Postharvest control of decay organisms in Muscadine grapes

Final Report
SRSFC Project #2009-12
Research Proposal

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Objective:

The objective of this study is to expand our current knowledge pertaining to the harvest, handling, and storage of Muscadine grape by evaluating the use of sulfur dioxide as a fumigant and BioSave 10 LP as a biofumigant for the postharvest control of fungal pathogens.

Justification and Description:

Muscadine grapes (*Vitis rotundifolia* Michx.) are native to the southeastern United States, and are well adapted to growth in hot, humid climates. Muscadine grapes differ from traditional bunch grapes by their large size, thick skins, and unique fruity aroma (Conner, 2006), and these differences may play a role in the post-harvest management of this crop. Postharvest temperature management (0ºC, 90-95% R.H.) is the primary tool available to growers for maintaining quality and controlling fungal pathogens postharvest (Smit et al., 1971). However, latent infections from the field results in the mycelial spread and sporolation of numerous pathogens during storage. The

majority of commercial muscadine cultivars are also extremely susceptible to stem-tear injury during harvest, which contributes significantly to the susceptibility of the fruit to fungal infection (Ballinger and Nesbitt, 1982). As a result, the spread of these pathogenic organisms is the single most important limiting factor in the long-term storage and quality of muscadine grapes.

The major pathogens resulting in significant postharvest losses are all fungal. The three most important are ripe, bitter, and macrophoma rots, caused by the fungi *Glomerella cingulata*, *Greeneria uvicola*, and *Botryosphaeria dothidea*, respectively (Krewer et al., 2002). All of these diseases can appear quite suddenly during storage, and generally affect fruit as they ripen. These diseases spread rapidly once established, which will generally occur after approximately 1-2 weeks of storage (James et al., 1997). The extent of infection is largely dependent on the maturity of the fruit at harvest, as well as the degree of bruising and stem-tear injury. Limited management options are available for control of these pathogens. Currently, temperature management (0-1ºC, 90-95% R.H.) and chlorine washes (100 ppm) are the only two tools that are routinely used, with the latter generally only being used immediately prior to transport.

For nearly a century, sulfur dioxide has been used as a very effective postharvest fumigant of table grapes (*Vitis vinifera*) for the control of gray mold (*Botrytis cinerea*). In the past, a few experiments have been conducted investigating the use of SO_2 on Muscadine grapes, with varying conclusions being drawn. Smit *et al.* (1971) reported that treatment with SO_2 resulted in an acceptable product after storage for 2 months (0ºC). In contrast, Takeda (1981) found that grapes can not be stored greater than 2 weeks (1ºC), and that shelf-life can not be extended beyond these two weeks by SO_2 due to bleaching and off-flavor production. Finally, Ballinger and Nesbitt (1982) found that SO_2 was effective in controlling decay, and that placing newspaper into the lined boxes reduced the SO_2 damage by 74%. The results from these studies suggest that treatment with SO_2 is beneficial for muscadine grapes, and based on recent findings on table grapes, the required concentration for effective control may be significantly lower than those used in the aforementioned studies.

Small paragraph about BioSave 10 LP

The combination of the ubiquitous presence of inoculum, limited availability of resistant cultivars, limited pre- and post-harvest chemical control options, and substantial postharvest losses ascribed to the activity of these fungal pathogens in a high-valued product such as muscadine, all contribute to the candidacy of the grape as an appropriate crop for testing of BioSave 10 LP biopesticide.

It is the objective of this study to evaluate these potential technologies for use in the muscadine grape industry. The loss of fruit in storage due to pathogens is the greatest limiting factor in the expanded production of the grape. If an effective control option is available, it is likely that a significant expansion in acreage will result not only in Georgia, but in all muscadine producing regions in southeastern U.S.

Materials and Methods:

Experiment #1: Storage Trial

Cultivars and Harvest:

Muscadine grapes were harvested by a local grower (Paulk Vineyards, Ocilla, Ga.). Samples of grapes were collected both before and after fruit had passed through the commercial cooling, washing (100 ppm chlorine spray), grading and packing lines. The common commercial cultivars 'Fry' and 'Supreme' were used. Fruit were transported to the Postharvest facility at the Vidalia Onion Research Laboratory, University of Georgia, Tifton Campus, and placed immediately at 0-1ºC (90-95% R.H.) until treatments were applied.

Sulfur dioxide treatment: After cooling, fruit (pre and post chlorine wash) were fumigated with 100 ppm SO_2 (100 ppm-hour) for 1 hour in a sealed room with high air-circulation. After the completion of the fumigation, the room was vented with air for 3-4 hours until SO_2 levels were reduced below detectable levels, then fruit were placed in regular air storage of 0-1ºC (90-95% R.H.).

Biosave treatment: After cooling, fruit (pre and post chlorine wash) were immersed in BioSave 10P biofungicide cultures of *Pseuodomonas syringae* as per manufacturer's instructions at a rate of 16.67g per gallon of water, permitted to dry, then placed in regular air storage of 0-1ºC (90-95% R.H.).

Storage and evaluations: Fruit were removed after 10 and 20 days, permitted to warm for 24 hours at room temperature (21ºC) and evaluated 1, and 4 days post-removal for physiochemical and quality evaluations including: berry size, weight, total soluble solids content (ºBrix), titratable acids content (Mettler-Toledo DL15 automatic titrator), firmness (Bioworks Firmtech II), as well as for the incidence of storage disorders, such as: shrivel, bruises, skin crack, and molds. These latter evaluations were also performed on fruit 9 days post-removal.

