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Antimicrobial activity of volatile organic compounds and their effect on lipid peroxidation and electrolyte loss in *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* mycelia

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Anthracnose is an important plant disease and is the main disease affecting guava (*Psidium guajava*) post-harvest, leading to economic losses and limiting fruit export. Because of the indiscriminate use of fungicides, along with environmental and food safety concerns, alternative methods of disease control are warranted. The yeast *Saccharomyces cerevisiae* produces a mixture of volatile organic compounds (VOCs) that show *in vitro* antimicrobial activity against various phytopathogens, with the compounds 3-methyl-1-butanol (3M1B) and 2-methyl-1-butanol (2M1B) being primarily responsible for this activity. Considering the potential of using VOCs for controlling post-harvest anthracnose, this study aimed to evaluate the *in vitro* effect of 3M1B and 2M1B on the development of *Colletotrichum gloeosporioides* and *C. acutatum*, the causal agents of the disease in guava, and to elucidate the possible modes of action of these antimicrobials. The mycelial growth of the plant pathogens was inhibited similarly by 3M1B and 2M1B, and complete inhibition occurred at doses ≥1 μL·mL^{-1} of air. Exposure of fungi to the VOCs increased the peroxidation levels of membrane lipids, indicating the occurrence of oxidative stress, in addition to increasing the non-selective permeability of the plasma membrane. Therefore, both 3M1B and 2M1B show potential to control *C. gloeosporioides* and *C. acutatum*.

Key words: anthracnose, inhibition, plasma membrane peroxidation, post-harvest, volatile compounds.

INTRODUCTION

Anthracnose is a disease caused by *Colletotrichum* spp. that impacts the growth of guava (*Psidium guajava* L.) in all guava-producing countries, causing considerable post-harvest losses and limiting fruit export. In the absence of

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control measures, the disease incidence can reach 70 - 100% (Lima Filho et al., 2003).

Although the causal agent of anthracnose in guava is globally accepted to be *C. gloeosporioides* (teleomorph: *Glomerella cingulata*) (Pandey, 1988; Menezes and Hanlin, 1996), there are reports of isolates of *C. acutatum* (teleomorph: *G. acutata*) causing fruit disease in India (Das and Bora, 1998) and Brazil (Peres et al., 2002). Therefore, guava anthracnose can be caused by single or multiple infections of these two fungal pathogens. Among fruits, where the symptoms are more severe, penetration of *Colletotrichum* sp. occurs in unripe fruits in the field. The pathogen can survive in quiescent form, and symptoms can occur at post-harvest. In severe cases, the fruits become mummified or rotten. These symptoms significantly compromise the market for fresh fruits.

The use of only a limited range of fungicidal and fungistatic compounds is allowed at post-harvest because of the risk of a residual effect. To date, no products have been registered for use in guava post-harvest in Brazil. In addition, knowledge of the potential impacts of traditional control practices on the environment and human health has stimulated the search for alternative control methods. In this context, biofumigation of fruits by using microorganisms that produce volatile organic compounds (VOCs) with antimicrobial activity or fumigation by using artificial mixtures of VOCs in closed chambers may be a viable alternative for controlling post-harvest pathogens. In addition to the treatment of fruits and vegetables, VOCs can be used to control pathogens associated with seeds and to fumigate contaminated soils (Strobel, 2011).

VOCs produced by microorganisms are metabolic compounds that are released as gases or exhibit a high vapor pressure and are released from cells under normal conditions (Tarkka and Piechulla, 2007). These compounds generally have a low molecular weight and are active at low concentrations, and can be categorized into different chemical classes (Wheatley, 2002). *Muscodor albus* is a known producer of bioactive VOCs, and the use of the organism itself or an artificial mixture of VOCs it produces has shown potential in controlling a wide range of post-harvest plant pathogens associated to soil, stored seeds, insect pests and plant nematodes (Strobel, 2011). Fialho et al. (2011) reported the potential use of *Saccharomyces cerevisiae* strains for the production of fungistatic VOCs. These compounds inhibited the mycelial growth of *Guignardia citricarpa*, the causal agent of citrus black spot, by up to 87%, in addition to controlling the white mold caused by *Sclerotinia sclerotiorum* in stored bean seeds. The main compounds responsible for the observed inhibition were identified as 3-methyl-1-butanol (3M1B) and 2-methyl-1-butanol (2M1B).

Little is known about the mode of action of antimicrobial VOCs. However, there is evidence that they cause DNA damage in the target organism (Mitchell et al., 2010) and alter the expression of enzymes associated to morphogenesis (Wheatley, 2002), which may affect growth. Moreover, they may alter the properties of cell membranes and trigger oxidative stress. Understanding these mechanisms of action is essential for elucidating the relationship of VOCs with microorganisms and for developing safe fumigants for the control of post-harvest diseases (Fialho et al., 2014). In this context, the objectives of the present study were to evaluate the *in vitro* effect of the VOCs 3M1B and 2M1B, isolated from *S. cerevisiae*, on the development of *C. gloeosporioides* and *C. acutatum* and to elucidate the possible modes of action involved in the observed inhibition, such as changes in membrane permeability and lipid peroxidation resulting from oxidative stress.

