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In vitro Effects of *Muscodor albus* and Three Volatile Components on Growth of Selected Postharvest Microorganisms

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Abstract. **Biofumigation by volatiles of *Muscodor albus* Worapong, Strobel & W.M. Hess, an endophytic fungus, was investigated for the biological control of three postharvest fungi, *Botrytis cinerea* Pers., *Penicillium expansum* Link, and *Sclerotinia sclerotiorum* (Lib) de Bary, and three bacteria, *Erwinia carotovora* pv. *carotovora* (Jones) Bergey et al., *Pseudomonas fluorescens* Migula (isolate A7B), and *Escherichia coli* (strain K12). Bacteria and fungi on artificial media in petri dishes were exposed to volatiles produced by *M. albus* mycelium growing on rye seeds in sealed glass 4-L jars with or without air circulation for up to 48 hours. The amount of dry *M. albus*–rye seed culture varied from 0.25 to 1.25 g·L^{-1} of jar volume. Fan circulation of volatiles in jars increased efficacy and 0.25 g·L^{-1} with fan circulation was sufficient to kill or suppress all fungi and bacteria after 24 and 48 hours, respectively. Two major volatiles of *M. albus,* isobutyric acid (IBA) and 2-methyl-1-butanol (MB), and one minor one, ethyl butyrate (EB), varied in their control of the same postharvest fungi and bacteria. Among the three fungi, IBA killed or suppressed *S. sclerotiorum, B. cinerea,* and *P. expansum* at 40, 25, and 45 µL·L^{-1}, respectively. MB killed or suppressed *S. sclerotiorum, B. cinerea,* and *P. expansum* at 75, 100, and 100 µL·L^{-1}, respectively. EB was only able to kill *S. sclerotiorum* at 100 µL·L^{-1}. Among the three bacteria, IBA killed or suppressed *E. coli* (K12), *E. carotovora* pv. *carotovora,* and *P. fluorescens* at 5, 12.5, and 12.5 µL·L^{-1}, respectively. MB killed or suppressed *E. coli* (K12), *E. carotovora* pv. *carotovora,* and *P. fluorescens* at 100, 75, and 100 µL·L^{-1}, respectively. EB did not control growth of the three bacteria. This study demonstrates the need for air circulation in *M. albus*, MB, and IBA treatments to optimize the efficacy of these potential postharvest agents of disease control.**

Bacterial and fungal infections, which may occur at harvest, during handling, storage, transport and marketing, and after consumer purchase (Eckert and Ogawa, 1988), are a major cause of postharvest loss in horticultural crops. Several fungicides and volatile chemicals are available for postharvest treatment of fruits and vegetables, but some of them have been removed from the market due to possible toxicological risks or insufficient efficacy (Adaskaveg et al., 2002). At the same time, there is a growing interest in finding and developing more natural means of controlling postharvest diseases (Ezra et al., 2004a; Mercier and Jiménez, 2004; Strobel et al., 2001).

An interesting candidate for biological control is *Muscodor albus* isolate 620 (Worapong et al., 2001) an endophytic fungus isolated from a cinnamon tree (Ezra et al., 2004b; Ezra and Strobel, 2003; Strobel et al., 2001). *Muscodor albus* inhibits and/or kills microorganisms by production of a number of volatiles, mainly alcohols, acids, and esters. Antifungal activity is mainly associated with the production of isobutyric acid (IBA) 2-methyl-1-butanol (MB) and ethyl butyrate (EB) (Mercier and Jiménez, 2004). MB, IBA, and EB comprise 48.5%, 14.9%, and 0.14% of the volatiles produced by fresh rye culture of *M. albus* 1 h after placement in a container. Other compounds produced by *M. albus* such as ethyl propionate, ethyl isobutyrate and methyl isobutyrate, although less inhibitory, may also contribute to the antimicrobial activity. Many of these compounds are well known as natural constituents of fresh leaves, fruit, wine, rum and blue cheese aromas, other natural essential oils and olive and vegetable oil (Federal Register, 2004).

Stinson et al. (2003) reported that sugar beet stand establishment increased and disease severity decreased in sterile soil artificially infested with *Rhizoctonia solani* (Kühn) AG 2-2, V*erticillium dahliae* Kleb. and *Fusarium oxysporum* Schlech.:Fr. f. sp. *betae* (Steward) Snyd. & Hans after biofumigation with *Muscodor albus* (isolate 620) or *M. roseus* (isolate A3-5). They also demonstrated that eggplant seedlings grown in disease-infested soil and biofumigated with *M. albus* have significantly less disease, compared with nonbiofumigated soil. The effectiveness of *M. albus* in controlling soil-borne disease has also been reported by Mercier and Manker (2005).

In vitro exposure of a wide range of fungal and bacterial microorganisms to volatile compounds released from *M. albus* kills or inhibits spore germination and mycelial or colony growth, without physical contact, including *Sclerotinia sclerotiorum*, *Botrytis cinerea,* and *Penicillium expansum,* all major postharvest pathogens (Ezra and Strobel, 2003; Mercier and Jiménez, 2004; Strobel et al., 2001). Mercier and Jiménez (2004) have reported that biofumigation for 24 h with a culture of *M. albus* grown on autoclaved rye grain completely controls blue (*P. expansum*) and grey mold (*B. cinerea*) of apple, as well as brown rot of peaches [*Monilinia fructicola* (Wint.) Honey], in wound-inoculated fruit. Biofumigation with *M. albus* also controls sour rot (*Geotrichum citri-aurantii* Butler) and green mold [*P. digitatum* (Pers.:Fr.) Sacc.] of lemons (Mercier and Smilanick, 2005) as well as grey mold of grapes (Mlikata Gabler et al., 2005). In the case of lemons, whole room biofumigation was attempted and shown to be successful in reducing green mold (Mercier and Smilanick, 2005).

