Comparison of *Muscodor albus* Volatiles with a Biorational Mixture for Control of Seedling Diseases of Sugar Beet and Root-Knot Nematode on Tomato

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ABSTRACT

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A biorational synthetic mixture of organic components mimicking key antimicrobial gases produced by *Muscodor albus* was equivalent to the use of live *M. albus* for control of seedling diseases of sugar beet (*Beta vulgaris*) caused by *Pythium ultimum*, *Rhizoctonia solani* AG 2-2, and *Aphanomyces cochlioides*. The biorational mixture provided better control than the live *M. albus* formulation for control of root-knot nematode, *Meloidogyne incognita*, on tomato (*Lycopersicon esculentum*). The biorational mixture provided control of damping-off equal to a starch-based formulation of the live fungus for all three sugar beet pathogens, and significantly reduced the number of root-knot galls on tomato roots compared with a barley-based formulation. Rate studies with the biorational mixture showed that 2 and 0.75 µl/cm³ of soil were required to provide optimal control of *Rhizoctonia* and *Pythium* damping-off of sugar beet, respectively. Five microliters of biorational mixture per milliliter of water was required for 100% mortality in 24 h for *Meloidogyne incognita* in in vitro studies. In in vivo studies, 1.67 µl of the biorational mixture/cm³ of sand resulted in fewer root-knot galls than a *Muscodor albus* infested ground barley formulation applied at 5 g/liter of sand.

Additional keywords: biological control, biorational formulation, soil fumigation

Soil fumigation in the United States is highly dependent on the use of methyl bromide-chloropicrin mixtures (MBC). These mixtures are highly efficacious preplant soil fumigants in field and greenhouse production for control of soilborne diseases, insects, nematodes, and weeds (28). Methyl bromide-containing products are currently being phased out due to their role in depletion of the ozone layer (37). The loss of methyl bromide products for preplant soil fumigation will seriously reduce options for controlling soilborne pathogens such as *Pythium* sp., *Fusarium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Verticillium dahliae*, and *V. albo-atrum*, and the root-knot nematode *Meloidogyne incognita* (23,28,36). Current research on methyl bromide alternatives includes developing management systems utilizing alternative chemicals (2,10,12,23,35), solarization (6,7,19,26), biofumigation with *Brassica* spp. (31,33), organic amendments (1,5,16), and biological control (8,20,29). Currently, these alternatives

do not offer the broad-spectrum activity and efficacy of MBC.

Many fungal species are known to produce different concentrations of volatile organic compounds (VOC) (4). Dennis and Webster (11) demonstrated that *Trichoderma* sp. emit VOC that inhibit other fungi, whereas other wood inhabiting fungi generate volatile products that have little or no antimicrobial properties. *Muscodor albus*, isolate 620 (39), an endophytic fungus obtained from *Cinnamomum zeylanicum* Bl. in Honduras, produces a mixture of VOC that effectively inhibits and kills a wide range of microorganisms (14,18,32). The control of microorganisms with volatiles produced by fungi such as *Muscodor albus* and *Muscodor roseus* (38) has been termed "mycofumigation" (32,40,41). The VOC produced include chemicals in the following classes: alcohols, esters, ketones, acids, and lipids, all of which exhibit low toxicity to mammals and higher plants including representatives from the plant families Asteraceae, Chenopodiaceae, Cruciferae, Cucurbitaceae, Gramineae, Leguminaceae, Rosaceae, and Solanaceae (22,24,34). In early experiments, each of these classes of VOC were examined separately for mycelial growth inhibition against a range of fungi, but no single class of VOC were as effective as the VOC mixture produced by *M. albus* (34).

Stinson et al. (32) introduced the biological management of soilborne pathogens using mycofumigation. Reductions of sugar beet (*Beta vulgaris* L.) diseases caused by the soilborne pathogens *Aphanomyces cochlioides*, *R. solani*, and *P. ultimum* were shown in greenhouse assays with *M. albus* and its close relative *M. roseus* (15,32,38). In field experiments with *M. albus*, mycofumigation has reduced damping-off caused by *A. cochlioides* and *P. ultimum* (15). Mycofumigation reduced disease severity and soil colony forming units of *V. dahliae* on eggplants (*Solanum melongena* L.) and chrysanthemum (*Chrysanthemum* × *grandiflorum* (Ramat.) Kitam.) (15,32). Mycofumigation using *Muscodor* sp. is a new concept of soil fumigation with potential for application, and it is critical to understand the effects of the mycofumigant fungi and their VOC on plant pathogenic and beneficial organisms. Prior to this research, mycofumigation with *M. albus* had not been tested on any nematodes.