MATERIALS AND METHODS

Colletotrichum sp. isolates

C. gloeosporioides and *C. acutatum* were isolated from lesions in symptomatic guava fruits and grown in potato dextrose agar (PDA) at 25°C under a 12-h photoperiod. The cultures were deposited in the Department of Plant Pathology and Nematology at ESALQ-USP, Piracicaba, São Paulo, Brazil.

Antifungal activity

The VOCs 3M1B and 2M1B were purchased from Sigma-Aldrich (USA).

For the antimicrobial activity assays, commercially available two-section divided polystyrene plates (90 mm x 15 mm, 60 mL volume) were used. On one side of the plate containing 10 mL of PDA medium, a 5-mm culture disc containing pathogen mycelia was applied aseptically. On the opposite side of the plate, different volumes (10, 25, 50, and 75 µL) of 3M1B and 2M1B were added to a cotton ball to yield final concentrations of 0.2, 0.5, 1.0, and 1.5 $\mu L \cdot mL^{-1}$ of air, respectively. The compounds 3M1B and 2M1B were evaluated individually or using a mixture ratio of 1:1 (v/v). After the addition of these compounds, the plates were immediately sealed with plastic film and incubated at 25°C under a 12-h photoperiod. The control plates contained the pathogen without the VOCs.

Mycelial growth was assessed when the colonies on the control plates reached the plate edges (72 h), after averaging two diametrically opposed measures of the colony. The data were used to calculate the MIC_{50}, which is the minimum inhibitory concentration capable of inhibiting 50% of pathogen growth. Upon completion of the experiment, the culture discs containing mycelia (initial inoculum) were transferred to plates containing PDA medium without VOCs to assess pathogen viability.

Assessment of electrolyte leakage

Commercially available two-section divided polystyrene plates were used for these assays. On one side of the plate, containing 10 mL of PDA medium, cellophane was aseptically applied to the surface of the medium and a 5-mm culture disc containing pathogen mycelia was transferred on top of the cellophane. The plates were sealed with plastic film and incubated at 25°C under a 12-h photoperiod. After 72 h, 50 µL of 3M1B and 2M1B (1.0 $\mu L \cdot mL^{-1}$ of air), either individually or using a mixture ratio of 1:1 (v/v), was transferred to a cotton ball on the opposite side of the plate. The

plates were sealed with plastic film and incubated for 72 h. The control plates contained the pathogen without the VOCs.

The mycelium was collected, weighed, and transferred to 110-mL plastic cups containing 10 mL of a 0.2 M sucrose solution. For the positive control, 10 µL of the anionic detergent Triton X-100 was added to the sucrose solution. After 60 min, the solutions were transferred to test tubes, and their electrical conductivity was measured with a conductivity meter; the results were expressed as $mS.g^{-1}$ of fresh mycelium.

Assessment of lipid peroxidation

Lipid peroxidation was assessed based on the production of metabolites (primarily malondialdehyde - MDA) that were reactive to 2-thiobarbituric acid (TBA), as described by Heath and Packer (1968) and Cakmak and Host (1991). Fungi were grown as described above by using a dose of 1.0 $\mu L \cdot mL^{-1}$ of air of 3M1B and 2M1B, either individually or using a mixture ratio of 1:1 (v/v). The cellophane containing the mycelium was collected and weighed, and 300 mg of this material was homogenized in 1.3 mL of 0.1% (w/v) trichloroacetic acid (TCA) by using a mortar and pestle in the presence of liquid nitrogen. After centrifugation at 10,000 *g* for 15 min at 4°C, an aliquot containing 500 µL of the supernatant was added to test tubes containing 1.5 mL of 0.5% TBA (w/v) in 20% TCA (w/v). The test tubes were subsequently incubated in a water bath at 90°C for 20 min and cooled in an ice bath. The samples were then centrifuged at 16,000 *g* for 4 min, and the absorbance was measured at 535 nm. The concentration of the MDA-TBA mixture was measured using the molar extinction coefficient of MDA, which corresponds to 155 $mM^{-1} \cdot cm^{-1}$, and data were expressed as $nmol.g^{-1}$ of fresh mycelium.

All experiments were carried out under a completely randomized design, with four replicates per treatment, and each replicate consisted of a single plate.

RESULTS

Mycelial growth

The exposure of *C. gloeosporioides* and *C. acutatum* to different concentrations of 3M1B and 2M1B significantly inhibited mycelial growth (Figure 1). The degree of inhibition caused by these two compounds was similar for the two fungal species and was proportional to the increase in the concentration. Growth inhibition occurred at concentrations ≥0.2 $\mu L \cdot mL^{-1}$ of air and varied between 15 and 20%. In addition, 100% inhibition was observed at concentrations ≥1.0 $\mu L \cdot mL^{-1}$ of air.