Strobel et al. (2001) tested several of the major individual volatiles produced by *M. albus* and found that they had less effect on the growth of the tested pathogens than the *M. albus* culture.

The optimum amount of *M. albus* which may provide economical control of a specific microorganism without injuring stored product is not fully understood. Therefore, more research work is needed to clarify application methods and rates for *M. albus* and/or individual *M. albus* volatile compounds when used as biofumigants to control postharvest diseases. In this paper we investigate the in vitro biofumigation conditions needed for *M. albus* to effectively control selected major postharvest bacteria (*Erwinia carotovora* pv. *carotovora*, *Pseudomonas fluorescens* (isolate A7B), and *Escherichia coli* (K12)) and fungi (*B. cinerea, P. expansum, and S. sclerotiorum*). The effective anti-microbial concentrations of two major volatiles (IBA and MB) and one minor volatile (EB) produced by *M. albus* were also investigated.

Materials and Methods

Muscodor albus and chemicals. Dry, *M. albus* strain 620–colonized rye seed (from J. Mercier, AgraQuest Inc., Davis, Calif.) was stored at 4 °C and warmed to room temperature for 2 h before being used in experiments. The volatile chemicals, IBA, MB, and EB were purchased from Sigma-Aldrich (Oakville, Ont., Canada).

Bacterial and fungal inoculum production. Cultures of *E. carotovora* pv. *carotovora, P. fluorescens* (A7B) and a nonpathogenic strain of *E. coli* (K12) were grown on nutrient agar (NA; Difco) for 24 to 48 h at 27 °C before use. Bacterial suspensions with an absorbance value of 0.05 units at 600 nm (Abs_{600}) were prepared in 10 mL of sterile distilled water (SDW), then diluted 1 to 1000 before being applied to fresh NA with a spiral plater (Spiral Biotech., Bethesda, Md.).

Inhibition of *S. sclerotiorum* growth by *M. albus* or chemicals was tested on mycelium. Two disks of agar containing mycelium were

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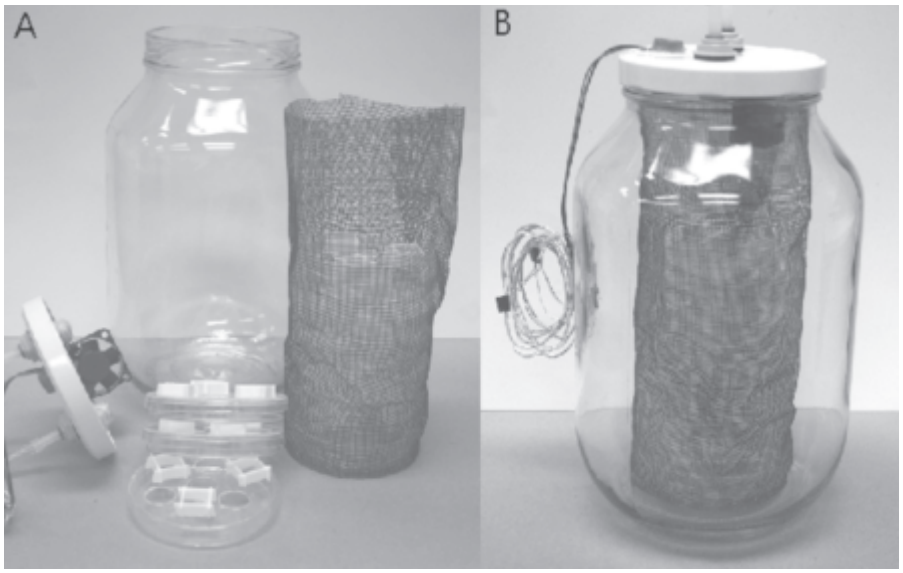

Fig. 1. The *Muscodor albus* and *M. albus* volatile treatment chamber consisting of a 4-L glass jar with a sealing lid. (**A)** A 4-cm^2 fan for air circulation was attached to the inside of the lid. Petri dishes of test microorganism spores, mycelium or cells on media were fitted with lids containing holes and spaces to facilitate air circulation. Dishes were stacked in nylon mesh sleeves to permit easy insertion and withdrawal from the chamber. (**B)** The assembled chamber with stacked petri dishes in the nylon sleeve.

cut with a flame sterilized corkborer from the margin of a 2-d-old colony and transferred to fresh 9-cm-diameter petri dishes of PDA and equally spaced with the mycelial side of the disk in contact with the PDA surface.

Inhibition of *P. expansum* and *B cinerea* growth was tested on spore germination. *P. expansum* inoculum was prepared by cutting an 8-mm-diameter disk from a sporulating culture and transferring it to 10 mL of SDW in a 15-mL Falcon centrifuge tube. The tube was capped tightly and shaken vigorously to dislodge and break up the chains of spores. *B. cinerea* spores were collected by flooding a sporulating culture with 10 mL of SDW and dislodging the spores by rubbing the culture with a bent glass rod. Spore suspension (100 µL) was transferred with a micropipetter to the center of fresh 9-cm-diameter petri dishes of PDA and spread over the media with a bent glass rod.