Statistical Analysis: Data was analyzed using Statistical Analysis Software v. 9.2 and the Proc Mixed procedure in conjunction with SAS Enterprise Guide v. 4.2.

Experiment #2: Controlled Inoculations

Fruit: Muscadine grapes, cultivars 'Fry' and 'Supreme' were harvested by hand at Paulk Vineyards, Ocilla, Ga, on October 1, 2009. After harvest, fruit were sorted and treated with 100 ppm chlorine prior to being placed in short-term cold storage (5ºC, 90% R.H.) for less than 2 hours. Fruit were then transported to the University of Georgia Vidalia Onion Research Laboratory, Tifton, Ga., where the fruit were subsequently warmed to room temperature using a fan to remove condensed moisture.

Culture and inoculations: Isolates of bitter rot (*Melanconium fuligineum, Greeneria uvicola*) and ripe rot (*Glomerella cingulata*) were cultured on potato dextrose agar (PDA) at 20ºC for 10-14 days until fungi commenced spore formation. Spores were removed using sterile water (containing Triton X-100), and suspension density determined using a hemacytometer and a light microscope. Controlled inoculations of fruit with ripe and bitter rot were performed using a small cosmetic spray bottle. In order to mimic stem tear injury, an ethanol-flamed sterilized blade was used to remove the stem scar and surrounding tissues (approximate diameter 10 mm). Seven fruit per repetition were placed in a Petri dish, with cut-surface facing upwards. Suspensions of ripe or bitter rots ($1x10^6$ and $2x10^6$, respectively) were sprayed uniformly on the exposed flesh. After the surface dried (approximately 1 hour), Petri plates were placed into a 9.6-L polypropylene container attached to a pump supplying humidified air at a rate of 24-L/day at room temperature (21ºC). This rate ensured high humidity in the container, while maintaining sufficient air exchanges. Each replication of 7 fruit each was placed into three separate polypropylene containers.

BioSave 10 LP treatment: The treatment with BioSave 10 LP was the same as above, except that after the spray inoculations of bitter and ripe rot had dried, fruit were sprayed in a similar manner with BioSave 10 LP at a rate of 16.67 g per gallon of water (the highest registered rate of the product). The spray was then permitted to dry prior to being placed in similar polypropylene containers in triplicate.

Sulfur dioxide treatment: Treatment conditions followed those described by Palou *et al.* (2002). In brief, a sulfur dioxide gas distribution manifold was attached to the polypropylene containers in order to achieve inlet gas concentrations of 0, 2.5, 5, and 10 µL/L. The desired concentrations were obtained by mixing a stock 40 µL/L sulfur dioxide source with pure compressed air. This air source was humidified by passing it through a closed water bubbler system prior to mixing with the sulfur dioxide. This flow through system was maintained at 20ºC to evaluate the ability of a continuous exposure to sulfur dioxide a controlling the nesting and sporolation of the pathogens. Flow rates through each chamber were monitored using a flow meter (24 L/day). Inlet and outlet sulfur dioxide concentrations were monitored using dosimeter tubes and gas sampling pump (Matheson, Kitawaga).

Evaluations and Statistical Analysis: Fruit were subjectively scored for percent surface infected (1 - 0-20%, 2 - 21-80%, 3 - 81-100%), spread of fungus beyond cut surface to exterior or interior of fruit (Y/N), surface rupture (Y/N), and presence of mycelium (Y/N) and spores (Y/N). Results were analyzed using SAS version 9.2 (Cary, N.C.) and SAS Enterprise Guide 4.2 using PROC Mixed.

Results:

Experiment #1: Storage Trial

In general, the firmness of the berries decreased over the course of the 20 days of cold storage (figure 1), with firmness also decreasing significantly upon removal from storage

during the 1 or 4 day simulated marketing period (figure 2). Across all treatment effects, 'Supreme' was approximately 12% more firm than 'Fry', while firmness dropped 21% over the course of the experiment. Similarly, firmness dropped 26% during the 4-days at room temperature, providing further support of the importance of maintaining the cold chain through to market. The 100 ppm treatment of chlorine did have a significant effect on the retention of firmness, with an overall 6% gain in retention as a result of the treatment.