No synergistic effect on fungal inhibition was observed between the two compounds compared to the inhibition exhibited by the isolated compounds (Figure 2). The 3M1B/2M1B mixture also promoted fungal inhibition starting at the lowest concentration tested (0.2 $\mu L \cdot mL^{-1}$ of air), and maximal inhibition was achieved at ≥1.0 $\mu L \cdot mL^{-1}$ of air. The MIC_{50} values were also measured and differed significantly only for the comparisons of *C. gloeosporioides* grown in the presence of the 3M1B/2M1B mixture with the other treatments (Table 1).

Based on the results shown in Figures 1 and 2 and in Table 1, no significant difference in pathogen inhibition was observed between the treatments in which 3M1B and 2M1B were applied individually. However, when considering the MIC_{50} values, *C. acutatum* was more sensitive than *C. gloeosporioides* when exposed to the VOCs mixture at the 1:1 ratio (v/v), indicating that the compounds at this ratio exert a synergistic effect. Both pathogens resumed growth when transferred to PDA medium in the absence of the tested VOCs, indicating the fungistatic activity of the VOCs.

It was observed that *C. gloeosporioides* produced a higher biomass compared to *C. acutatum*. However, the two fungi were inhibited similarly when exposed to the VOCs (Figure 3), and no significant difference in their responses was observed when the compounds were applied either individually or as a mixture. The mean percentages of *C. gloeosporioides* and *C. acutatum* inhibition after treatment were 64.9 and 55.4%, respectively.

Electrolyte leakage

The mycelium of *C. gloeosporioides* exposed to the VOCs showed electrolyte losses, based on the observed increase in electrical conductivity, by an average of 66% compared to control fungi (Figure 4). Similarly, exposure to the VOCs caused electrolyte losses in *C. acutatum*, as the electrical conductivity of the exposed fungi was three times higher than that of the control. No significant differences in electrolyte losses were observed when the two compounds were tested individually or as mixture.

Lipid peroxidation

Regarding lipid peroxidation, Figure 5 shows that the levels of MDA in *C. gloeosporioides* mycelium increased approximately 260% when the fungus was exposed to the VOCs. Similarly, lipid peroxidation in *C. acutatum* increased upon exposure to the VOCs, though to a lesser extent; 233% on average compared to the control.

DISCUSSION

There is a high potential in the use of volatile metabolites produced by microbes to control plant pathogens in fruits, grains, and stored seeds (Strobel, 2011). Preliminary studies have shown the inhibition potential of increasing doses of an artificial mixture of VOCs isolated from *S. cerevisiae* against the mycelial growth of *G. citricarpa*, with this inhibition reaching up to 88% (Fialho et al., 2010).

According to the authors, this artificial mixture comprised six compounds and exhibited an MIC_{50} of 0.48 $\mu L \cdot mL^{-1}$; the same MIC_{50} was observed herein for *C. gloeosporioides* exposed to the 3M1B/2M1B mixture.