Treatment with Muscodor volatiles and M. albus. To assist the passage of volatiles over the surface of the media in stacks of Petri dishes the lids were ventilated with holes and separated by spacers. In a sterile laminar flow hood the lids of all the freshly inoculated Petri dishes were replaced with lids containing four, 2 cm diameter holes arranged in a triangle with one hole in the center. In the spaces between the holes, three 2-cm polystyrene squares, 0.8 mm tall, were attached with methylene chloride (Fig. 1). Two dishes of each test microorganism were randomly stacked in a sleeve constructed of nylon fly screening (9.7 cm in diameter × 25 cm tall). Bacteria and fungi were tested separately. The sleeves of stacked dishes were lowered into 4-L glass jars with lids. To assist the distribution of volatiles within the sealed jar a 12V DC fan (Mode Electronics, Burnaby, B.C., Canada) measuring 40 × 40 × 20 mm with a 5.2 cubic foot per min capacity was attached to the inside of each lid.

A range of volatile chemical concentrations was used to determine the effective concentration to control each of the individual fungal and bacterial organisms tested. For EB and MB, 0, 100, 200, 300, and 400 µL was added to jars containing bacterial or fungal cultures. IBA was applied at 0, 50, 100, 150, and 200 µL per jar for fungi and 0, 10, 20, 30, and 40 µL per jar for bacteria. The chemicals were applied to 4.25 cm Whatman 1 filter paper circles in 5 cm diameter glass Petri dishes placed on the top of the stack of cultures in the nylon sleeve within the jar. The lid was immediately screwed tight and sealed with Parafilm and the chemicals were allowed to completely volatilize. The jars were placed in a controlled environment room at 20 °C. The fans were connected to a 12-v DC power supply and the fungal and bacterial cultures were incubated in the dark for 24 and 48 h, respectively. Similarly, to determine the weight of *M. albus*-colonized grain required to control the test fungi and bacteria, 0, 1, 2, 3, 4, and 5 g of colonized grain were weighed on to 9-cm circles of Whatman 1 filter paper in the lids of 9-cm petri dishes and then placed on top of the stack of fungal or bacterial cultures in nylon sleeves within the jars. A volume of SDW equal to the weight of the grain was added to each dish to moisten and activate the *M. albus* (Jiménez and Mercier 2005) plus an additional 1 mL to saturate the air in the jar. The control consisted of 1 mL of SDW without *M. albus*. The jars were immediately closed, sealed and incubated as described for the chemicals except that a second set of jars and cultures was treated with *M. albus* without fan activity.

After the prescribed incubation period the jars were returned to a vented laminar flow hood and the sleeves of cultures were removed from the jars. The dishes which contained the chemicals or *M. albus* were removed. After the fungal and bacterial cultures were ventilated

Table 1. Germination and growth and survival of *Botrytis cinerea*, *Penicillium expansum*, *Sclerotinia sclerotiorum*, *Erwinia carotovora* pv. *carotovora*, *Pseudomonas fluorescens,* and *Escherichia coli* following exposure to *Muscodor albus* volatiles in 4-L glass jars, with or without circulation fans (+Fan or –Fan).

Muscodor albus[z]	*Botrytis cinerea*[y]		*Penicillium expansum*		*Sclerotinia sclerotiorum*		*Erwinia carotovora* pv. *carotovora*		*Pseudomonas fluorescens*		*Escherichia coli*	
(g·L^{-1})	–Fan	+Fan	–Fan	+Fan	–Fan	+Fan	–Fan	+Fan	–Fan	+Fan	–Fan	+Fan
Germination or growth at removal (%)[x]												
0	100 a[w]	100 a	100 a	100 a	100 a	100 a	100	100	100	100	100	100
0.25	87 ab	92 a	31 b	10 b	17 b	17 b	0	0	0	0	25	0
0.5	85 b	34 b	5 c	1 c	14 bc	12 bc	0	0	0	0	0	0
0.75	50 c	6 c	1 c	0 c	11 bc	5 cd	0	0	0	0	0	0
1.0	47 c	0 d	1 c	0 c	10 bc	3 cd	0	0	0	0	0	0
1.25	41 c	0 d	0 c	0 c	8 c	0 d	0	0	0	0	0	0
Survival after removal and 24 h in ambient air (%)												
0	100	100	100	100	100	100	100	100	100	100	100	100
0.25	0	0	31	0	100	0	0	0	0	0	25	0
0.5	0	0	0	0	100	0	0	0	0	0	0	0
0.75	0	0	0	0	100	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	0	0	0	0

[z]Weight of rye grains per liter of treatment chamber volume.
[y]The fungi (*B. cinerea*, *P. expansum*, and *S. sclerotiorum*) and bacteria (*E. carotovora* pv. *carotovora*, *P. fluorescens*, and *E. coli*) were exposed to *M. albus* volatiles for 24 and 48 h, respectively.
[x]Germination or growth (%) was assessed at removal from *M. albus* volatiles and was compared to controls. For *B. cinerea* and *P. expansum*, growth is defined as the germ tube length being 2× spore diameter; for *S. sclerotiorum,* growth was measured as the colony diameter. For bacteria, visible colonies were counted.
[w]Means within a column not having the same letter are significantly different at $P = 0.05$.