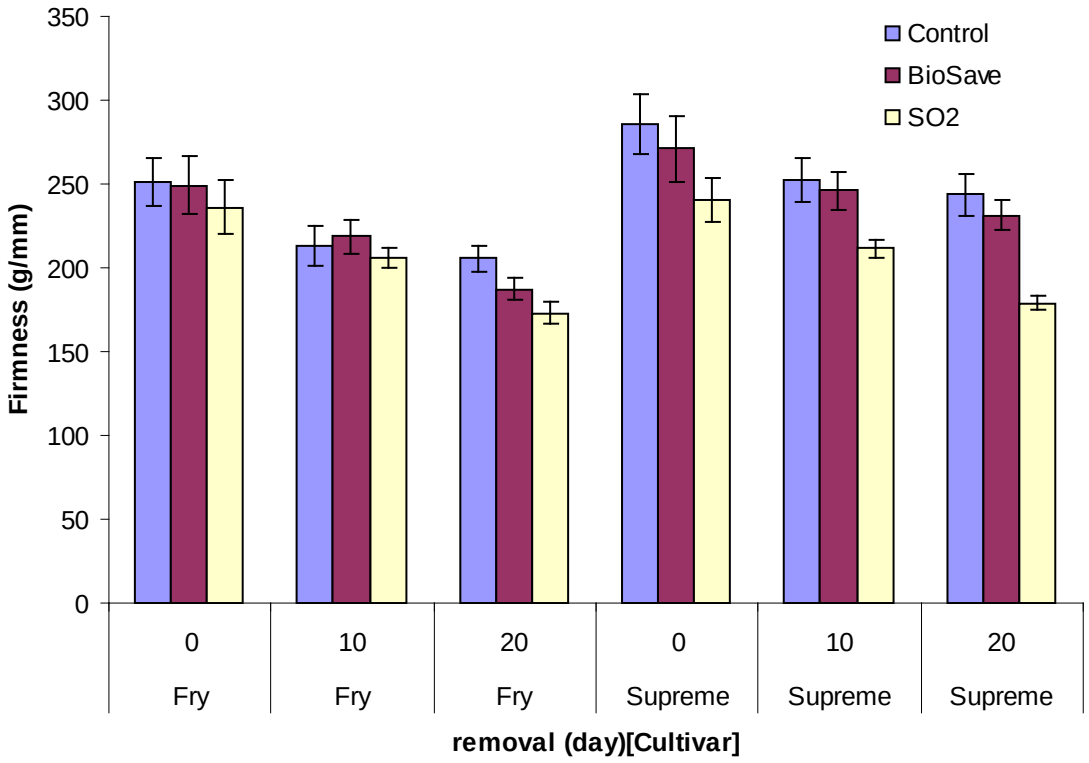

Figure 1. Change in firmness (g/mm) of muscadine grapes 'Fry' and 'Supreme' after 0, 10 or 20 days of cold storage (0-1ºC, 90-95% R.H.) and a treatment of BioSave 10 LP or 100 ppm-hr fumigation of SO_2, over all other treatment effects ($p < 0.01$).

With respect to the effect of the treatments, BioSave 10 LP did not result in either a significant retention, or contribute to a significant loss in firmness. In comparison, the sulfur dioxide treatment did result in a significant loss of firmness. This loss in firmness is likely due to the damage that occurred as a result of the rate (100 ppm-hr) of the sulfur dioxide treatment. This rate was used based on the similar rate used by the table grape industry. The stem scar is particularly susceptible to injury from the gaseous treatment. In our experiment, a white halo would appear concentrically around the stem scar, subsequently leading to the development of a depression around the stem scar, ultimately contributing to increased pathogenicity and concomitant loss in firmness (figure 3). Subsequent experiments used lower rates in effort to determine a suitable treatment concentration.

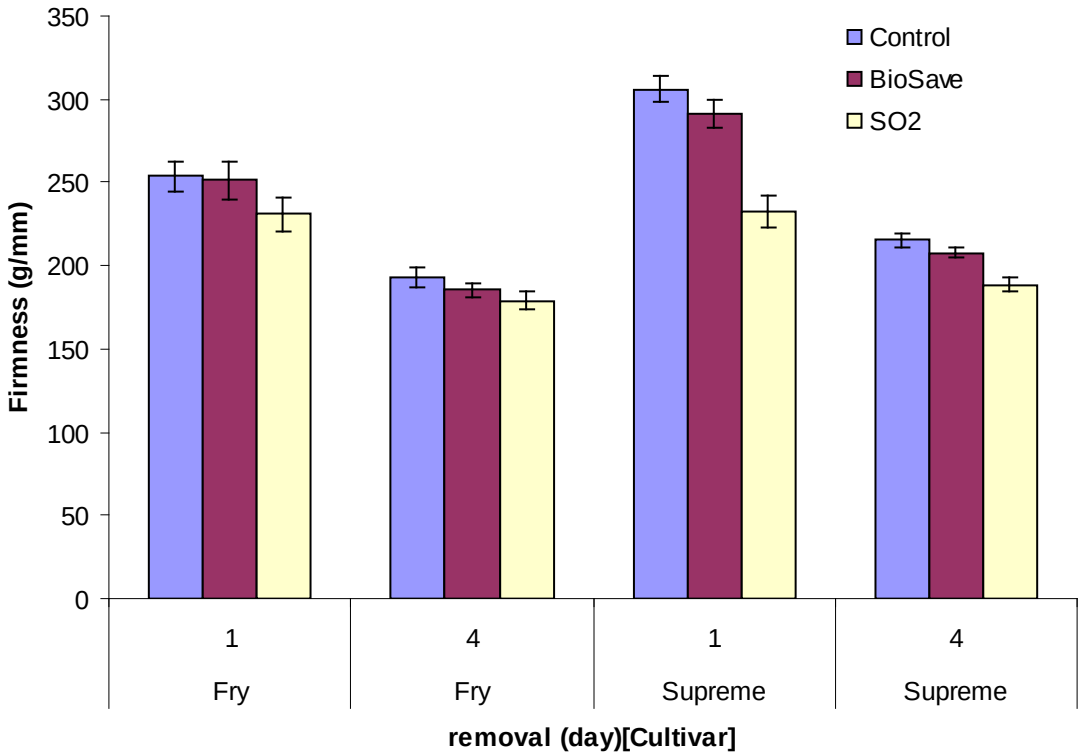

Figure 2. Change in firmness (g/mm) of muscadine grapes 'Fry' and 'Supreme' after 1 or 4 days of room temperature storage (21ºC) and a treatment of BioSave 10 LP or 100 ppm-hr fumigation of SO_2, over all other treatment effects ($p < 0.01$).

Figure 3. Sulfur dioxide injury on 'Fry' muscadine grape after a 100 ppm-hr treatment with SO_2, and 20 days of cold storage (0-1ºC, 90-95% R.H.).

