Mycofumigation with *Muscodor albus* and *Muscodor roseus* for Control of Seedling Diseases of Sugar Beet and Verticillium Wilt of Eggplant

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ABSTRACT

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Mycofumigation is the use of antimicrobial volatiles produced by fungi such as *Muscodor albus* and *M. roseus* for the control of other organisms. Sugar beet (*Beta vulgaris* L.) stand establishment was increased and disease severity decreased by mycofumigation with *M. roseus* and *M. albus* in autoclaved soil infested with *Rhizoctonia solani*, *Pythium ultimum*, or *Aphanomyces cochlioides*. Eggplant seedlings (*Solanum melongena* L.) transplanted into autoclaved soil infested with *Verticillium dahliae* and mycofumigated with *M. albus* and *M. roseus* had significantly less disease ($P < 0.05$) after 4 and 5 weeks compared with nonmycofumigated *Verticillium*-infested soil. The effect of formulation on efficacy of mycofumigation with *M. roseus* was tested using potato dextrose agar strips, alginate capsules, ground barley, pesta granules, and stabilize granules. The stabilize and ground barley formulations of *M. roseus* resulted in the best control of *P. ultimum* damping-off. The best control of *A. cochlioides* damping-off was with the stabilize formulation, and the stabilize, ground barley, and agar strip formulations provided similar control of *R. solani* damping-off. In soil infested with *P. ultimum*, mycofumigation with *M. albus* stabilize formulation resulted in stand establishment similar to that in the autoclaved soil. Mycofumigation was ineffective in controlling Fusarium wilt of sugar beet. Neither *M. albus* nor *M. roseus* affected sugar beet or eggplant growth or appearance except in the stabilize formulation, where stunting was noticed. Mycofumigation with *M. albus* and *M. roseus* shows promise for control of soilborne diseases caused by *P. ultimum*, *A. cochlioides*, *R. solani*, and *V. dahliae*.

Additional keywords: biological control

This research introduces "mycofumigation" as an alternative concept for the biological control of soilborne pathogens. Mycofumigation refers to the use of antimicrobial volatiles produced by fungi, including the endophytic fungi *Muscodor albus* (isolate 620) (24) and *Muscodor roseus* (isolate A3-5) (23) for the control of microorganisms. In vitro effects of mycofumigant gases produced by *Trichoderma* spp. and *M. albus* have been described by Dennis and Webster (7) and Strobel et al (21), respectively. Strobel et al. (21) identified five classes of compounds (alcohols, esters, ketones, acids, and lipids) as the key components of the mycofumigant gas volatiles. The compounds within each class

were tested individually and combined into an artificial mixture in vitro for biological activity against *Pythium ultimum*, *Rhizoctonia solani*, *Phytophthora cinnamomi*, *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *betae*, and *Sclerotinia sclerotiorum* (21). No single chemical class was as toxic independently to test fungi as the natural volatiles from *M. albus*. The combination of synthetic gas components, however, were equally toxic to test microbes, indicating an additive or synergistic effect among the classes of volatiles. The most effective single component was the esters (21). Volatile compounds produced by *M. albus* killed all test fungi except *F. oxysporum* f. sp. *betae* after 3 days of exposure. *Fusarium oxysporum* f. sp. *betae* was only inhibited.

Management of soilborne pathogens is a major reason for the use of soil fumigants. Annual agricultural use of the broad-spectrum pesticide methyl bromide in the United States totals about 19,000 metric tons, with soil fumigation accounting for 85% of the total use (27). For over 20 years, preplant soil fumigation with methyl bromide-chloropicrin (MBC) has been the standard method used by strawberry growers to prevent weed and soilborne pest and disease problems (4). Due to its implica-

tions in ozone depletion, methyl bromide is scheduled to be phased out by the year 2005 in developed countries and 2025 in developing countries (27). Currently, both chemical and nonchemical alternatives to methyl bromide are being investigated for management of soilborne pathogens (8). Chemical alternatives include chloropicrin, iodomethane, 1,3-dichloropropene + chloropicrin (Telone C-35), metam sodium (Vapam), dazomet (Basamid), and various combinations of the above products with sodium tetrathiocarbamate (Enzone) (15).

Nonchemical alternatives to soil fumigation with methyl bromide include crop rotation, solarization (5), biofumigation with *Brassica* spp. (2,5,18,20), and incorporation of organic composts or biological controls (9). Solarization is not a practical alternative in most temperate climates and is highly weather dependent. Regions where high summer temperatures coincide with rainy seasons, such as the southeastern United States, may have less potential for successful solarization because of the cooling effect of frequent rain showers and the extended cloud cover reduces solar radiation captured under the plastic tarp (5). However, none of these have proven to be acceptable replacements for methyl bromide + chloropicrin in all environments. This research explores the potential for *M. albus* or *M. roseus* as an alternative for control of soilborne plant pathogenic fungi.