with 2 min of compressed air to remove treatment volatiles the vented lids were replaced with standard petri dish lids. Bacterial colony numbers were estimated from counts using a spiral plate counting grid (Spiral Biotechnology, Bethesda, Md.) and the per cent colony survival compared with the controls was calculated. *Sclerotinia sclerotiorum* growth was quantified by taking two measurements, perpendicular to each other, of the diameter of mycelial growth from each of the two mycelial disks applied to each dish of media and a percent growth relative to the control was calculated. The percent spore germination was recorded for *P. expansum* and *B. cinerea* cultures by counting 100 spores near the center of the petri dish and recording the number of spores with germ tubes 2× longer than the width of the spore. All counts were made immediately after the end of the treatment period and again after an additional 24 h of incubation on a lab bench. All experiments were repeated three times.

Statistical analysis. For each organism, the five levels of *M. albus* (fixed factor 1) were randomly assigned to chambers with or without a circulation fan (fixed factor 2) in a two-way randomized complete design structure. Where the data were normally distributed, the germination, growth or survival means (n = 6) for each organism treated with or without a fan at each level of *M. albus* were separated by the Waller Duncan k ratio *t* test, where k = 100 approximates $P = 0.05$, of SAS's Proc GLM (SAS Institute, 1994). The relationship between the dependent variables, fungal spore germination or mycelial growth, or bacterial colony establishment (Y), and the levels of the independent variables, IBA, MB, or EB (X), was established by logistic regression (SigmaPlot, 2004). Unless noted otherwise, only results significant at $P = 0.05$ are discussed.

Results

Muscodor albus volatiles had a significant effect on the germination, growth and survival of the three postharvest fungi (*B. cinerea, P. expansum, and S. sclerotiorum*) and bacteria (*E. carotovora* pv. *carotovora, P. fluorescens* A7B, and *E. coli* K12) examined in this study (Table 1). The fungal results clearly show that increasing the weight of *M. albus*-colonized grain from 0.25 to1.25 g·L^{-1} had a significant effect ($P = 0.05$) on the ability of *M. albus* volatiles to inhibit spore germination of *B. cinerea* and *P. expansum* and colony diameter increase of *S. sclerotiorum*. While spore germination of *P. expansum* and colony growth of *S. sclerotiorum* were nearly completely inhibited in the presence of 1.25 g·L^{-1} of *M. albus*-colonized grain, *B. cinerea* in jars without air circulation (–Fan) had 41% of the spores germinated after 24 h. However, *B. cinerea* spores in jars with air circulation fans (+Fan) stopped germinating at 1.0 g·L^{-1} of *M. albus*-colonized grain. This difference between the efficacy of *M. albus* volatiles with and without air circulation was not evident with *P. expansum* or *S. sclerotiorum*.

The results of the fungal spore and mycelium survival or growth 24 h after removal from the treatments and placement in ambient air are markedly different than the germination and growth results measured immediately after removal. None of the germinated spores or mycelial colonies exposed to *M. albus* volatiles continued to grow except for *P. expansum* at 0.25 g·L^{-1} without air circulation (–Fan) and *S. sclerotiorum* at 0.25 to 1.0 g·L^{-1} (–Fan) (Table 1).

All three of the test bacteria were killed or suppressed after a 48 h exposure to *M. albus* volatiles even at the lowest weight of colonized rye grain except for *E. coli* (Table 1). Of the *E. coli* colonies, 25% at 0.25 g·L^{-1} without air circulation (–Fan) survived the *M. albus* volatiles but none survived or grew after the same exposure with air circulation (+Fan).

The three test volatiles, MB, IBA, and EB, had an inhibitory effect on one or more of the six test organisms, as measured immediately after removal from treatment (data not shown) and again 24 h later (Figs. 2, 3, and 4, respectively). Preliminary research showed that, without fan-assisted circulation of the volatiles, their inhibitory effects were restricted to culture surfaces immediately below the openings in