Synthetic mimics of natural pesticidal compounds are termed biorational or biochemical pesticides. Examples include various mitochondrial respiration inhibiting strobilurin class fungicides that mimic strobilurin A produced by *Strobilurina tenacellus* (Pers. Ex Fr.) Sing. (3) and fludioxonil that mimics an antibiotic from *Pseudomonas* sp. (21). Mycofumigation with *M. albus* is the first example of a potential biorational fumigant based on use of a fungus.

The objectives of this research were to determine if a synthetic mixture of components of *M. albus* VOC could result in control of soilborne pathogens of sugar beet and root-knot nematode on tomato (*Lycopersicon esculentum* Mill.) comparable to a live formulation of *M. albus*. The effects of mycofumigation with a biorational mixture based on *M. albus* VOC and *M. albus* on sugar beet damping-off caused by *A. cochlioides*, *P. ultimum*, and *R. solani*, and root-knot of tomato caused by *Meloidogyne incognita* are reported herein. The soilborne pathogens of sugar beet and the root-knot nematode were chosen as representatives of soilborne pathogens that have been traditionally controlled through preplant soil fumigation with MBC mixtures. The sugar beet pathosystem was chosen because assay protocols were es-

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established in our lab (32), and tomato was chosen for root-knot because it is an excellent host and suffers significant economic loss from this pest (25).

MATERIALS AND METHODS

Artificial mixture of volatile compounds of *M. albus* and storage. The VOC produced by *M. albus* were quantitatively and qualitatively determined using gas chromatography and mass spectroscopy (13,34). The synthetic biorational mixture (Table 1) was made by using authentic standard chemicals, obtained from Sigma-Aldrich (St. Louis, MO), found in *M. albus* gases and propanoic acid esters that were synthesized in the Montana State University Chemistry Department under the supervision of G. A. Strobel (14). The components of the mixture were adjusted to the same proportions as the components identified in the naturally occurring VOC (13–15,34) (Table 1). The liquid artificial mixture was stored in vials at -20°C until used.

Preparation of *M. albus* ground barley formulation. *M. albus*, isolate 620, was grown and maintained in 10-cm petri plates on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) at $23 \pm 1^{\circ}\text{C}$ for 2 weeks. Fifteen 5-mm-diameter agar plugs were transferred into 2-liter flasks with 1 liter of autoclaved potato dextrose broth (PDB, Difco Laboratories) and placed on a rotary shaker (250 rpm) for 2 weeks at $23 \pm 1^{\circ}\text{C}$. The ground barley formulation was prepared by adding 60 ml of colonized PDB to mason jars containing 300 g of twice autoclaved barley (*Hordeum vulgare* L.). After growth on the substrate for 3 weeks at $23 \pm 1^{\circ}\text{C}$, the colonized seeds were dried for 48 h in a fume hood. The barley seeds were ground using a Waring blender, resulting in particles ranging from 30 to 1,500 μm with a mean particle size of 300 μm , and stored in sealed plastic bags at 4°C until used.

Preparation of plant-pathogen and nematode inocula. Cultures of *R. solani* AG (Anastomosis Group) 2-2, *A. cochlidioides*, and *P. ultimum* were grown and maintained in 10-cm petri plates containing potato dextrose agar at $22 \pm 1^{\circ}\text{C}$. All of these isolates were obtained from sugar beets grown in Montana. Inoculum of *R. solani* was prepared as infested ground barley according to the methods of Ruppel et al. (30). Inoculum of *A. cochlidioides* and *P. ultimum* was prepared by chopping 2-week-old PDA cultures into 1- to 2-mm pieces with a sterile tool. The *R. solani* inoculum consisted of dormant mycelium, whereas the *A. cochlidioides* and *P. ultimum* inocula consisted of mycelia, sporangia, and oospores.