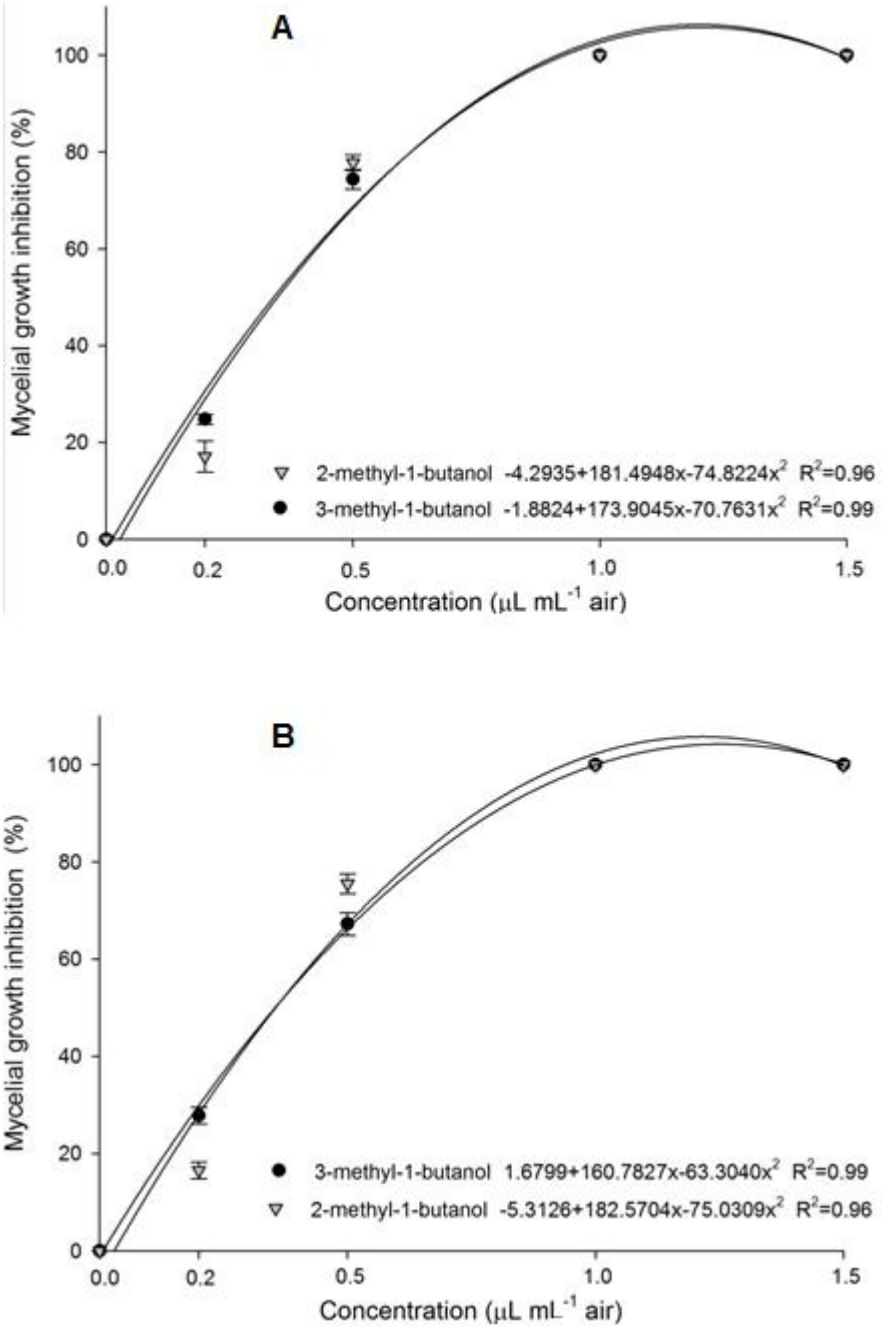

Figure 1. Inhibition of the mycelial growth of *C. gloeosporioides* (A) and *C. acutatum* (B) exposed to the volatile organic compounds 3-methyl-1-butanol (●) and 2-methyl-1-butanol (▽). The mean values of four replicates were calculated as the percentage of inhibition compared to the control without the addition of volatile compounds.

Similar to the results of the present study, the plant pathogens *Botrytis cinerea* and *Penicillium expansum* affecting post-harvest processes were shown to be inhibited by the fungus *Nodulisporium* sp. CF016, when exposed to VOCs for three days (Park et al., 2010). The volatile compounds dimethyl disulfide, dimethyl trisulfide, and acetophenone, isolated from *Streptomyces globisporus,* completely inhibited the growth of the plant pathogen *Penicillium italicum* after five days of exposure to a concentration of 0.1 µL·mL^{-1} of air (Li et al., 2010). In the same way, an artificial mixture of VOCs, consisting of 20 compounds isolated from the fungus *M. albus*, was demonstrated to impair the development of various microorganisms, including *C. gloeosporioides*. The

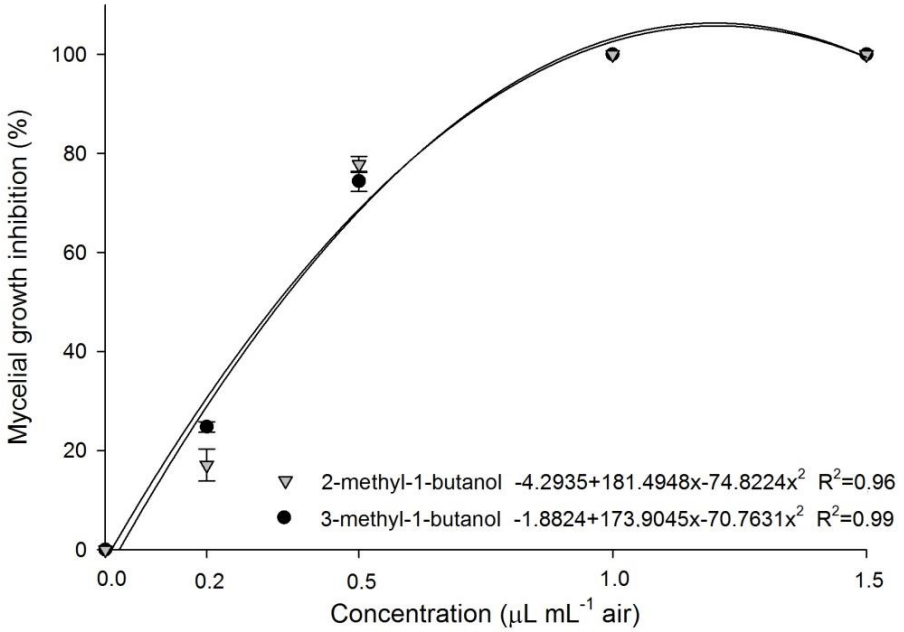

Figure 2. Inhibition of the mycelial growth of *C. gloeosporioides* (●) and *C. acutatum* (▽) grown on PDA medium when exposed to a mixture of the volatile organic compounds 3-methyl-1-butanol and 2-methyl-1-butanol [1:1 ratio (v/v)]. The mean values of four replicates were calculated as the percentage of inhibition compared to the control without the addition of volatile compounds.

