

Muscodor albus, a biological fumigant with the potential to replace chemical fumigation in managing apple replant disease

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Introduction

Apple replant disease, caused by a complex of micro-organisms, is a serious problem in many apple growing regions of the world. *Pythium* and *Cylindrocarpon* spp. have been identified as primary causal agents in North America (Braun, 1991; Mazzola, 1998). Broad-spectrum chemical fumigants have been the most effective and widely used control strategy. However, chemical fumigants pose a threat to ozone depletion, ground water contamination and applicator health. The biofumigant, *Muscodor albus* is a fungal endophyte of the cinnamon tree and produces more than 20 volatile organic compounds (Strobel et al., 2001). These relatively innocuous volatiles kill or suppress a wide range of fungal pathogens (Mercier and Manker, 2004). The purpose of this study was to determine if *M. albus* has the ability to ameliorate the effects of apple replant disease.

Materials and Methods

Four replicate soil samples were collected from an old orchard site which had been identified as having an apple replant disease problem. The soil was passed through a 6 mm sieve to remove roots and stones. One liter sub-samples were either sterilized by autoclaving, or had 0, 5, 10 or 20 grams of rye grain colonized by *M. albus* (Fig. 1) added to it and thoroughly mixed. The treated soils were used to fill 4" pots that were

Figure 1. Dry *Muscodor albus* colonized rye grains on the left and moist grains after 4 days of incubation at 20°C on the right.

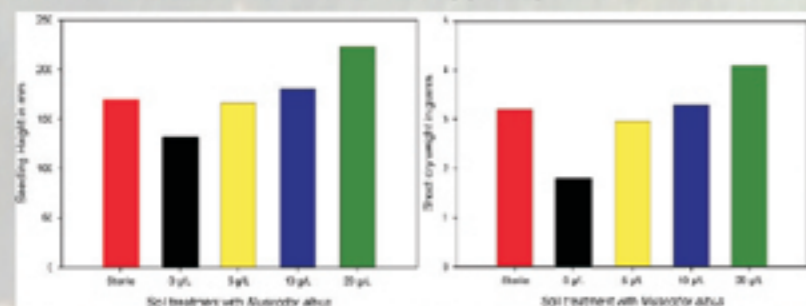


placed on a greenhouse bench and watered twice daily for 2 days. Then one, 2-week-old apple seedling was planted into each of two pots of each replicate soil treatment. Seedlings were watered twice daily and fertilized with 20-20-20 N:K:P biweekly for eight weeks. Seedling heights were measured the day of planting and after 8-weeks of growth. The seedling tops were removed, weighed, oven-dried and weighed again. The roots were gently washed to remove soil. One of the two root systems for each replicated and treatment was also dried and weighed. Samples taken from the remaining roots were surface sterilized and placed onto media to isolate plant pathogenic fungi. Additional root samples were stained to visualize and quantify mycorrhizal colonization and a final root sample was ground in liquid nitrogen and the DNA of the plant and all associated micro-organisms was collected and purified. A nested polymerase chain reaction (PCR) using primers specific to fungi was used to amplify a small piece of a highly conserved DNA region. The outside primers were the universal primer pair UN-UP18S42 and UN-LO28S22. The internal primer pair was fungal specific primers ITS1F-GC and ITS2. The DNA fragments collected are species specific and can be used to identify fungi by sequencing. They can be separated by Denaturing Gradient Gel Electrophoresis (DGGE) to monitor differences in fungal community populations between treated soils. Pure cultures of *Cylindrocarpon lucidum*, *C. destructans*, *Pythium irregulare*, *P. sylvaticum* and *Phytophthora cactorum*, known apple root pathogens, were exposed to *M. albus* (1g/L) volatiles in sealed jars for 48 h at 20°C. The effect of the volatiles on spore germination or mycelial growth was recorded.

Results

Seedling height and dry weight were significant ($P < 0.05$) in a linear relationship between *M. albus* concentration and increased growth (Fig. 2).

Figure 2. Apple seedling root and shoot growth response to *Muscodor albus* soil treatments for apple replant disease.



The root dry weight did not show a linear relationship between *M. albus* concentration and growth. At 20 g *M. albus*/L soil, the root dry weight decreased although shoot height and weight increased. In addition, there was no significant difference in mycorrhizal colonization among *M. albus* treatments but roots in sterilized soil contained no mycorrhizae (Fig. 3, 4 and 5).

Figure 3. Response of root dry weight and mycorrhizal colonization of apple seedling roots to soil sterilization or the addition of increasing amounts of *Muscodor albus*.