Tomato roots with galls containing *Meloidogyne incognita* were obtained from Ken Barker, Department of Plant Pathology, North Carolina State University, and maintained on tomato in the greenhouse at

Montana State University. *M. incognita* juveniles (J2) were harvested from 'Delicious' tomato plants with established root-knot galls using modifications to the method of Hussey and Barker (17). Galled roots were homogenized in a Waring blender for 20 s in a small amount of tap water. This suspension was placed in a mason jar with 500 ml of 1.5% NaOCl and shaken vigorously for 3 min, then poured into a large container with 4 to 5 liters of cold tap water. The entire solution was then poured through a series of sieves in the following order: 250, 100, 45, and 25 μm . The eggs were collected on the 25- μm sieve and transferred onto a tissue-covered screen and placed into a shallow covered dish for hatching. Water from the dish was passed through the 25- μm sieve daily to collect juvenile hatchlings, which were then placed in tap water in a beaker with a stream of fresh air bubbling through the aqueous suspension.

Greenhouse bioassays for testing the efficacy of *M. albus* against soilborne pathogens. Greenhouse bioassays were designed to measure the efficacy of the gas producing fungus *M. albus* in stabilize formulation, a starch and corn-oil based formulation described by Quimby et al. (27) and the biorational mixture of the VOC found in gases produced by *M. albus* for control of *R. solani*, *A. cochlidioides*, and *P. ultimum* on sugar beet (*Beta vulgaris* L.). All experiments were carried out in a greenhouse with 24°C day and 18°C night temperatures with a 16-h photoperiod. The growing substrate (Bozeman silt loam: washed concrete sand:Canadian sphagnum peat, 1:1:1) used for all sugar beet experiments was sieved (2 mm mesh opening) and autoclaved at 121°C and at a pressure of 21 kg/cm^2 for 60 min. For soil infestation, 5 g of ground barley inoculum of *R. solani* was mixed thoroughly in 1 kg of soil. One 2-week-old colonized petri plate culture of *A. cochlidioides* or *P. ultimum* was chopped into small pieces (1 to 2 mm) with a sterile tool and mixed with 6.4 kg of soil.

Pathogen-infested soil was treated with either the stabilize formulation or the biorational formulation in 10-cm petri plates. The stabilize formulation of *M. albus* was added at a rate of 0.5 g to 60 cm^3 of pathogen-infested soil. This rate was based on in vivo results reported by Stinson et al. (32). The biorational fumigation treatment was added at a rate of 60 μl of the biorational mixture into plastic minicups (6 mm diameter), which were nestled with the top open into the middle of 60 cm^3 of pathogen-infested soil in petri plates. Minicups were used to assure that only VOCs were in contact with the pathogen-infested soil. The dosage of the biorational mixture was based on previous in vitro observations showing the LD_{50} concentration of the mixture for microbe inhibition was 60 $\mu\text{l}/50$ ml of air space in a standard petri

plate (34). For the pathogen controls, 60 cm^3 of infested soil was placed in petri plates, and an equal volume of noninfested soil was used for the noninfested control. All petri plates were sealed with two layers of Parafilm (Pechiney Plastic Packaging, Inc., Neenah, WI) and stored for 1 week at $22 \pm 1^{\circ}\text{C}$ for fumigation. After the 1-week fumigation period, the soil treatments were added as an approximately 0.6-cm-thick layer (60 cm^3) on top of 400 cm^3 of autoclaved soil in 10-cm plastic square pots (10 $\times$ 10 $\times$ 8.6 cm). Twenty-five sugar beet seeds (cv. Beta 8754) without fungicide seed treatment were placed on the mycofumigated, biorational fumigated, pathogen-infested, or nontreated soil layer, covered with approximately 1.0 cm (80 cm^3) of sterilized soil, and placed in a greenhouse with 22°C day, 18°C night temperature and a photoperiod of 16 h. The pots of the treatments with the pathogens, *A. cochlidioides* and *P. ultimum*, were placed in shallow saucers with standing water for 3 days to favor infection (32). Pots of the noninfested control were also placed in saucers for the same time period. Seedling establishment was monitored for 28 days after planting and expressed as percent nonvisibly diseased sugar beet seedlings of the total number of seeds that were planted. Both bioassays were arranged in randomized complete designs with five replications. Experiments with all pathogens were repeated two times. Statistical analysis of the data was conducted by analysis of variance (ANOVA) using the general linear model procedure of the SAS program (SAS Institute, Cary, NC). The treatment means were separated using Fisher's least significant difference test at $P = 0.05$.