With respect to the role of chlorine as in indirect contributor to the maintenance of fruit firmness, across all treatment effects, the treatment with 100 ppm of chlorine resulted in a 6% retention in firmness. The benefit from the treatment was most apparent in the 'Fry' fruit during the simulated marketing period at room temperature (figure 4), and in 'Supreme' during the 20-days of cold storage (figure 5). It is important to note that there was no significant interaction between the BioSave 10 LP and the treatment with chlorine. It has been duly noted by the manufacturer that improper use of chlorine when use in conjunction with the BioSave 10 LP can have deleterious results on the activity of the biofungicide.

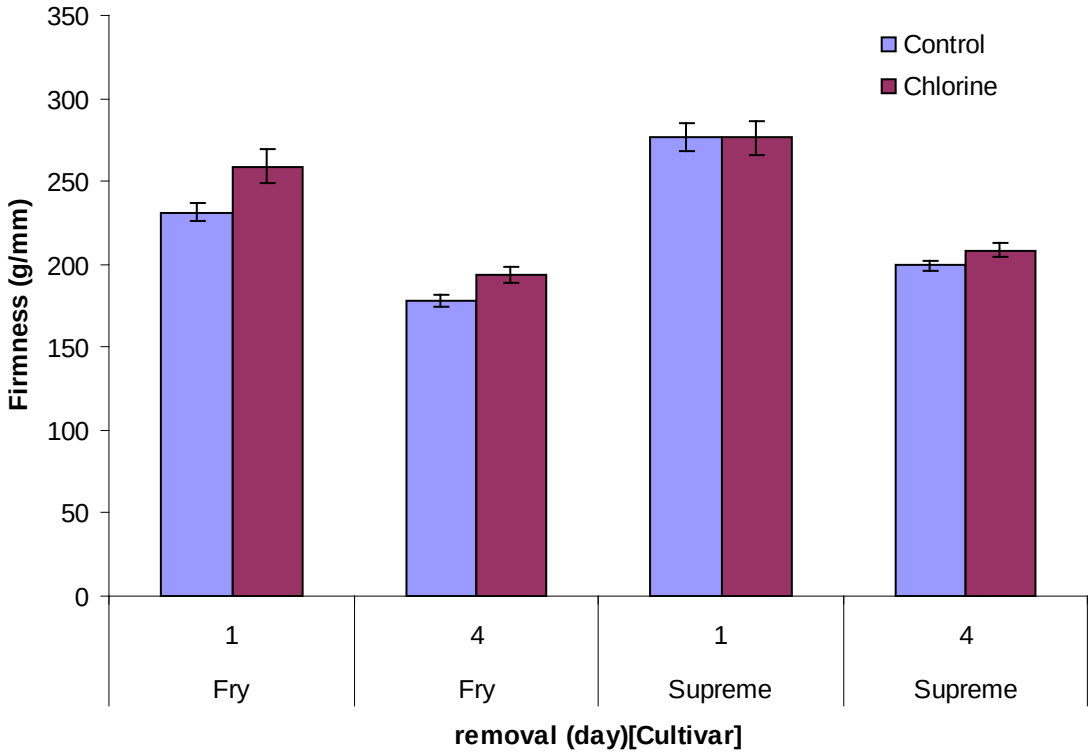

Figure 4. Change in firmness (g/mm) of muscadine grapes 'Fry' and 'Supreme' after 1 or 4 days of room temperature storage (21ºC) and a postharvest treatment of 100 ppm of chlorine, over all other treatment effects ($p < 0.01$).

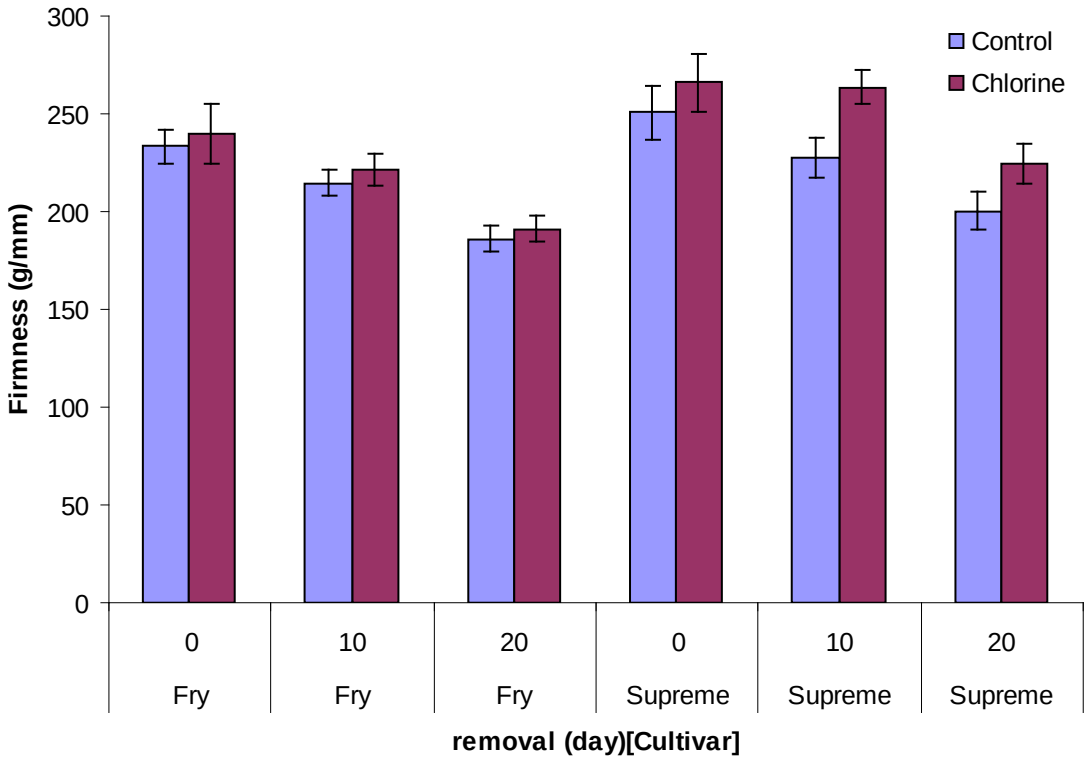

Figure 5. Change in firmness (g/mm) of muscadine grapes 'Fry' and 'Supreme' after 0, 10 or 20 days of cold storage (0-1ºC, 90-95% R.H.) and a postharvest treatment of 100 ppm of chlorine, over all other treatment effects ($p < 0.01$).