The objective of this research was to determine if the mycofumigant effects of *M. albus* or *M. roseus* observed on phytopathogenic fungi in vitro in petri plate assays could be effectively transferred to in vivo assays using sugar beet (*Beta vulgaris* L.) and eggplant (*Solanum melongena* L.) pathosystems and infested soil. The sugar beet pathosystem was selected because assay protocols were established in our lab, and eggplant was selected because of its susceptibility to *V. dahliae* and because it develops symptoms rapidly in the glasshouse. *Pythium ultimum*, *Aphanomyces cochlioides*, *R. solani*, *F. oxysporum* f. sp. *betae*, and *V. dahliae* were chosen as test pathogens because they are ubiquitous soil inhabitants with resistant survival structures and are representative of the pathogens controlled through preplant soil fumigation with MBC mixtures. *P. ultimum*, *A. cochlioides*, and *R. solani* commonly result in damping off of seedlings and root rot. *F. oxysporum* f. sp. *betae* and *V. dahliae* are

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responsible for causing systemic vascular wilts in sugar beet and eggplant, respectively.

The effects of mycofumigation on disease incidence and severity, population densities of *V. dahliae*, and the growth of sugar beet seedlings are reported here. Five different formulations of *M. roseus* were evaluated as mycofumigants.

MATERIALS AND METHODS

Inoculum production of phytopathogenic fungi. *R. solani* AG 2-2 and a chlorate-resistant nitrate-nonutilizing (*nit*) mutant (11) of *V. dahliae* (provided by R. Rowe, Ohio State University) inoculum was produced by transferring these fungi separately from potato dextrose agar (PDA) cultures to sterile, moist whole barley grains in 1-liter Erlenmeyer flasks (16,19). The inoculated barley was kept at $23 \pm 1^\circ\text{C}$ and shaken every day during a 2-week incubation period, followed by air drying at $23 \pm 1^\circ\text{C}$ for 48 h. Inoculum was ground to a sawdust texture (30 to 1,500 μm , with a mean particle size of approximately 300 μm) with a Waring blender. Inoculum was stored at 4°C for up to 9 months. Inoculum consisted primarily of sclerotia, microsclerotia, and dormant mycelium. Two different inoculum batches (A and B) of *R. solani* were produced as described above were used as inoculum. Batch B resulted in an increased amount of disease when used at the same inoculum level as batch A, even though they were produced under the same conditions and from the same stock culture. *P. ultimum* and *A. cochlidioides* were grown in 9-cm-diameter petri dishes on PDA at 23°C until the agar surface was covered. The resulting colonized agar contained mycelia, sporangia and oospores. *F. oxysporum* f. sp. *betae* was grown in potato dextrose broth (PDB) on a shaker at room temperature ($23 \pm 1^\circ\text{C}$) for 2 weeks. Inoculum was primarily microconidia and chlamydospores.

Preparation of mycofumigant. *M. albus* and *M. roseus* was applied as colonized agar strips, ground pesta, and alginate formulations and in the stabilize formulation. *M. albus* and *M. roseus* were grown in 9-cm-diameter petri plates of PDA at $23 \pm 1^\circ\text{C}$ for 2 weeks. Four agar strips (0.5 by 8.0 by 0.5 cm) per 375 g of soil were used as agar strip inoculum. For the initial formulation trial, *M. roseus* was grown in 250 ml of PDB, after which the following formulations were prepared: pesta, a formulation including semolina flour and kaolin clay (6); alginate capsules, composed of sodium alginate and kaolin clay (22); and stabilize, a mixture of water-absorbent starch, corn oil, sucrose, and fumed silica (17). Pesta and alginate formulations were ground in a Waring blender to a particle size of approximately 1 to 2 mm. The formulations were dried under the hood for 48 to 72 h and stored at 4°C . The stabilize formulation was prepared in

a small food processor, resulting in granules 0.5 to 2 mm in size. Excess silica was removed from the stabilize formulation using a 500- μm sieve, retaining the material on the top of the sieve for use. *M. roseus* also was grown on barley following the protocol previously described for *R. solani* and *V. dahliae*. *M. albus* was grown in 250 ml of PDB on a shaker for 2 weeks, then prepared using the stabilize formulation (17). CFU per gram of the stabilize formulation were estimated by adding 0.1 g of stabilize formulation to 9 ml of 0.1% agar, serially diluting the suspension, and culturing 1-ml aliquots on PDA supplemented with streptomycin sulfate at 30 $\mu\text{g}/\text{ml}$ and tetracycline at 12 $\mu\text{g}/\text{ml}$. Plates were incubated in a plastic box at 23°C for 5 days, after which colonies were counted. To determine whether the colonized barley or stabilize inoculum had antifungal activity, they were autoclaved and placed on petri plates containing PDA with 5-mm *P. ultimum* or *R. solani* plugs for 7 days, at which time colony growth was compared with nonamended plates.