Testing different rates of *M. albus* biorational mixture for controlling *P. ultimum*, *R. solani*, and *Meloidogyne incognita*. Soil fumigation was performed by adding 15, 30, 45, 60, 90, or 120 μl of

Table 1. Volatile organic components of *Muscodor albus* biorational mixture

Chemical compound	% ^z
Octane	0.47
Acetone	1.36
Acetic acid, methyl ester	0.99
Acetic acid, ethyl ester	11.14
Propanoic acid, 2-methylethyl ester	3.03
Propanol, 2-methyl	3.01
1-Butanol, 3-methyl-, acetate	32.47
Propanoic acid, 2-methyl, 3-methylbutyl ester	2.24
1-Butanol, 3-methyl-	33.58
2-Nonanone	0.60
Propanoic acid, 2-methyl	8.86
Caryophyllene	0.71
Phenylethyl alcohol	1.54

^z Relative percentage of each compound used in the mixture was based on the gas chromatography and mass spectroscopy analysis of the volatiles produced by 10-day-old cultures of *M. albus* grown on potato dextrose agar.

the biorational mixture into plastic mini-cups (6 mm diameter), which were nestled with the top open into the middle of 60 cm³ of pathogen-infested soil in petri plates. For the pathogen controls, 60 cm³ of infested soil were placed in petri plates, and an equal volume of noninfested soil was used for the noninfested control. All petri plates were sealed with two layers of Parafilm and stored for 1 week at 22 ± 1°C for fumigation. The methods, experimental design, and statistical analysis following the fumigation period were similar to the methods described earlier. EC₅₀ values were calculated from the linear regression model which was calculated using the REG procedure in the SAS program.

M. albus was grown on PDA in two of four quadrants of a four-quadrant segmented plate (VWR, West Chester, PA) for 7 days; 5 ml of tap water was added to the other two quadrants. The plates were sealed with two layers of Parafilm and incubated for 5 days. Noninfested PDA was used as the nontreated control. After 5 days incubation at room temperature, ca. 25 *M. incognita* juveniles (J2) were added to each water-filled section. Mobility of juveniles was assessed using visual observation for mobility after 24 and 72 h, and 1 week. At each time, juveniles from each treatment replicate were collected using a 25-µm sieve, resuspended in fresh tap water, and evaluated for mobility after 30 min. The experimental design was a complete randomized block with five replicates, and the described experiment was repeated twice.

In vitro assays for nematicidal activity of volatile components from *M. albus*. Assays were performed by adding 1 ml of fresh tap water containing ca. 50 *Meloidogyne incognita* juveniles (J2) to 24-well cell culture plates (Nalg Nunc International, Rochester, NY). The biorational mixture previously described was added to each well at a rate of 0 to 8 µl/ml. Mobility of juveniles was assessed after 1, 2, and 24 h. At each time, juveniles from each treatment were collected as described above and evaluated for mobility after 30 min. The experimental design was a complete randomized block with three replicates, and the described experiment was repeated twice.

Means were separated using analysis of variance and the Fisher's test for least significant differences. EC₅₀ and EC₉₀ values were calculated for the 1-h time period from the linear regression model, which was calculated using the REG procedure in SAS (SAS).