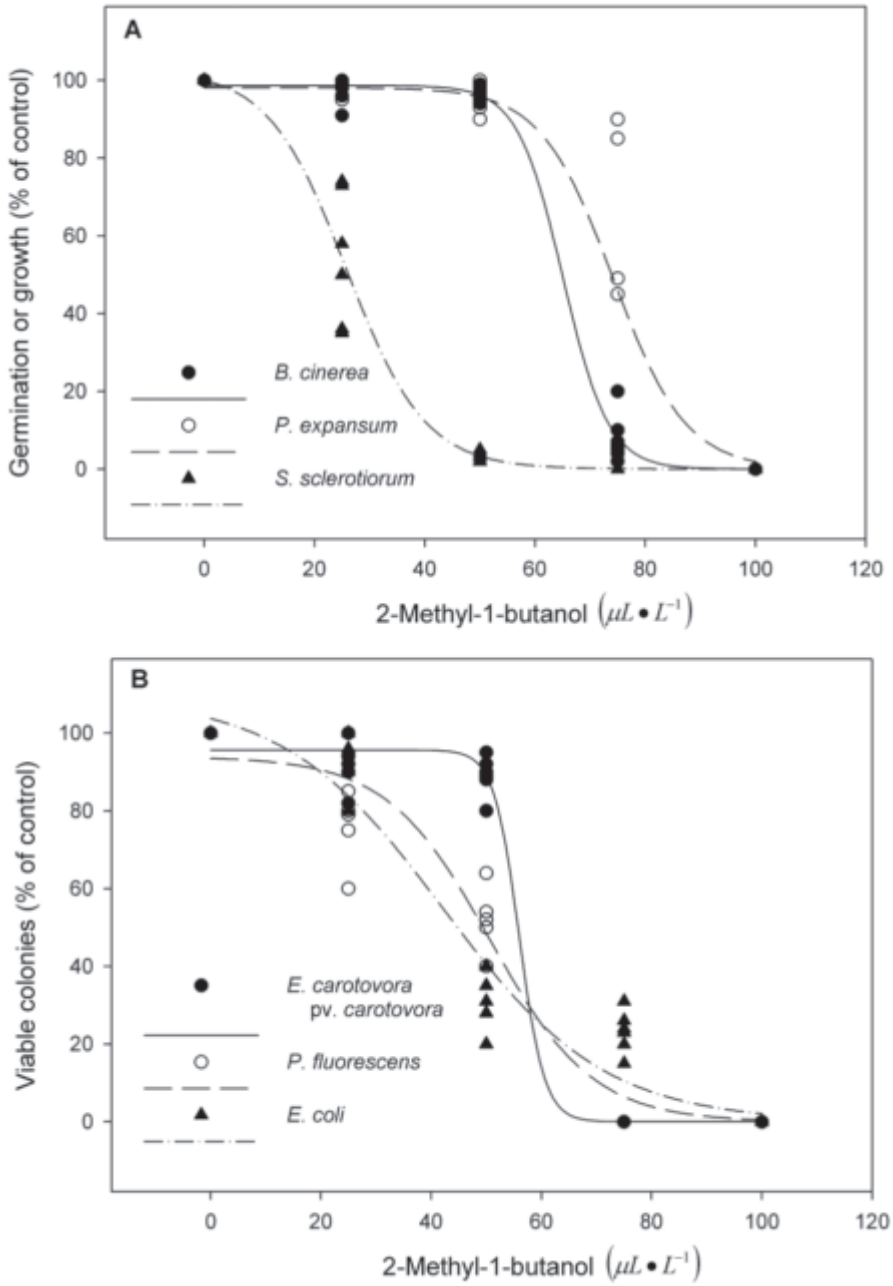

Fig. 2. Influence of 2-methyl-1-butanol (MB) after 24 h postremoval on (**A**) germination (%) of *Botrytis cinerea* [germination (%) = 98.7/(1 + exp(– (x – 65.1)/–4.1)), $R^2 = 0.99$], *Penicillium expansum* [germination (%) = 98.1/(1 + exp(– (x – 74.2)/–6.6)), $R^2 = 0.96$], and *Sclerotinia sclerotiorum* [mycelial growth (%) = 102.6/(1 + exp(– (x – 25.8)/–7.1)), $R^2 = 0.98$] or (**B**) viable colonies (%) of *Erwinia carotovora* pv. *carotovora* [viable colonies (%) = 95.7/(1 + exp(– (x – 56)/–2.3)), $R^2 = 0.99$], *Pseudomonas fluorescens* [viable colonies (%) = 93.9/(1 + exp(– (x – 50.6)/–9.3)), $R^2 = 0.98$], and *Escherichia coli* [viable colonies (%) = 109.4/(1 + exp(– (x – 42.3)/–14.6)), $R^2 = 0.98$].

the petri dish lids (Fig. 5). Thus, only data generated from chambers with fan circulated atmospheres are presented and discussed. For the three fungi treated with MB, there was no germination (*B. cinerea* and *P. expansum*) and no growth (*S. sclerotiorum*) at 100 $\mu L \cdot L^{-1}$ (Fig. 2a). The 50% inhibitory concentration (IC_{50}) for germination or growth was 27, 65, and 75 $\mu L \cdot L^{-1}$ for *S. sclerotiorum*, *B. cinerea*, and *P. expansum*, respectively. The slopes of the linear portion of the regression lines were similar for the three fungi (–3.8, –3.5, and –5.5, respectively). *Erwinia carotovora* pv. *carotovora* was killed or suppressed at 75 $\mu L \cdot L^{-1}$ of MB and there was no growth of *P. fluorescens* or *E. coli* at 100 $\mu L \cdot L^{-1}$ of MB (Fig. 2b). The IC_{50}s for *P. fluorescens*, *E. coli* and *E. carotovora* pv. *carotovora* were 50, 45 and 56 $\mu L \cdot L^{-1}$, respectively. The slopes of the regression lines for *P. fluorescens* and *E. coli* were –2.3 and –1.8, respectively, but *E. carotovora* pv. *carotovora* was steeper with a slope of –8.6 indicating that the latter pathogen was markedly more sensitive to MB over the concentration range tested.

