, then re-fumigated at weekly intervals during storage. Values are the means of 4 replicate boxes and the entire experiment was repeated twice.