Greenhouse bioassays for testing the efficacy of *M. albus* and the biorational mixture for control of *Meloidogyne incognita* on tomato. The efficacy of the live *M. albus* barley formulation and the biorational mixture was tested against *Meloidogyne incognita* on tomato. Treatments included a control with sterile ground barley, *M. albus* grown on ground barley, and the biorational mixture at a concentration of 5 µl/ml in aqueous solution. For the *M. albus* and barley control treatments, 3 g of barley formulation per pot was hydrated with 1 ml of water 24 h before use. Fifteen-centimeter-diameter pots were filled with 600 cm³ of quartz sand, and ca. 5,000 juveniles (J2) were added per pot. Barley formulations were spread across the surface of the sand, and the biorational treatment was added by applying a total of 200 ml of an aqueous solution to each pot, resulting in a concentration of 1.67 µl/cm³ of the biorational mixture in the sand. Fifty grams of sand was spread over the surface of the pots, covering the barley formulation. Two hundred milliliters of tap water was added to the barley control and the *M. albus* treated pots. Saucers were placed under each pot to catch the excess water. One day after initiating the mycofumigation treatments, 6-week-old tomato seedlings cv. Delicious were transplanted into the treated sand and watered using tap water. Fertility was managed by using 5 g of Osmocote 19-6-12 N-P-K (Scotts, Marysville, OH) per pot and fertilizing weekly with Peters water soluble fertilizer 21-5-20 N-P-K at 100 ppm N (Scotts). The number of galls per root, and fresh and dry weights of whole plants were determined.

RESULTS

In soil infested with *R. solani*, mycofumigation with live *M. albus* growing on the stabilize formulation and fumigation with the biorational mixture at 1 µl/cm³ of soil resulted in a significantly higher percent-

age of healthy seedlings 28 days postplanting ($P = 0.0001$) relative to the *R. solani*-infested control. Percent healthy seedlings was statistically equivalent to the noninfested control (Table 2). Mycofumigation of *P. ultimum*- or *A. cochlidioides*-infested soil using live *M. albus* in the stabilize formulation and the biorational chemical mixture resulted in a greater percentage of healthy seedlings 28 days postplanting ($P = 0.0001$) than the pathogen-infested controls. *M. albus* formulated as stabilize was equivalent to the noninfested control (Table 2). There were no significant differences in percentages of healthy seedlings between the *M. albus* stabilize formulation and the biorational chemical mixture treatment; however, the percentage of healthy seedlings in the biorational treatment was significantly lower than in the noninfested control.

The *M. albus* stabilize formulation resulted in stunted sugar beet seedlings 14 days after planting (E. Grimme, unpublished). However, no differences in growth were observed 21 and 28 days after planting when compared with seedlings grown in sterilized soils. No reduction of growth was observed for seedlings grown in the biorational fumigation treatment. This observation supports the suggestion that the stabilize formulation itself was affecting the seedling growth, not the *Muscodor* fungus (32) or VOC produced by the fungus.

The biorational mixture at rates of 0.5, 0.75, 1.0, 1.5, and 2 µl/cm³ of soil for fumigation of *Pythium*-infested soil resulted in significantly higher percentages of healthy seedlings 28 days postplanting ($P = 0.0001$) relative to the *P. ultimum*-infested control with the rates 0.75 to 2 µl/cm³ of soil being statistically equivalent (Table 3). Fumigation using 0.5 µl/cm³ of the biorational was statistically different from the *P. ultimum* control, but the percentage of healthy seedlings was significantly lower than in the noninfested control or in the treatments using higher rates. Stands in the 0.25 µl/cm³ rate were similar to the pathogen control. Based on our data, the EC₅₀ for *P. ultimum* was calculated to be 0.5 µl/cm³ of soil.

Fumigation of *R. solani*-infested soil using the rates of 0.5, 0.75, 1.0, 1.5, and 2 µl/cm³ of soil of the biorational mixture resulted in significantly higher percentages of healthy seedlings 28 days postplanting ($P = 0.0001$) relative to the pathogen control with the 2 µl/cm³ rate providing stands equivalent to the noninfested control (Table 3). The rate of 0.25 µl of biorational mixture resulted in a significantly lower percentage of healthy seedlings compared with the other treatments, and was statistically similar to the pathogen control. For the control of *R. solani*, the EC₅₀ was calculated to be 1.50 µl/cm³ of soil.