When IBA was used, all three fungi were reduced to 0% germination or growth at <45 $\mu L \cdot L^{-1}$ (Fig. 3a). *Botrytis cinerea* (IC_{50} = 10) and *S. sclerotiorum* (IC_{50} = 8) appeared to be more sensitive than *P. expansum* (IC_{50} = 38), however, the slopes of the linear portions of the regression lines were much steeper for *B. cinerea* (–23) and *P. expansum* (–30) than for *S. sclerotiorum* (–5.5). All three bacteria were more sensitive to IBA than the three fungi (Fig. 3b). The IC_{50} for *P. fluorescens*, *E. coli,* and *E. carotovora* pv. *carotovora* were 2.3, 2.9, and 3.1 $\mu L \cdot L^{-1}$, respectively. In this instance the slope of the linear portion of the regression curve was much steeper for *E.coli* (–200) than for *P. fluorescens* (–15) and *E. carotovora* pv. *carotovora* (–16.7).

Fumigation with EB at 100 $\mu L \cdot L^{-1}$ did not affect any of the six organisms (data not shown), except for *S. sclerotiorum* (Fig. 4). There was no mycelial growth of *S. sclerotiorum* at 100 $\mu L \cdot L^{-1}$ and the IC_{50} value was 35 $\mu L \cdot L^{-1}$ with a regression curve slope of –1.7.

Discussion

Muscodor albus volatiles show great potential for controlling important postharvest diseases both in vitro and in vivo. All bacterial and fungal postharvest organisms tested were killed or suppressed by *M. albus* under the conditions defined in this study (Table 1). The bacteria seemed to be more sensitive than the fungi to the *M. albus* volatiles; however, they were exposed to the volatiles for 48 h while the fungi could only be exposed for 24 h. The rapid mycelial growth of the fungi threatened to overgrow the media making it impossible to count germinated spores or measure radial growth at sublethal exposures of >24 h. While there was considerable germination of *B. cinerea* and mycelial growth of *S. sclerotiorum* even at 1 $g \cdot L^{-1}$ of *M. albus*-colonized grain, these spores and mycelia were shown to be dead or exhibited inhibited growth in most treatments 24 h postremoval, even at the lowest weight of *M. albus* (Table 1). This discrepancy between effects of volatiles on germination and survival is likely related to the relative rates of spore germination and production of volatiles by *M. albus*. *Muscodor albus* is preserved as an air-dried mycelium on grains and is activated at the start of the experiment with an application of an equal weight of water (Jiménez and Mercier, 2005). Once hydrated, *M. albus* will produce maximum concentrations of volatiles within 24 h (Mercier and Jiménez, 2005), similar to fresh culture recently placed in a closed container (Mercier and Jiménez, 2004). *Botrytis cinerea* germinated in 3 to 4 h at 20 °C (data not shown) and *S. sclerotiorum* can completely colonize a 10 cm petri dish in just 72 h. Therefore, lethal concentrations of the volatiles may not be reached in the treatment chamber until after spore germination or mycelial growth has been initiated. In addition, it can be seen that spore germination of *B. cinerea* or survival of *S. sclerotiorum* mycelium without fan-assisted air circulation was greater. None of the six microorganisms survived even the lowest amount of *M. albus* in jars with air circulation after 24 h postremoval, and *B. cinerea* germination was reduced. The increased efficacy of *M. albus* volatiles in response to air circulation was