Aside from firmness, there were no noteworthy trends to report regarding the changes in total soluble solids content or titratable acids content over the various storage, shelf-life and treatment effects.

With respect to the presence of molds during the postharvest storage and marketing of the fruit, there was a significant increase in the percent incidence as time progressed over the course of the 20 days of storage. As seen in figure 6, the incident rate after 1 day at room temperature post-removal was very low. However, as fruit were maintained at room temperature for another 4 or 9 days, the rate of incidence increased dramatically, culminating with 75% after 20+9 days. With the exception of 10-day 'Fry', the incidence of mold was reasonably well controlled for up to 4 days post-removal for the first two removals. Although cold storage remained very effective throughout the experiment, the post-removal control was lost after this extended period of storage.

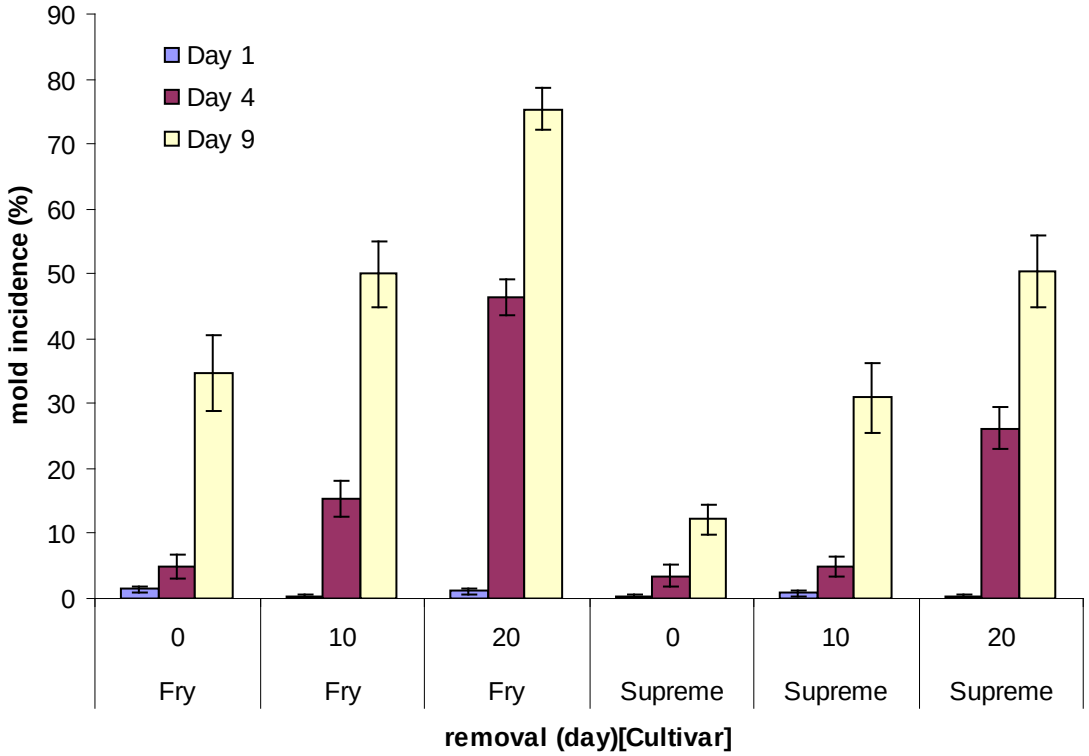

Figure 6. Percent incidence of mold (%) of muscadine grapes 'Fry' and 'Supreme' after 0, 10 or 20 days of cold storage (0-1ºC, 90-95% R.H.) and a post-removal simulated marketing period of 1, 4 or 9 days at room temperature (21 ºC), over all other treatment effects ($p < 0.01$).

With respect to the effect of the fumigant and biofumigant on the reduction of molds, there was a significant ($p < 0.0001$) effect of BioSave 10 LP at reducing the incidence rate (figure 7). This benefit was especially evident after the initial treatment (removal 0) offering promise of the use of the technology for controlling pathogen growth during short-term storage of the fruits prior to packaging and shipping. As mentioned previously, the only practice for the postharvest control of muscadine grape fungal pathogens is through the use of hypochlorous surface disinfectant. This practice is not effective against pathogens that are present under the surface of the fruit, and has no residual effect on the product. Depending on market demand, fruit will often be harvested and placed into cold storage for numerous days. The collaborating grower has expressed great value in applying BioSave 10LP as soon after harvest as possible, and allow it to outcompete latent field infection pathogens (BioSave 10LP competes better as temperatures are reduced) as the fruit await packing (i.e. market). Conversely, the grower could also apply BioSave to fruit that will be shipped to distant markets, thereby increasing the likelihood of an acceptable load upon arrival at inspection authorities.