In *M. albus* petri plate assays, a greater percentage of *Meloidogyne incognita* ju-

Table 2. Percent healthy sugar beet seedlings after 28 days following mycofumigation of soil infested with *Rhizoctonia solani*, *Pythium ultimum*, or *Aphanomyces cochlidioides* using live *Muscodor albus* (stabilize formulation) or a biorational mixture of chemicals found in volatiles emitted by *M. albus*

Treatment	Percent healthy seedlings		
	<i>R. solani</i> ^y	<i>P. ultimum</i>	<i>A. cochlidioides</i>
Live <i>M. albus</i> -stabilize formulation, 0.008 g/cm ³ of soil	94.4 a ^z	92.0 ab	88.8 ab
Biorational chemical mixture, 1.0 µl/cm ³ of soil	89.2 a	77.2 b	78.0 b
Pathogen-infested control	71.2 b	2.8 c	0.8 c
Noninfested control	98.4 a	97.6 a	93.6 a

^y Soil was infested as follows: *R. solani*, 5 g of dry ground barley inoculum/kg soil; *P. ultimum* and *A. cochlidioides*, one homogenized, completely colonized 10-cm petri plate/6.4 kg of soil.

^z Means followed by the same letter are not significantly different based on Fisher's LSD at $P = 0.05$. There was no treatment-experiment interaction.

veniles in plates with *Muscodor albus* were inactive after 24 and 72 h and 1 week compared with juveniles from the control plates (Table 4). After 1 week, 100% of the juveniles were immobile and did not regain mobility after exposure to fresh water for 30 min compared with only 25% mortality in the control plates.

In the nematode experiment, varying concentrations of the biorational mixture, *Meloidogyne incognita* juveniles from all treatments (1 to 8 µl/ml) immediately curled in response to the addition of the biorational mixture to assay wells compared with no curling in the water controls. These differences were not apparent at the 1- and 2-µl/ml rates after 1 h and resuspension in water for 30 min, whereas 56% were straight and immobile in the 3 µl/ml treatment and 100% straight and immobile in the 6- to 8-µl/ml treatments (Table 5). After 2 h exposure followed by resuspension in water for 30 min, 100% of the juveniles in the 5-µl/ml treatment were straight and immobile. After 24 h exposure followed by resuspension in water for 30 min, some motility returned in the 3- and 4-µl/ml treatments, but in the 5- to 8-µl/ml treatments, 100% were straight and immobile (Table 5). The EC₅₀ for *M. incognita* juveniles was 2.9 µl/ml and the EC₉₀ was 5.54 µl/ml.

In the tomato root-knot experiment, the biorational mixture reduced the average number of galls per plant by nearly 84% compared with the barley control (Table 6). While the *M. albus* barley formulation reduced the number of galls per root, it was not significantly different from the barley control ($P < 0.05$). Neither dry nor fresh root weights were affected by any treatment.

DISCUSSION

The chemical and beneficial biological properties of *Muscodor* sp. were described previously in vitro (14,15,34) and in vivo (15,18,32,40,41). The improved seedling health in the present study with the live *M. albus* stabilize formulation was similar to the results reported by Stinson et al. (32), and fumigation with the biorational chemical mixture resulted in similar disease

control over the 28-day test period. Comparison of live formulations of *M. albus* and the biorational chemical mixture for soil disinfestation is an important step for the development of large-scale field and greenhouse sanitation strategies. Based on results of these studies and others from our laboratory, the biorational mixture tested would be used on a broadcast basis at 1,120 liters/ha compared with 9,363 kg/ha for the stabilize formulation of live *M. albus*. These rates stand in comparison to 701.2 liter/ha (highest labeled rate for all crops) for the widely used soil fumigant Vapam HL (sodium methyldithiocarbamate; AMVAC, Los Angeles, CA) or 392 kg/ha (highest labeled rate for all crops) for the granular soil fumigant Basamid (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione; BASF Corp., Research Triangle Park, NC). The above rates for the commercially formulated products are for control of soilborne fungi such as *R. solani* and *P. ultimum* and for root-knot nematodes including *Meloidogyne incognita*. While the rates for the biorational mixture or the stabilize formulation of *Muscodor albus* reported herein are much higher than comparable registered chemical fumigants, we have not yet attempted to examine the lowest efficacious rate of either the biorational or stabilize formulation for use in field soils. Ezra and Strobel (14) reported that the use of 0.26 µl of the biorational mixture per cubic centimeter of air space provided 100% mortality of *R. solani* and *P. ultimum* in in vitro studies. In addition, Ezra et al. (13) and Ezra and Strobel (14) demonstrated the effective range of *M. albus* VOC to be between 100 and 200 ppb. Thus, it may be possible to reduce the rate of the biorational formulation by as much as 43%, although a lower reduction might be possible in soil. The use of the biorational formulation eliminates the consideration of introducing a nonindigenous fungus into soils, and the rates used appear to be within the realm of feasibility. In addition, the use of the biorational mixture eliminates the variability associated with live formulations of *M. albus*, which were greatly affected by storage time and tem-

perature (15). In our studies, we have noted a greater than 50% CFU/g reduction in the stabilize formulation even when stored at -4 or 4°C for 6 months (data not shown).