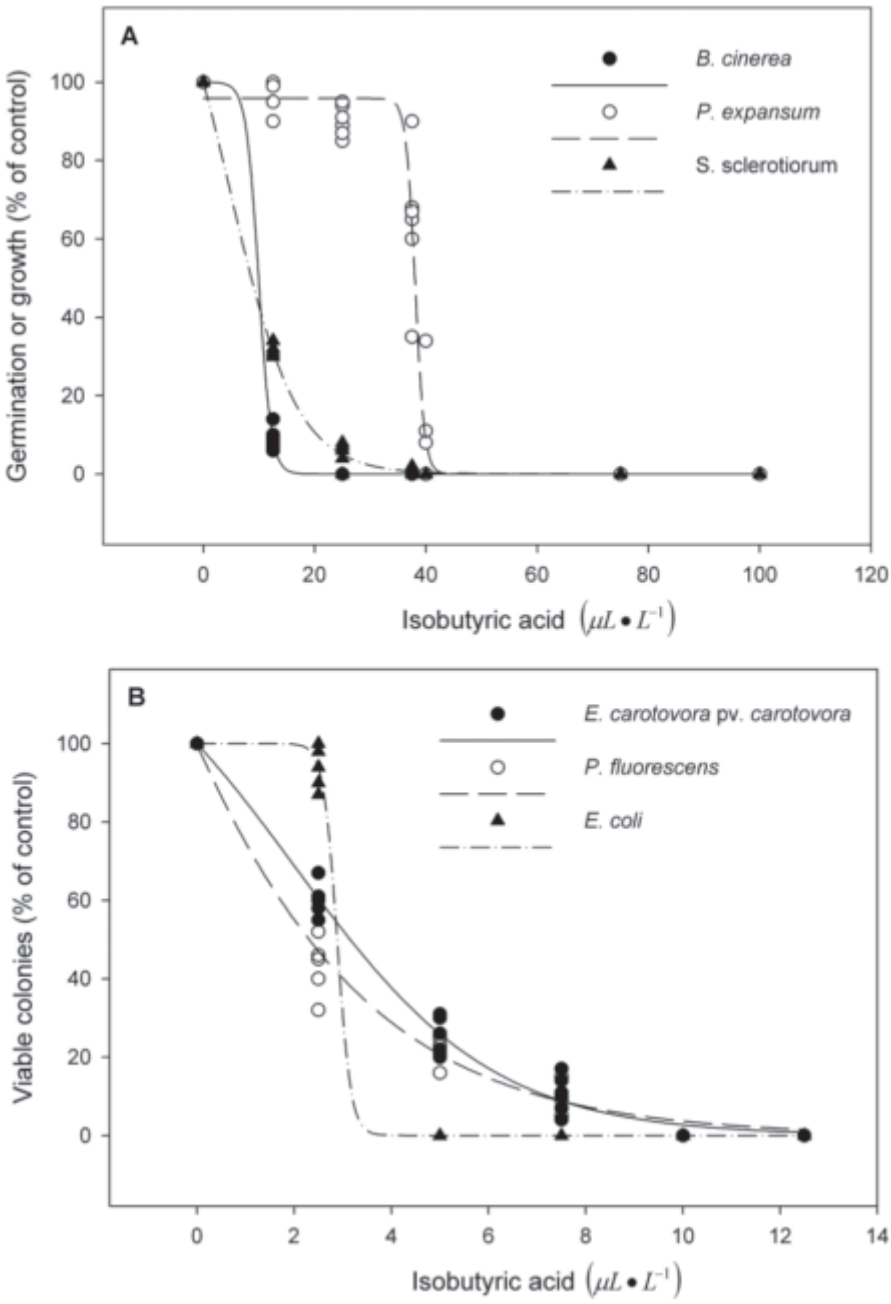

Fig. 3. Influence of isobutyric acid (IBA) after 24 h postremoval on (**A**) germination (%) of *Botrytis cinerea* [germination (%) = 100/91 + exp(– (x – 9.9)/–1.1)), R^2 = 0.99], *Penicillium expansum* [germination (%) = 95.8/(1 + exp(– (x – 38.1)/–0.8)), R^2 = 0.99], and *Sclerotinia sclerotiorum* [mycelial growth (%) = 145.8/(1 + exp(– (x – 4.7)/–6.0)), R^2 = 0.99] or (**B**) viable colonies (%) of *Erwinia carotovora* pv. *carotovora* [viable colonies (%) = 137.5/(1 + exp(– (x – 2.0)/–2.1)), R^2 = 0.99], *Pseudomonas fluorescens* [viable colonies (%) = 469.2/(1 + exp(– (x + 3.69)/–2.8)), R^2 = 0.99], and *Escherichia coli* [viable colonies (%) = 100/(1 + exp(– (x – 2.88)/–0.14)), R^2 = 0.99].

likely the result of heavier-than-air volatiles settling to the bottom of the containers if not stirred. Also, the petri dishes of microorganisms were stacked inside nylon mesh sleeves with only a few holes in the lids of the dishes, which may have restricted air movement over the surface of the media. This was particularly evident when *P. fluorescens* was exposed to IBA (7.5 $\mu L \cdot L^{-1}$) without fan-circulated air and colony inhibition only occurred directly below the holes in the lid (Fig. 5). Similar, but less dramatic, results were observed for all the fungal and bacterial organisms tested. Therefore, circulation of the volatiles appears to be required to maximize efficacy and will likely be important in biofumigation of bulk-stored fruits and vegetables.

Previously, it had been established that the main volatile compounds responsible for the inhibitory activity of *M. albus* against microorganisms were esters, alcohols and acids (Ezra et al., 2004; Strobel et al., 2001). In several studies, artificial combinations of a few to 20 of the identified volatiles have been tested for antimicrobial activity comparable to *M. albus* (Ezra et al., 2004; Ezra and Strobel, 2003; Strobel et al., 2001). Mercier and Jiménez (2004) determined that IBA, MB, and EB had the greatest antimicrobial activity; however, the conditions, methods of application and the optimum rates were not published. In our work, IBA had greater antimicrobial activity than MB, and EB had little activity except against *S. sclerotiorum*. While all fungi and bacteria were killed or suppressed by exposure to <45 and <12.5 $\mu L \cdot L^{-1}$ of IBA and MB, respectively, these calculated concentrations were considerably higher than the 4.6 and 15.5 $nL \cdot L^{-1}$ measured in the headspace above *M. albus* cultures (Mercier and Jiménez, 2004). Discrepancies in the response to individual *M. albus* volatiles compared with the natural suite of volatiles produced by *M. albus* cultures has been attributed to synergistic activity. However, Strobel et al. (2001) report that the optimum concentration of an artificial mixture of *M. albus* volatiles giving comparable inhibitory and biocidal effects was 1200 $\mu L \cdot L^{-1}$, which is higher than the individual volatile activities demonstrated in this study. This fact does not support a theory of synergistic activity and suggests that other unidentified volatiles may be involved. While the fungi or bacteria in this study had similar IC_{50} values for individual chemicals, some had very different regression line slopes. *Botrytis cinerea*, *P. expansum*, and *E. coli* had steeper slopes of their IC_{50} regression curves than the other test microorganisms (Fig. 3). These very sensitive reactions to the individual volatiles around the organisms' IC_{50} values made it impossible to determine if combinations of chemicals were synergistic, since the Abbott technique for measuring synergism examines the effect on growth or survival of the organism for the combination of two chemicals at their IC_{50} values compared with the individual chemical response (Gisi, 1996). Just the slightest variance in volatile chemical application resulted in missing the IC_{50} and rendering the results out of range and not suitable for analysis (data not shown).