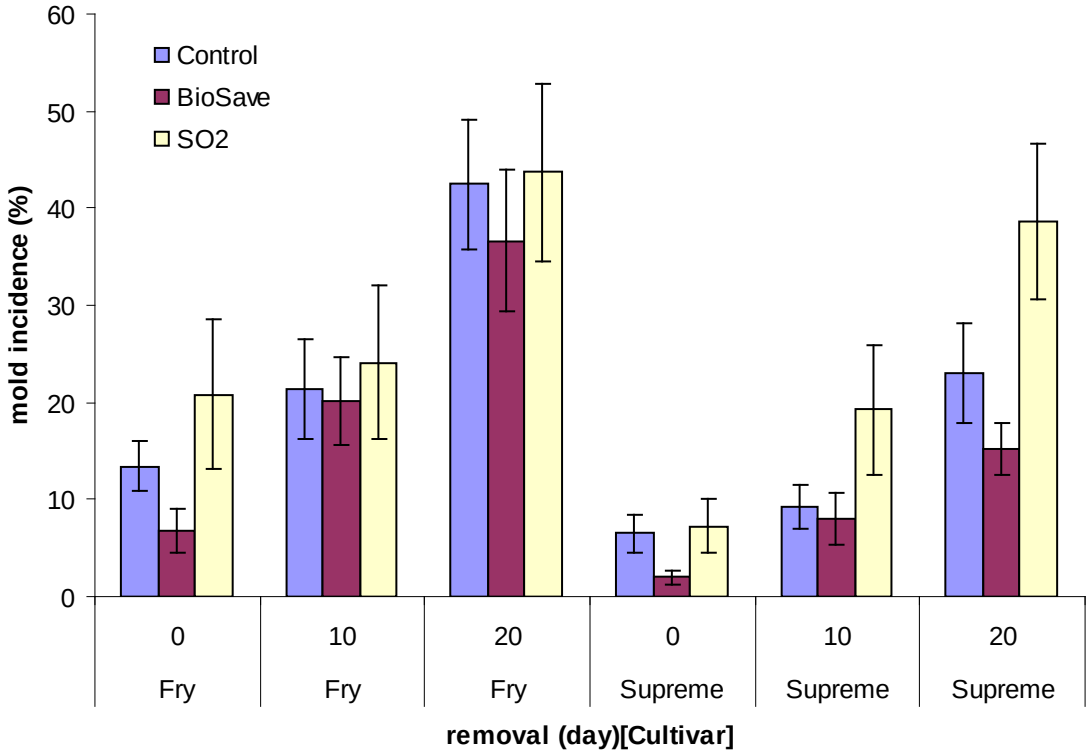

Figure 7. Percent incidence of mold (%) of muscadine grapes 'Fry' and 'Supreme' after 0, 10 or 20 days of cold storage (0-1ºC, 90-95% R.H.) and a treatment of BioSave 10 LP or 100 ppm-hr fumigation of SO_2, over all other treatment effects ($p < 0.01$).

Experiment #2: Controlled Inoculations

The rate of inoculum that was applied to the fruit was very high. This was performed in order to expedite any treatment effects of this preliminary study. However, despite this challenge, the BioSave 10 LP was able to offer significant benefit in the control of bitter rot. Across all experimental factors, Biosave treatment resulted in a highly significant reduction in the incidence of fungal activity (F-value 146.14, *p*-value <0.0001). The interaction between pathogen type and BioSave treatment was also highly significant (F-value 38.69, *p*-value <0.0001), with a 61% reduction of bitter rot and a 20% reduction in the incidence of ripe rot. BioSave was equally effective against 'Fry' and 'Supreme', with a significant 24% and 36% reduction in the incidence of fungal activity, respectively (figure 8).

As seen in figure 9a, the inoculated control fruit of both 'Fry' and 'Supreme' had significant amounts of mycelial growth on the cut surface of the fruit, and the rot symptoms migrated down through the entire fruit. This softening and browning is especially evident in 'Fry', where the control fruit are brown throughout, while the BioSave 10 LP fruit were still predominantly green and firm. Though not as evident in the figure, significant softening of the grape was evident in the control 'Supreme'

treatment, where two of the fruit began to leak into the Petri dish. In contrast, the BioSave 10 LP treatment for 'Supreme' resulted in nearly 100% control of the bitter rot fungus, and fruit remained very firm and intact (Figure 9).