Mycofumigation with the fungus *M. albus* or the biorational mixture was effective in improving healthy seedling establishment in experiments with soils infested with *A. cochliformis*, *P. ultimum*, and *R. solani*. The treatment with live *M. albus* formulated as stabilize or the biorational chemical mixture resulted consistently in a significantly higher sugar beet seedling survival relative to the respective pathogen treatments. The biorational treatment was statistically as effective against the tested soilborne pathogens as the live fungus formulation. These results support the in vitro results of Strobel et al. (34) that the fungal mycelium was not responsible for microbial inhibition, but the VOC produced by *M. albus* were effective in killing or inhibiting the other microorganisms. It is interesting that the biorational mixture was equal to the noninfested control for Rhizoctonia damping-off but was statistically less effective for Pythium- and Aphanomyces damping-off. Whether this

Table 4. An in vivo assay of *Muscodor albus* volatile organic compounds on activity of *Meloidogyne incognita* second-stage juveniles in an in vitro assay

Treatment	Percent immobile juveniles ^y		
	24 h	72 h	1 week
Noninfested control (PDA)	12 a ^z	21 a	25 a
<i>Muscodor albus</i>	31 b	74 b	100 b

^y Nematode juveniles were observed using a dissecting scope and recorded as mobile or immobile.

^z Means followed by the same letter are not significantly different based on Fisher's LSD at $P = 0.05$.

Table 5. Effect of volatile compound concentrations produced by *Muscodor albus* on viability of *Meloidogyne incognita* second-stage juveniles

Treatment concentration	Percent immobile juveniles ^y		
	1 h	2 h	24 h
0	0 d ^z	0 d	0 d
1 µl/ml	0 d	0 d	0 d
2 µl/ml	0 d	0 d	0 d
3 µl/ml	56 c	73 c	26 c
4 µl/ml	83 b	89 b	63 b
5 µl/ml	94 b	100 a	100 a
6 µl/ml	100 a	100 a	100 a
7 µl/ml	100 a	100 a	100 a
8 µl/ml	100 a	100 a	100 a

^y Nematode juveniles were observed using a dissecting scope and recorded as mobile or immobile. Immobile juveniles failed to regain activity after resuspension in fresh tap water.

^z Means followed by the same letter are not significantly different based on Fisher's LSD at $P = 0.05$.

Table 3. Percent healthy sugar beet seedlings after 28 days following mycofumigation of soil infested with *Rhizoctonia solani* or *Pythium ultimum* using the *Muscodor albus* biorational mixture

Concentration of <i>M. albus</i> biorational mixture (µl/cm ³ of soil)	Percent healthy seedlings	
	<i>P. ultimum</i> ^y	<i>R. solani</i>
2.00	93.2 a ^z	75.2 ab
1.50	92.4 a	67.6 bc
1.00	94.4 a	56.4 c
0.75	91.6 a	38.8 d
0.50	52.0 b	36.0 de
0.25	6.0 c	24.0 ef
Pathogen-infested control	6.4 c	22.4 f
Noninfested control	90.8 a	84.8 a

^y Soil was infested as follows: *R. solani*, 5 g of dry ground barley inoculum/kg soil; *P. ultimum*, once homogenized, completely colonized 10-cm petri plate/6.4 kg of soil.

^z Means followed by the same letter are not significantly different based on Fisher's LSD at $P = 0.05$.

is a statistical effect or a reflection that the biorational mixture was not equivalent to the live *M. albus* gases is unknown at this time. It is possible that other components of the gases produced by live *M. albus* are effective on these nonfungal pathogens. In addition to the volatile antimicrobial compounds produced by *M. albus*, we have evidence that water-soluble antimicrobial compounds also are produced. In well plate assays, *P. ultimum* is strongly inhibited and sometimes killed by water-soluble filter-sterilized compounds produced by *M. albus* (N. K. Zidack, unpublished). This implies that mycofumigation with *M. albus* may have a broader spectrum of activity than can be explained by the activity of the VOCs alone.

