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Control of green mold and sour rot of stored lemon by biofumigation with *Muscodor albus*

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Abstract

Control of postharvest lemon diseases by biofumigation with the volatile-producing fungus *Muscodor albus* was investigated. In vitro exposure to *M. albus* volatile compounds for 3 days killed *Penicillium digitatum* and *Geotrichum citri-aurantii*, causes of green mold and sour rot of lemons, respectively. Lemons were wound-inoculated with *P. digitatum* and placed in closed 11-L plastic boxes with rye grain cultures of *M. albus* at ambient temperature. There was no contact between the fungus and the fruit. Biofumigation for 24–72 h controlled green mold significantly, even when treatment began 24 h after inoculation. Effectiveness was related to the amount of *M. albus* present. In tests conducted inside a 11.7-m^3 degreening room with 5 ppm ethylene at 20 °C, green mold incidence on lemons was reduced on average from 89.8 to 26.2% after exposure to *M. albus* for 48 h. Ethylene accelerates color development in harvested citrus fruit. *M. albus* had no effect on color development. Biofumigation in small boxes immediately after inoculation controlled sour rot, but was ineffective if applied 24 h later. *G. citri-aurantii* may be less sensitive to the volatile compounds than *P. digitatum* or escapes exposure within the fruit rind. Biofumigation with *M. albus* could control decay effectively in storage rooms or shipping packages.

Keywords: Biofumigation; *Citrus*; Fumigant; *Geotrichum citri-aurantii*; Lemon; *Muscodor albus*; *Penicillium digitatum*; Postharvest; Sour rot; Volatile

1. Introduction

Harvested citrus fruit are very susceptible to wound infection by *Penicillium digitatum* (Pers.:Fr.) Sacc, which causes green mold of citrus (Sommer et al., 2002). Minimizing wounds on fruit, proper temperature management, and postharvest fungicide treatments are the main methods of reducing losses by this pathogen (Eckert and Eaks, 1989). Continuous use of fungicides such as imazalil, thiabendazole, and *o*-phenylphenol in citrus packing facilities in California has resulted in the appearance of *P. digitatum* isolates with multiple fungicide resistances within natural populations (Holmes and Eckert, 1999), which further complicates the management of green mold. In addition, maximum residue limits for imazalil, the most important fungicide in use, are lower in most citrus importing countries than in the United States (United States Department of Agriculture-Foreign Agricultural Service, 2004). Because of these constraints, new approaches to protect citrus fruit from diseases in domestic and foreign markets are of interest to the citrus industry.

Sour rot of citrus, caused by *Geotrichum citri-aurantii* Butler (syn = *Geotrichum candidum* Link), is another potentially devastating storage disease. Although less common than green mold, it can cause significant losses in high rainfall years. Sour rot is not controlled with the currently registered fungicides imazalil and thiabendazole,

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and only partially controlled by sodium *o*-phenylphenate, which is not commonly used due to risk of fruit injury (Eckert and Eaks, 1989). Sour rot can be partially managed by sanitation and minimizing fruit storage temperatures after harvest, but chilling injury to the fruit and temperature variations during transport and marketing limit the effectiveness of this approach. Inoculum from infected fruit may create large nests of infected fruit during storage and transportation.

There have been considerable efforts to develop methods to control fungal decay in citrus that are considered to be safer to consumers, workers, and the environment than the fungicides now in use. These include biological control (Janisiewicz and Korsten, 2002), low toxicity chemicals (Smilanick et al., 1997, 1999), and physical treatments such as heat and UV-C, which can enhance disease resistance (D'hallewin et al., 1993, 2000; Porat et al., 2000). Currently, the biofungicides Aspire and BioSave are registered for postharvest use on citrus in the United States (Janisiewicz and Korsten, 2002), although both have shown limitations in efficacy as stand-alone treatments for citrus fruit (Brown and Chambers, 1996; Brown et al., 2000; Droby et al., 1998). Because most alternative treatments need further efforts in research and development to be implemented, there is still a need in the citrus industry for effective and environmentally friendly means of postharvest decay control.