Table 1. MIC_{50} for the mycelial growth of *C. gloeosporioides* and *C. acutatum* grown in the presence of the volatile organic compounds 3-methyl-1-butanol (3M1B) and 2-methyl-1-butanol (2M1B).

Pathogen	Treatment	MIC_{50}*	SD
C. gloeosporioides	3M1B	0.33a	0.039
	2M1B	0.34a	0.052
	3M1B:2M1B (1:1, v/v)	0.48b	0.076
C. acutatum	3M1B	0.34a	0.052
	2M1B	0.35a	0.034
	3M1B:2M1B (1:1, v/v)	0.27a	0.057

*$\mu L.mL^{-1}$ of air. The values represent the means of four replicates (mean ± SE). Means followed by the same letter did not differ by the Tukey test ($p \leq 0.05$).

mycelial growth of the pathogen was totally inhibited, although the effect was not lethal at a concentration of 1.2 $\mu L \cdot mL^{-1}$, with a MIC_{50} of 0.3 $\mu L \cdot mL^{-1}$ (Atmosukarto et al., 2005), and similar results were obtained in the present study. Previous studies demonstrating the antimicrobial effect of VOCs produced by bacteria against important pathogens in the post-harvest period also appear promising. The effect of VOCs and secondary metabolites produced by strains of *Bacillus subtilis* on the mycelial growth of *P. digitatum* revealed that exposure of the fungus to VOCs caused inhibition of 30 -70%. However, as observed in the present study, pathogen growth was recovered when the mycelium was cultured in plates containing only PDA medium, demonstrating the fungistatic effect of the compounds (Leelasuphakul et al., 2008).

The mean inhibition of mycelial biomass production observed by using the evaluated VOCs was 64.9% for *C. gloeosporioides* and 55.4% for *C. acutatum*. Similar results were obtained by Humphris et al. (2001). These authors reported that *Trichoderma* species, which produce several VOCs showing inhibitory activity against plant pathogens, also produce 2M1B, and they found that biomass production was decreased by up to 80% in wood-decay fungi when exposed to 2M1B at 2,500 $\mu g \cdot mL^{-1}$ of air. However, in the present study, fungal inhibition with 2M1B was achieved at a concentration of just 1 $\mu g \cdot mL^{-1}$ air.

In contrast to the findings of the present study, when Tunc et al. (2007) investigated the VOCs ethanol and carvacrol, they observed a synergistic effect between these compounds in the inhibition of *P. notatum*. In *M. albus*, the VOCs naphthalene, propanoic acid, and 3M1B were shown to display biological activity against the plant pathogens *Pythium ultimum*, *R. solani*, and *S. sclerotiorum*. The use of artificial mixtures demonstrated that all three compounds had to be present to produce this inhibitory activity, indicating the synergistic effects of

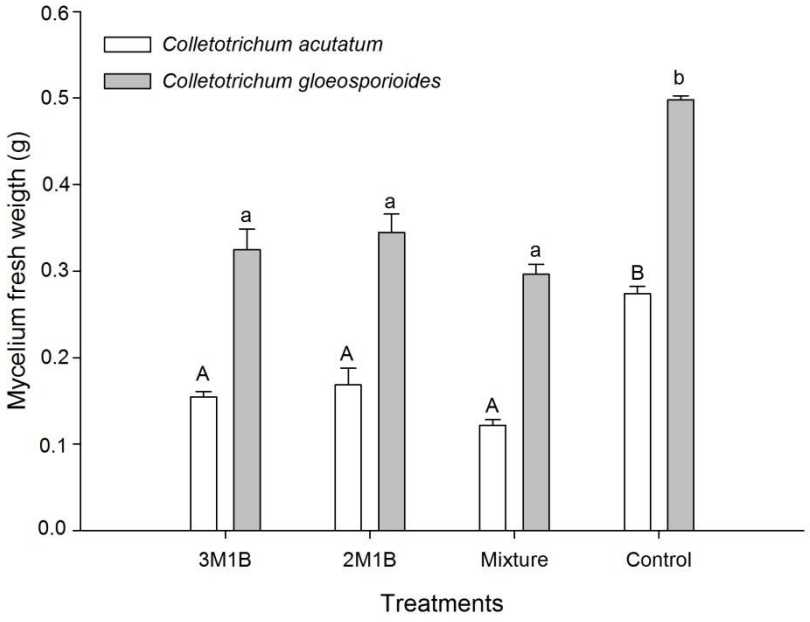

Figure 3. Effect of the volatile organic compounds 3-methyl-1-butanol (3M1B) and 2-methyl-1-butanol (2M1B) and their mixture (1:1 ratio; v/v) at a dose of 1.0 $\mu L \cdot mL^{-1}$ of air on mycelium production by *C. gloeosporioides* and *C. acutatum* after 72 h of exposure. The control plates contained the pathogen without the addition of VOCs. The values represent the means of four replicates (mean ± SE). Means followed by the same letter did not differ by the Tukey test ($p \leq 0.05$).