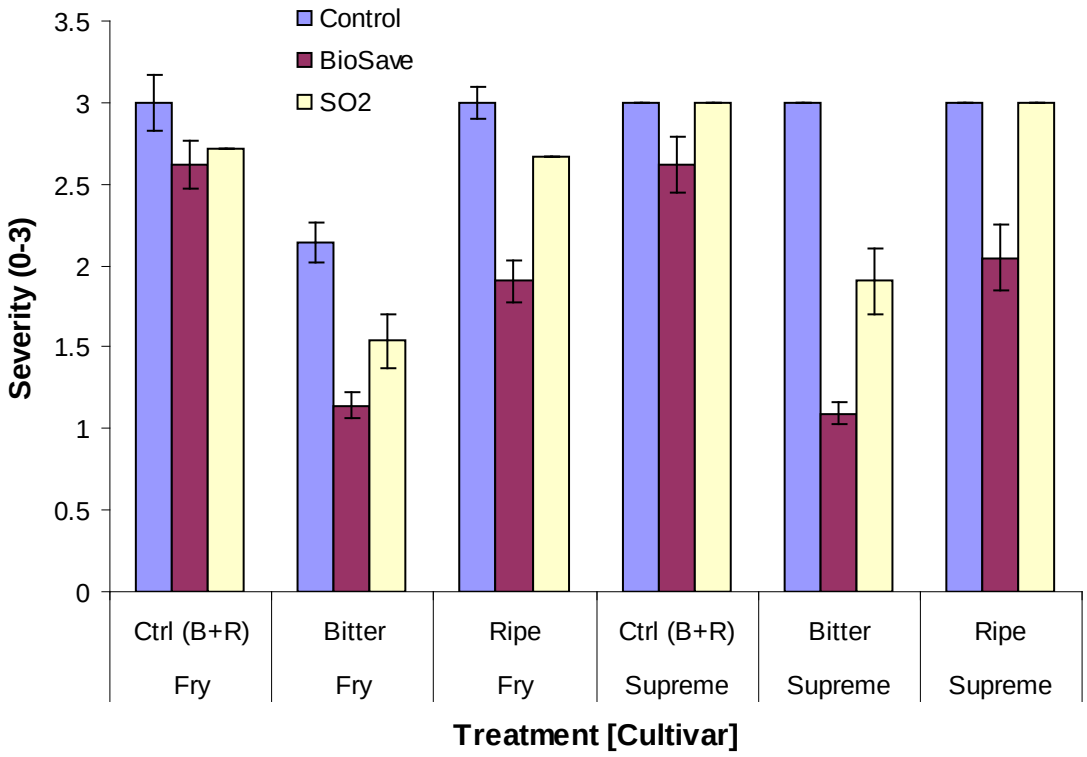

Figure 8. Severity (0, 0%; 1, 0-20%; 2, 21-80%; 3, 81-100%) scores of mold of muscadine grapes 'Fry' and 'Supreme' after artificial inoculations with ripe and bitter rots in combination (control) or alone after a treatment of BioSave 10 LP or 10 ppm of continuous fumigation of SO_2, over all other treatment effects ($p < 0.01$).

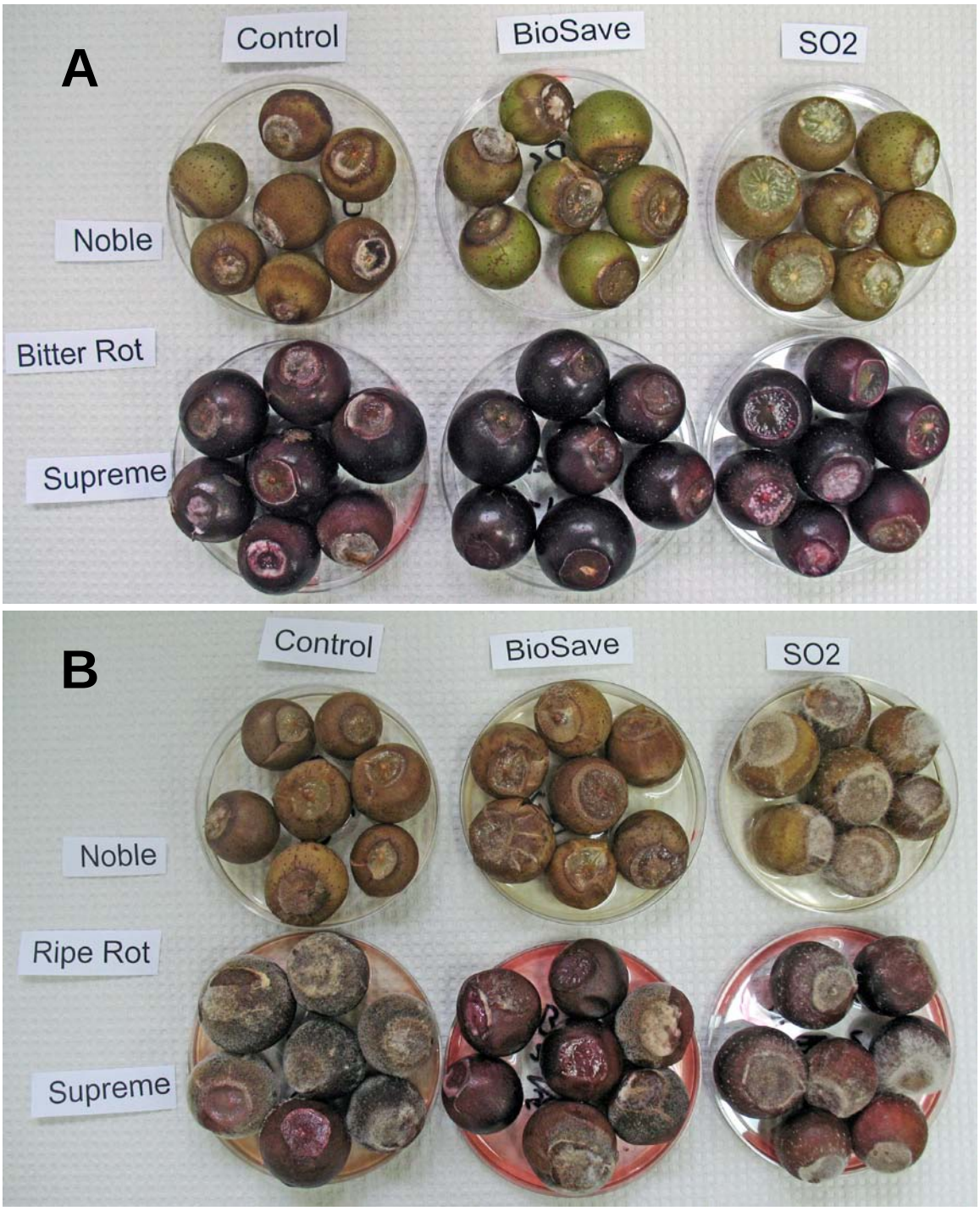

Figure 9. Effect of Biosave (and SO2) on the control of bitter rot (A) or ripe rot (B) in 'Fry' (mislabeled as 'Noble') and 'Supreme' muscadine grapes. Fruits were inoculated with $2x10^6$ or $1x10^6$ spores, respectively, using a spray bottle and permitted to dry before the spray application of BioSave 10 LP. Fruit were placed in a humidified container for one week (21ºC).