The lethal effect of gases produced by *M. albus* on *Meloidogyne incognita* juveniles (J2) came as somewhat of a surprise. Daisy and colleagues (9) demonstrated that a related organism, *Muscodor vitigenus*, produces naphthalene and has repellent activity against wheat stem sawfly larvae (*Cephus cinctus*). *M. vitigenus* produces significantly more naphthalene than *M. albus*, but has very little activity against *Meloidogyne incognita* juveniles (data not presented), while gases produced by *Muscodor albus* proved lethal to *Meloidogyne incognita* juveniles after 24 h exposure. The biorational mixture of VOCs proved to be acutely toxic to *M. incognita*, killing 100% of nematode juveniles at 2 h when applied in a 5- μ l/ml aqueous solution. The same concentration applied in a drench in root-knot tomato pot assays was significantly more effective than incorporation of the live *Muscodor albus* alone.

It should be pointed out that the biorational mixture, stabilize, and ground barley formulations do not contain the same spectrum of VOCs found in *M. albus* VOCs produced on PDA (14). This is not unexpected since the spectrum of VOC produced is greatly affected by the substrate on which *M. albus* is grown (14). While exhaustive research as to the optimal formulation of a biorational mixture has not been completed, the biorational formulation described in these experiments contains compounds such as 1-butanol, 2 methyl; 1, butanol, 2-methyl acetate; propanoic acid; and 2-methyl-, 2-methyl propyl ester found in the VOCs produced by the live *M. albus*. Work is currently underway to optimize the antimicrobial activity of the biorational formulation, and it is

clear from this work that a mixture of VOCs are necessary for activity approximating the VOCs produced by the live fungus.

The application of the *M. albus* biorational mixture in the field situation could be in the form of soil drenches or as injection-application with plastic cover as it is practiced with methyl bromide fumigation. It is possible that application rates could be reduced by using in-line row fumigation as opposed to broadcast fumigation since the VOCs are not phytotoxic. Future experiments will address the refinement of the biorational formulation and various formulations of live *M. albus* at various rates in the field. These experiments will provide information as to the practicality of mycofumigation or fumigation with a biorational formulation of *M. albus*-generated gases. We are also studying the toxicity of the water-soluble fungal products on a range of fungal pathogens.

Mycofumigation using *Muscodor* sp. or synthetic biorational mixtures of VOCs based on VOCs produced by the live fungus is a new concept of soil fumigation with potential broad application in the greenhouse and field. It is clear that mycofumigation or the use of *M. albus* based biorational formulations will not replace MBC since there will be no weed control. However, the use of live *M. albus* formulations or mimicking biorational formulations may provide control of important soil pathogens. On 22 September 2005, the US EPA granted a section 3 registration to AgraQuest (Davis, CA) for all soil and postharvest uses of *M. albus*. Due to the fact that methyl bromide and most of its chemical alternatives are extremely toxic to humans and animals, the relatively non-toxic nature of volatile gases of *M. albus* may promise to increase worker safety during soil fumigation and fewer adverse environmental effects (32,36,37). Compared with chemical treatments, soil fumigation with *M. albus* might not need a waiting period before planting. *M. albus* might find its place also in integrated pest management in combination with crop rotation, solarization, application of beneficial microorganisms like *Trichoderma* sp., and other strategies.

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Table 6. Effect of sterile barley, *Muscodor albus* barley formulation, and a biorational mixture based on the spectrum of volatiles produced by *M. albus* on root weight and galling of tomato by *Meloidogyne incognita*

Treatment	Fresh root weight (g)	Dry root weight (g)	No. of galls/ root system
Sterile barley control	33.5 a ²	3.6 a	63.0 a
<i>M. albus</i> barley formulation 3 g/liter sand	30.4 a	2.8 a	40.6 a
Biorational mixture 5 μ l/cm ³ sand	26.7 a	2.4 a	9.3 b

² Means followed by the same letter are not significantly different based on Fisher's LSD at *P* = 0.05.

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