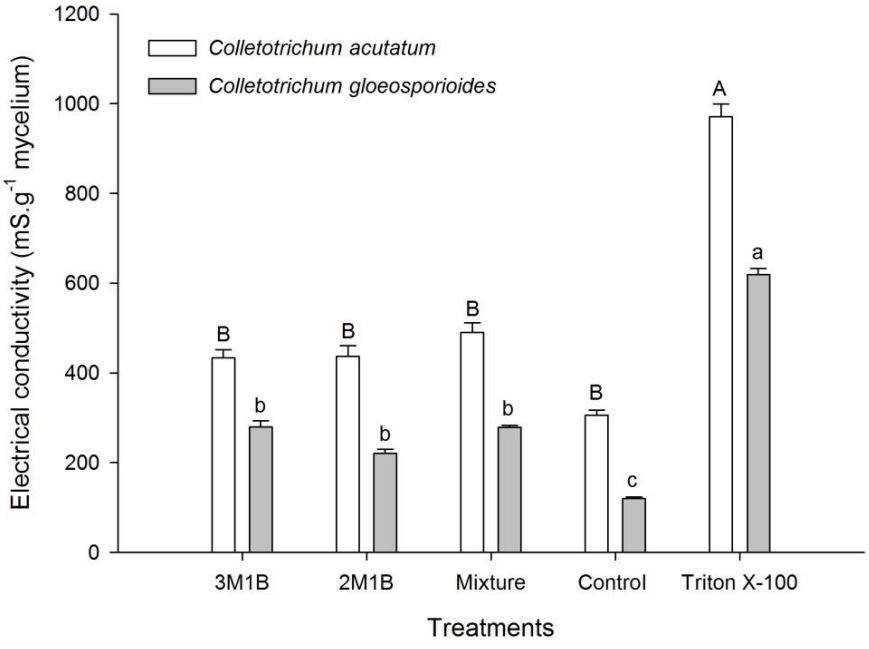

Figure 4. Effect of the volatile organic compounds 3-methyl-1-butanol (3M1B) and 2-methyl-1-butanol (2M1B) and their mixture (1:1 ratio; v/v) at a dose of 1.0 $\mu L \cdot mL^{-1}$ of air on electrolyte loss in *C. gloeosporioides* and *C. acutatum* mycelia after 72 h of exposure. The control and Triton X-100 treatments consisted of the pathogen without the addition of VOCs, and Triton X-100 was added to the sucrose solution at the time of evaluation. The values are the means of four replicates (mean ± SE). Means followed by the same letter did not differ by the Tukey test ($p \leq 0.05$).

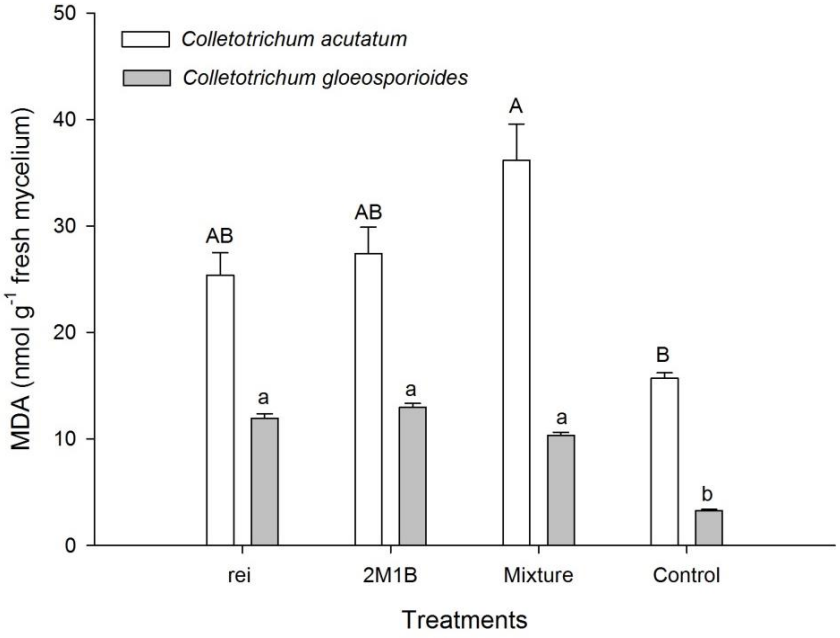

Figure 5. Effect of the volatile organic compounds 3-methyl-1-butanol (3M1B) and 2-methyl-1-butanol (2M1B) and their mixture (1:1 ratio; v/v) at a dose of 1.0 µL·mL^{-1} of air on lipid peroxidation (MDA content) in *C. gloeosporioides* and *C. acutatum* mycelia after 72 h of exposure. The control plates contained the pathogen without the addition of VOCs. The presented values are the means of four replicates (mean ± SE). Means followed by the same letter did not differ by the Tukey test ($p \leq 0.05$).

this mixture (Ezra et al., 2004). In the present study, no synergistic effect of the 3M1B/2M1B mixture was observed, most likely because these compounds are isomers and therefore exert similar effects on plant pathogens.