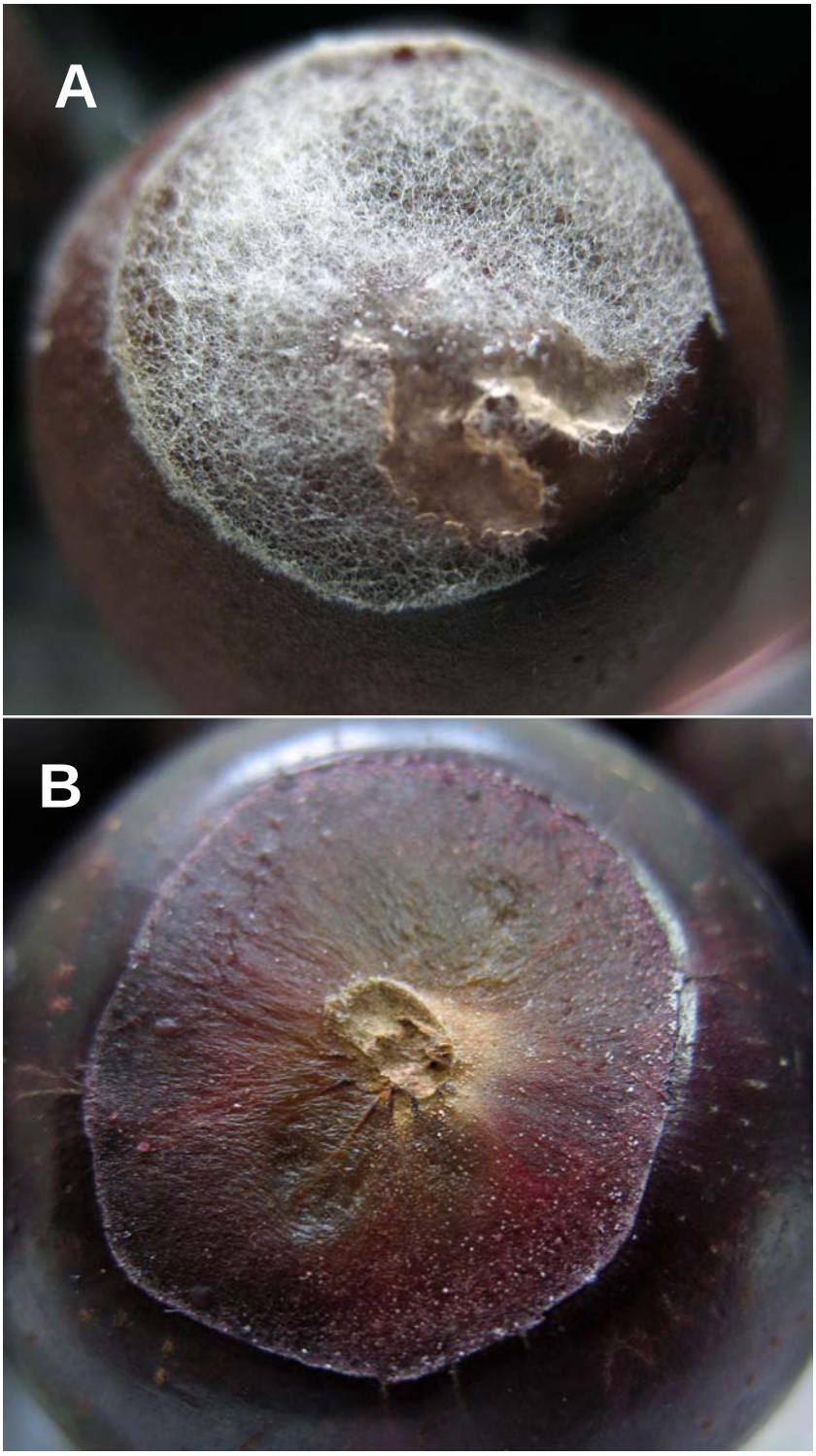

Figure 10. The mycelial growth and sporolation of bitter rot on ‘Supreme’ muscadine grape 7 days after inoculation (A), and BioSave 10 LP treatment (B).

Conclusion/Impact Statement

Controlling postharvest pathogens of muscadine grapes is important for market expansion and increasing consumer appeal. Two postharvest technologies were evaluated for their effectiveness at controlling ripe and bitter rots. These were a sulfur dioxide fumigation performed in a similar manner to the recommended table grape industry rates, and BioSave 10 LP, a biofumigant with known benefit for numerous other fruit and vegetable industries. In the postharvest storage trial, it was demonstrated that BioSave 10 LP has potential benefit for extending the postharvest storage and marketing windows of the muscadine grapes. Slight, but consistent suppression of the rate in the incidence of pathogen activity was found over most storage, shelf and cultivar effects. However, the sulfur dioxide rate used in this study was too high, and resulted in significant stem end damage. Future studies will need to explore lower rates to prevent damage, while still maintaining biological activity. In the controlled inoculation study, we did demonstrate that consistent low dose exposure to sulfur dioxide does offer some potential for control of storage pathogens. However, this same study demonstrated a much greater effectiveness of BioSave 10 LP under the same conditions. In the future, we will evaluate BioSave 10 LP for control of another major storage pathogen, macrophoma rot, which was not investigated in this preliminary study.

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