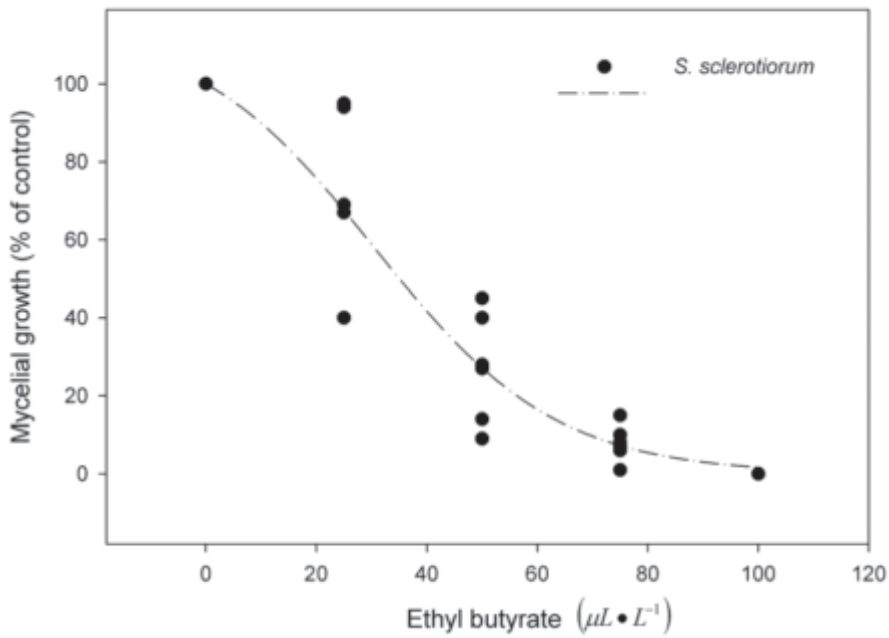

Fig. 4. Influence of ethyl butyrate (EB) after 24 h postremoval on square root mycelial growth (%) of *Sclerotinia sclerotiorum* [mycelial growth (%) = 115.3/(1 + exp(– (x – 30.7)/–16.4)), $R^2 = 0.96$].

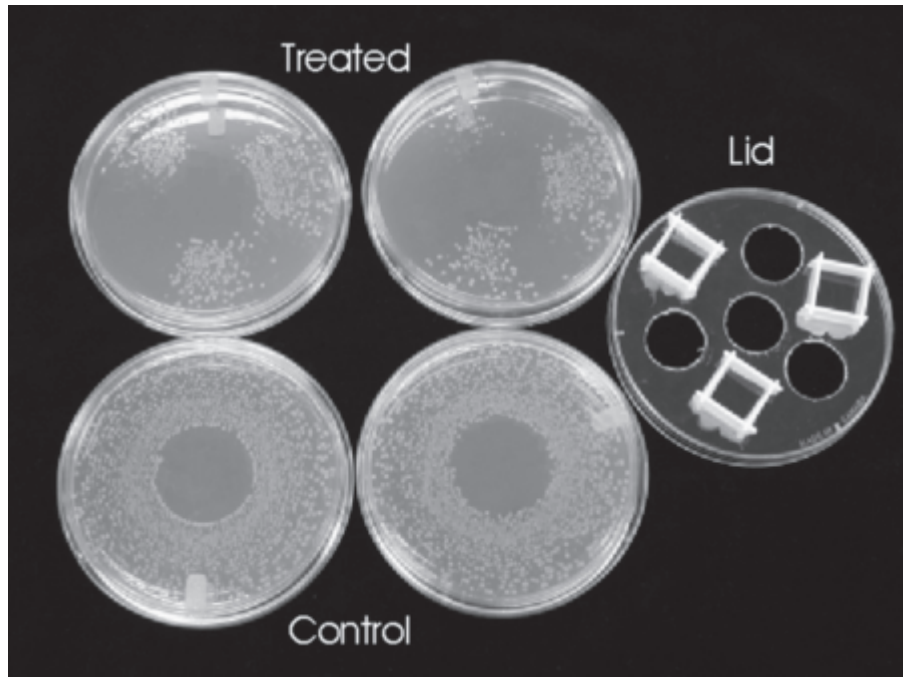

Fig. 5. Effects of isobutyric acid (7.5 $\mu L \cdot L^{-1}$) in jars without fan-circulated air on colony growth of *Pseudomonas fluorescens* below holes in the petri dish lids. The control petri dish received no treatment.

Recently, *Muscodor* spp. have been tested for use in certain agricultural settings to treat pathogen-infested plants, soil and seeds, with promising results (Mercier and Jiménez, 2004; Mercier and Manker, 2005; Mercier and Smilanick, 2005; Stinson et al., 2003; Strobel et al., 2001). Previous experiments indicated 1 to 13 $g \cdot L^{-1}$ of *M. albus*-colonized grain controlled blue and grey mold of apple (Mercier and Jimenez, 2004). However, our finding that only 0.25 $g \cdot L^{-1}$ with atmosphere circulation was adequate to control fungal and bacterial pathogens in vitro suggests that using lower quantities of *M. albus*-colonized grain may be feasible. In addition, more results will have to be obtained in vivo, as interaction with stored products and accessibility of the volatiles to infection sites might affect their efficacy.

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