The mechanisms involved in the antimicrobial activity of 3M1B and 2M1B remain unknown. In this study, exposure of *C. gloeosporioides* and *C. acutatum* to both alcohols resulted in increased electrolyte loss, indicating damage to the plasma membrane, which may have contributed to the impaired fungal development. To the best of our knowledge, this is the first study to evaluate the effect of alcohols on the plasma membrane of *C. gloeosporioides* and *C. acutatum* when in contact with these compounds in the gas phase.

In the case of alcohols, such as ethanol, the mechanisms involved in the antimicrobial activity have been elucidated and appear to be more strongly correlated with physicochemical characteristics than with the interaction with specific receptors on the target cells. Alcohols cause protein denaturation and affect the organization and stability of the lipid bilayer of the plasma membrane. Damage to the plasma membrane results in increased non-selective permeability, which leads to loss of ions and essential metabolites (Ingram and Buttke, 1984; Seward et al., 1996). Both 3M1B and 2M1B are higher aliphatic alcohols and consequently show an increased potential to damage cell membranes compared to less lipophilic substances, such as ethanol, for which high concentrations are needed to exert antimicrobial activity (Heipieper et al., 2000).

Alcohols can interfere with cellular growth, morphogenesis, transport systems, the loss of metabolites and the composition and biosynthesis of lipids. Alcohols cause deleterious effects on the arrangement of lipid components in the plasma membrane of *Escherichia coli* K-12 and inhibit its growth by 30-40%. However, bacterial cultures exposed to ethanol were observed to resume growth after recovery of the composition of the membrane fatty acids (Ingram, 1976). Heipieper et al. (2000) investigated the tolerance and adaptation of membrane fatty acids to ethanol in mutants of *Kluyveromyces lactis* and found that the MIC_{100} for the tested isolates was 8-10% ethanol (w/v).

Reactive oxygen species (ROS), such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), are potent oxidizing agents that can accumulate intracellularly at dangerous levels after exposure to physical and chemical factors such as UV radiation and heavy metals. When antioxidant mechanisms of cellular defense, such as catalase (CAT) and superoxide dismutase (SOD) activities, are not sufficient to detoxify ROS, oxidative stress can occur because ROS can react rapidly and non-specifically with macromolecules, resulting

in DNA mutation, protein oxidation, and lipid peroxidation of cell membranes (Angelova et al., 2005; Heller and Tudzynski, 2011). However, the available data on oxidative stress in organisms exposed to VOCs are limited. In the present study, the levels of MDA, a product of lipid peroxidation, increased in *C. gloeosporioides* and *C. acutatum* exposed to 3M1B and 2M1B, indicating that these VOCs caused an imbalance in the redox state of the fungi, given that lipid peroxidation is a marker of oxidative stress. Hence, lipid peroxidation may have caused the increased cell permeability observed herein because the effects of lipid peroxidation include decreased membrane fluidity and increased permeability to protons, ultimately leading to cell rupture. Furthermore, cytotoxic compounds such as hydroperoxides are formed during this process (Li et al., 2009). Peroxidation may affect the functionality of the cell membrane, resulting in irreversible damage to cellular functions. Therefore, lipid peroxidation may be indicative of plasma membrane damage. Although the growth of *C. gloeosporioides* and *C. acutatum* was shown to be inhibited by alcohols; this effect was not fungicidal. It is possible that these two fungal species employ antioxidant defense mechanisms, such as increased activity of the enzymes CAT and SOD, which can minimize the generation of ROS to some extent. For example, Fialho et al. (2014) observed increased activity of CAT and SOD in the mycelia of *G. citricarpa* exposed to an artificial mixture of VOCs isolated from *S. cerevisiae*. On the other hand, Splivallo et al. (2007) investigated the possible mode of action of VOCs produced by truffles (*Tuber* spp.), including 3M1B, on the growth of the plant *Arabidopsis thaliana*. Although plants and microorganisms are phylogenetically distinct organisms, some of their characteristics are similar, and they may therefore share the same targets of VOCs. Some 8-carbon compounds, such as 1-octen-3-ol and trans-2-octenal, were shown to be the most effective in inhibiting plant growth, inducing the production of ROS (H_2O_2), and increasing the activity of antioxidant enzymes.

Conclusion

Based upon the results of this study, the compounds 3-methyl-1-butanol and 2-metil-1-butanol show potential for controlling *C. gloeosporioides* and *C. acutatum* growth. Changes in pathogen plasma membrane permeability is one of the modes of action involved in the inhibition process. Therefore, the use of these volatiles as fumigants in the post-harvest treatment of guava fruits against anthracnose appears to be possible.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Angelova MB, Pashova SB, Spasova BK, Vassilev SV, Slokoska LS (2005). Oxidative stress response of filamentous fungi induced by hydrogen peroxide and paraquat. Mycol. Res. 109: 150-158.

Atmosukarto I, Castillo U, Hess WM, Sears J, Strobel G (2005). Isolation and characterization of *Muscodor albus* I-41.3s, a volatile antibiotic producing fungus. Plant Sci. 169: 854-861.