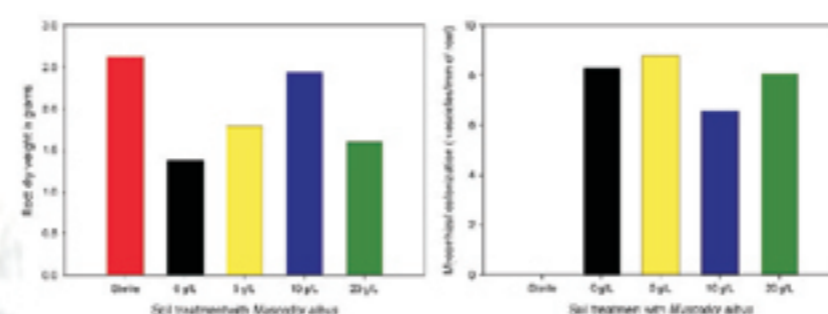
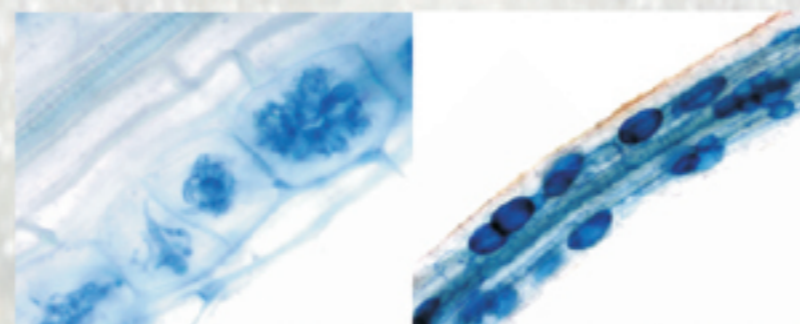


Figure 4. Apple seedling growth response to soil sterilization or *Muscodor albus* soil treatments for apple replant disease.



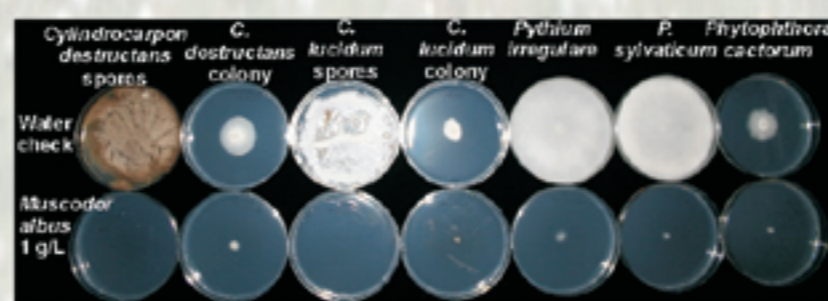
Figure 5. Arbuscules and vesicles of mycorrhizae colonizing the roots of apple seedlings treated with *Muscodor albus* to control replant disease.



Isolations of fungi from surface-sterilized root pieces resulted in very few fungal isolations from apple seedling roots grown in sterile soil and soil containing 20 g/L of *M. albus*-colonized rye. *Cylindrocarpon destructans*, a known cause of apple replant disease, was recovered from roots in non-treated field soil but not roots in sterilized or *M. albus* treated soils.

Mycelia or spores of known causal agents of apple replant disease and potential apple root pathogens were killed or suppressed when exposed to *M. albus* volatiles (Fig. 6).

Figure 6. Spores or mycelium of *Cylindrocarpon lucidum*, *C. destructans*, *Pythium irregulare*, *P. sylvaticum* and *Phytophthora cactorum* were killed or inhibited from growing when exposed to *Muscodor albus* volatiles for 48 h at 20°C in a sealed glass jar.



DNA extraction of the soils from the sterile, non-treated and *M. albus* treatments yielded small quantities of PCR product when a universal primer was used to amplify 18S rDNA and ITS regions. Using these PCR products as templates for more specific fungal primers (ITS1F - ITS2) of a small (~300 bp) region of ITS1 resulted in multiple bands for each sample (Fig. 7). Each band represents the unique DNA of a fungal species. DNA bands which appear only in the non-treated soil but not in the sterile or *M. albus* treated soils may belong to apple replant causal organisms which have been killed by *M. albus* or autoclaving. Identification of these organisms can be done by DNA sequencing but has not yet been completed.

Figure 7. DGGE separation of PCR fragments produced with fungal specific ITS1F and ITS2 primers on DNA extracted from sterilized, non-treated and *M. albus* treated apple replant soils. DNA bands appearing in the non-treated soil but not in other soils are potential causal agents of apple replant (arrow).



Conclusions

The biofumigant fungus, *Muscodor albus*, applied to apple replant disease soil in the greenhouse ameliorated disease symptoms.

M. albus increased shoot and root height and dry weight

Preliminary results indicate *M. albus* killed or suppressed the growth of apple replant disease causal organisms in vitro and in vivo.

M. albus did not appear to inhibit root colonization by beneficial mycorrhizal fungi.

M. albus has the potential to replace methyl bromide or chemical soil sterilants to control apple replant disease.

References

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