INTRODUCTION

Many fungi-producing VOCs exhibit antimicrobial activity (Mitchell et al., 2010). The VOC B23 is an isolate of the endophytic fungi Muscodor crispus found in the stem tissues of Ananas ananassoides, wild pineapple plants of the Bolivian Amazon Basin (Mitchell et al., 2008). The B23 isolate of M. crispus exhibits antimicrobial effects against a variety of pathogens including fungi, bacteria, and oomycetes (Krajacek et al., 2012). The components of B23 are also on the list of US Food and Drug Administration’s GRAS list of harmless substances allowing for the possibility of clinical applications in animals (Krajacek et al., 2012). Decanal, a bacterially produced VOC, also exhibits antimicrobial activity and has previously shown to inhibit P. destructans mycelial growth (Cornelson et al., 2014). This experiment explores the inhibitory effects of VOCs B23 and decanal on P. destructans mycelial growth by quantifying the growth of P. destructans mycelia exposed to various concentrations of pure B23 and an equimolar combination of B23/decanal and an equimolar combination of B23/decanal. Measurements of the mycelial plugs were taken throughout the experiment to determine which VOC, if any, had the greatest inhibitory effect. The combination of B23 and decanal at higher concentrations revealed that synergistic activity among the VOCs may be effective at mitigating the impact of WNS on impacted bat species. To further investigate the inhibitory effects of B23 and decanal on P. destructans growth, a slide agar overlay experiment was performed. The slide agar overlay experiment analyzed the inhibitory effects of VOCs on spore germination by exposing P. destructans conidia to various concentrations of pure B23 and an equimolar combination of B23/decanal and then comparing germination events between the various concentrations of VOCs and the control. The results of this experiment further confirm B23 and decanal to be effective inhibitors of P. destructans due to the level of inhibition exhibited in VOC-exposed mycelia compared to the control containing mycelia of VOCs. The combination of B23 and decanal also further supports the idea of potential synergistic interactions between B23 and decanal to increase inhibitory efficacy.

METHODS

Inhibition Assay

A small hole (~5 mm in diameter) was cut out of a 35 mm mycelial plate and dextrose agar (SDA) plate using a 1.5 cm diameter cork borer and a cork borer. The mycelial plug and dextrose agar (SDA) plate were placed on a glass Petri dish with the agar surface facing the plate. A sterile transfer device was then used to transfer the inoculum into the hole in the SDA plate. The Petri dish was then sealed with parafilm (Bemis, Neenah, WI) and a sterile cover slip was placed onto the Petri dish. The Petri dishes were then incubated at 25°C for 7 days before being photographed with a digital camera (Fujifilm, Tokyo, Japan).

Photographs of the plates were taken every 2 to 3 days throughout the experiment to determine the percent inhibition of each VOC on mycelial growth. A slide agar overlay experiment was performed. A circular transparent surface was placed onto the mycelial plug and the Petri dish was then sealed with parafilm (Bemis, Neenah, WI) and a microscope slide was placed on the transparent surface. The microscope slide was then sealed with parafilm (Bemis, Neenah, WI) and the Petri dish was then placed on a glass Petri dish with the agar surface facing the plate. A sterile transfer device was then used to transfer the inoculum into the hole in the SDA plate. The Petri dish was then sealed with parafilm (Bemis, Neenah, WI) and a sterile cover slip was placed onto the Petri dish. The Petri dishes were then incubated at 25°C for 7 days before being photographed with a digital camera (Fujifilm, Tokyo, Japan).

Photographs of the plates were taken every 2 to 3 days throughout the experiment to determine the percent inhibition of each VOC on mycelial growth. The growth pattern observed between the control plugs and the B23/decanal inoculated plugs suggest the potential of synergistic interactions between B23 and decanal to increase inhibitory efficacy.

RESULTS

The VOCs B23 and decanal were successful in inhibiting mycelial growth. Although both compounds seemed to succeed in inhibiting mycelial growth, the synergistic interaction of B23 and decanal displayed the most inhibitory effects. The size of the mycelial plugs inoculated with B23 and decanal of 2000 ppm decreased between day 1 and day 13 (Figure 1). The size of the mycelial plugs from the control, however, significantly increased between day 1 and day 13 (Figure 1). The size of the mycelial plugs from the control, however, significantly increased between day 1 and day 13 (Figure 1). The growth pattern observed between the control plugs and the B23/decanal inoculated plugs suggest the potential of synergistic interactions between VOCs is effectively mitigates the impact of WNS on impacted bat species.

CONCLUSION

It was found that B23 and B23/decanal together at equimolar ratios have inhibitory effects on P. destructans. The inhibitory effects of B23/decanal together at equimolar ratios demonstrate the potential of synergistic interactions between the two to effectively inhibit mycelial elongation and spore germination.