Biological fumigation, or biofumigation, with volatile compounds produced by the fungus *Muscodor albus* Worapong, Strobel, and Hess has shown promise for killing a wide range of storage pathogens and controlling fungal decay (Mercier and Jiménez, 2004). Biofumigation for 24 h or longer with rye grain culture of *M. albus* controlled brown rot of peaches, caused by *Monilinia fructicola*, and gray mold and blue mold of apple, caused by *Botrytis cinerea* and *P. expansum*, respectively (Mercier and Jiménez, 2004). Biofumigation of greenhouse soilless mix with rye grain culture of *M. albus* was also effective in controlling soil-borne diseases of vegetable seedlings (Mercier and Manker, 2005). *M. albus* was reported to produce 28 organic volatile compounds which together inhibited and killed various species of fungi, oomycetes, and bacteria (Strobel et al., 2001). The fungus, which is closely related to the endophytic fungus *Xylaria* (Ascomycetes), was isolated as an endophyte from a cinnamon tree (Worapong et al., 2001). *M. albus* is a sterile mycelium and can grow readily on ordinary culture media such as potato dextrose agar. The main objective of this research was to evaluate the potential of biofumigation with rye grain culture of *M. albus* to control green mold and sour rot of lemons, focusing on the biofumigant dose and treatment time required. Large-scale biofumigation of lemons during ethylene treatment of a storage room was also done to evaluate compatibility with the degreening process and potential for scaling up the treatment for commercial application.

2. Materials and methods

2.1. Fungal isolates

Muscodor albus isolate 620 was obtained from Dr. G. Strobel, Montana State University, and was described previously (Worapong et al., 2001). The fungus was grown on autoclaved rye grain as described previously (Mercier and Jiménez, 2004) and the fresh culture was used for the biofumigation experiments. In the case of large-scale experiments, air-dried rye culture was used after adding water at a 1:1 ratio (w/w). The air-dried rye culture of *M. albus* was shown to have disease control activity equivalent to fresh culture upon re-hydration when tested against blue mold of apple (J. Mercier, unpublished data).

Postharvest pathogens used in this study were *P. digitatum* isolate M6R, obtained from J.W. Eckert, and *G. citri-aurantii*, which was isolated from an infected lemon. The isolates were preserved as spore suspensions in 15% glycerol at −80 °C and re-cultured when needed. Inoculation methods were those recommended by Eckert and Brown (1986). The pathogens were grown on potato dextrose agar (PDA) at room temperature for 5–10 days. To make conidial suspensions of *P. digitatum*, the plates were flooded with water with 0.01% Tween 20 and rubbed gently with a glass rod. The resulting suspension was filtered through one layer of cheesecloth and the concentration was adjusted with a hemacytometer. Arthrospore suspensions of *G. citri-aurantii* were prepared similarly but were adjusted with a spectrophotometer to contain 1×10^8 arthrospores/ml.

2.2. In vitro effect of M. albus volatile compounds on P. digitatum and G. citri-aurantii

The effect of volatile compounds on the isolates of the postharvest pathogens used in this study was tested on PDA as described by Strobel et al. (2001). A strip of agar was removed across each plate to make a medium-free moat physically separating two areas with medium and preventing any metabolites from diffusing across the plate through the medium. After growing *M. albus* on one side of the plate for 7 days, a PDA agar plug (6 mm diameter) of a test pathogen culture was added to the other side before sealing with parafilm. Three replicate plates were used for each pathogen. Growth was rated after three days at 21–23 °C. Plugs that did not grow after three days were transferred to fresh PDA plates to assess their viability.

2.3. Control of green mold

Experiments were conducted with organically grown lemons cv. Eureka from California. Fruits were selected for absence of injuries and visible defects, to minimize