Cakmak I, Horst WJ (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean. Physiol. Plant. 83:463-468.

Das M, Bora KN (1988). Ultrastrutuctural studies on infection processes by *Colletotrichum acutatum* on guava fruit. Indian Phytopathol. 51:353-356.

Ezra D, Hess WM, Strobel GA (2004). New endophytic isolates of *Muscodor albus*, a volatile-antibiotic-producing fungus. Microbiology 150:4023-4031.

Fialho MB, Carvalho G, Martins PF, Azevedo RA, Pascholati SF (2014). Antioxidative response of the fungal plant pathogen *Guignardai citricarpa* to antimicrobial volatile organic compounds. Afr. J. Microbiol. Res. 8:2077-2084.

Fialho MB, Moraes MHD, Tremocoldi AR, Pascholati SF (2011). Potential of antimicrobial volatile organic compounds to control *Sclerotinia sclerotiorum* in bean seeds. Pesq. Agropec. Bras. 46:137-142.

Fialho MB, Toffano L, Pedroso MP, Augusto F, Pascholati SF (2010). Volatile organic compounds produced by *Saccharomyces cerevisiae* inhibit the *in vitro* development of *Guignardia citricarpa*, the causal agent of citrus black spot. World J. Microbiol. Biotechnol. 26:925-32.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloplasts. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125:189-1988.

Heipieper HJ, Isken S, Saliola M (2000). Ethanol tolerance and membrane fatty acid adaptation in *adh* multiple and null mutants of *Kluyveromyces lactis*. Res. Microbiol. 151:777-784.

Heller J, Tudzynski P (2011). Reactive oxygen species in phytopathogenic fungi: signaling, development and disease. Annu. Rev. Phytopathol. 49:369-390.

Humphris SN, Wheatley RE, Bruce A (2001). The effects of specific volatile organic compounds produced by *Trichoderma* spp. on the growth of wood decay basidiomycetes. Holzforschung 55:233-237.

Ingram LO (1976). Adaptation of membrane lipids to alcohols. J. Bacteriol. 125:670-678.

Ingram LO, Buttke TM (1984). Effects of alcohols on microorganisms. Adv. Microb. Phys. 25:253-300.

Leelasuphakul W, Hemmaneea P, Chuenchitt S (2008). Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit. Postharvest Biol. Technol. 48:113-121.

Li Q, Harvey LM, McNeil B (2009). Oxidative stress in industrial fungi. Crit. Rev. Biotechnol. 29: 199-213.

Li Q, Ning GP, Zheng L, Huang J, Li G, Hsiang T (2010). Fumigant activity of volatiles of *Streptomyces globisporus* JK-1 against *Penicillium italicum* on *Citrus microcarpa*. Postharvest Biol. Technol. 58:157-165.

Lima Filho RM, Oliveira SMA, Menezes M (2003). Enzymatic characterization and crossed pathogenicity of *Colletotrichum* spp. associated with postharvest diseases. Trop. Plant Pathol. 28:620-625.

Menezes M, Hanlin RT (1996). Apressoria of Brazilian isolates of *Colletotrichum gloeosporioides* (Penz.) Sacc. causal agent of anthracnose diseases. Braz. J. Microbiol. 27:247-251.

Mitchell AM, Strobel GA, Moore E, Robison R, Sears J (2010). Volatile antimicrobials from *Muscodor crispans*, a novel endophytic fungus. Microbiology 156:270-277.

Pandey RR (1988). Effect of foliar aplications of fungicides on the phylloplane mycoflora and fungal pathogens of guava. J. Phytopathol. 123:52-62.

Park MS, Ahn J, Choi JG, Choi YH, Jang KS, Kim JC (2010). Potential of the volatile-producing fungus *Nodulisporium* sp. CF016 for the control of postharvest disease of apple. Plant Pathol. J. 26:253-259.

Peres NAR, Kuramae EE, Dias MSC, Souza NL (2002). Identification and characterization of *Colletotrichum* spp. affecting fruit after harvest in Brazil. J. Phytopathol. 150:128-134.
Seward R, Willetts JM, Dinsdale MG, Lloyd D (1996). The effects of ethanol, hexan-1-ol, and 2-phenylethanol on cider yeast growth, viability, and energy status; synergistic inhibition. J. Inst. Brew. 102:439-443.
Splivallo R, Novero M, Bertea CM, Bossi S, Bonfante P (2007). Truffle volatiles inhibit growth and induce an oxidative burst in *Arabidopsis thaliana*. New Phytol. 175:417-424.
Strobel G (2011). Muscodor species-endophytes with biological promise. Phytochem. Rev. 10: 165-172.
Tarkka MT, Piechulla B (2007). Aromatic weapons: truffles attack plants by the production of volatiles. New Phytol. 175:381-383.
Tunc S, Chollet E, Chalier P, Preziosi-Belloy L, Gontard N (2007). Combined effect of volatile antimicrobial agents on the growth of *Penicillium notatum*. Int. J. Food Microbiol. 113:263-270.
Wheatley RE (2002). The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie van Leeuwenhoek 81:357-364.