Soil infestation and greenhouse bioassay. Greenhouse bioassays were conducted in a steam-pasteurized (80°C for 30 min) potting mix containing equal parts of Bozeman silt loam soil:washed concrete sand and Canadian sphagnum peat moss (vol/vol). AquaGro 2000G wetting agent was blended in at 2.2 kg per 0.078 m^3 of soil mix. The potting mix was then screened through a 2-mm mesh sieve and autoclaved for 75 min. Ground barley inoculum was thoroughly mixed at 5 and 2.5 g of inoculum/kg of soil for *R. solani* and *V. dahliae*, respectively. Initial inoculum levels of *V. dahliae* were estimated by dilution plating on selective media consisting of 2% water agar, 0.2% glucose, and potassium chlorate at 25 g/liter (12) supplemented with streptomycin sulfate at 30 $\mu\text{g}/\text{ml}$ and tetracycline at 12 $\mu\text{g}/\text{ml}$ to control bacterial contamination. *P. ultimum*- and *A. cochlidioides*-colonized agar was homogenized in a Handy Chopper (Black and Decker, Chicago) and mixed at a rate of 1 petri plate/6.4 kg of soil. Soil was infested with *F. oxysporum* f. sp. *betae* by mixing 1 ml of liquid culture per 375 g of soil. Initial and 1-week-after-fumigation inoculum levels were estimated by soil dilution on Nash-Snyder (14) medium.

The experimental design was a randomized complete block with five replications of the following treatments: untreated control (UTC), pathogen only, pathogen plus *M. albus*, and pathogen plus *M. roseus*. Each treatment replication consisted of a square pot (10 by 10 by 8.6 cm) lined with a paper towel. In the controls (untreated, pathogen only) 375 g of autoclaved soil was added per pot. For *M. albus* and *M. roseus* treatments, 325 g of pathogen-infested soil was added, either four agar strips (0.5 by 8.0 by 0.5 cm) or 2 g of the other mycofumigant formulations spread

uniformly in a layer, then covered with 50 g of pathogen-infested soil. Each pot was placed in a 3.78-liter plastic bag containing 100 ml of water and the bag was twist tied. The treatments then were stored in a dark room at $23 \pm 1^\circ\text{C}$ for 1 week to complete the mycofumigation process. Then, 25 untreated Beta 8754 sugar beet (*B. vulgaris* L.) seed were planted into each pot. The pots were placed in a greenhouse at day and night temperatures of 22 and 18°C , respectively (16-h photoperiod). Pots treated with *P. ultimum* and *A. cochlidioides* were placed in shallow saucers with standing water for 3 days immediately after planting to favor infection. Percent seedling emergence was recorded at 7 and 14 days after planting and final stand 1 week later. Individual seedlings then were removed and the roots were washed and rated for disease severity with a disease index (DI) calculated from the formula $\text{DI} = [\Sigma(\text{number of plants in each severity class} \times \text{class number})] \times 100 / (\text{mean number of plants grown in autoclaved soil} \times \text{number of disease classes})$. Seedling disease severity classes used were 0 = healthy, 1 = root or hypocotyls discolored, 2 = root or hypocotyls rotted but top green, and 3 = seedlings dead.

For eggplant disease assays, 3-week-old eggplant (cv. Black Beauty) seedlings were transplanted into pots (1 per pot) containing soil infested with *V. dahliae* at 0 and 1.64×10^4 CFU/g of oven-dry soil. Infested soil was sampled to determine CFU/g before and after the 1-week fumigation period. *V. dahliae* populations were determined by placing 1 g of soil into 9 ml of 0.1% agar; the soil solution then was vortexed for 45 s, then poured through one layer of cheesecloth. Three replicates of each dilution were plated per treatment. The plates were stored in a 24°C incubator for 7 days and colonies were counted. The same plates were read again at 10 days to be sure all CFU were counted. The transplanted eggplant seedlings were observed weekly and a DI calculated for each treatment. The DI was based on a scale of 0 to 3, where 0 = symptoms absent; 1 = symptoms slight, may have small areas of chlorosis, transplant not stunted; 2 = symptoms moderate, leaves yellowing, partial wilt of a couple of leaves, slightly stunted; and 3 = complete wilt, all leaves affected, and transplant severely stunted. DI was calculated using the previously mentioned DI formula.

All experiments were conducted two or three times with similar results and data presented represents pooled experiments. Data was analyzed by analysis of variance using the general linear model procedure of SAS and means were separated using Fisher's least significant difference test.

RESULTS

In repeated experiments designed to test the effect of mycofumigation by *M. albus*

and *M. roseus* on sugar beet emergence and growth, no effect on sugar beet emergence or plant appearance was observed when compared with emergence and appearance of seedlings in nonmycofumigated autoclaved soil, except where the stabilize formulation was used (*data unpublished*). Stunting was noted whenever this formulation was used. When *M. albus* or *M. roseus* ground barley or stabilize formulations were tested in agar plate assays after autoclaving, no growth reductions were noted for either *P. ultimum* or *R. solani* compared with total growth inhibition when nonautoclaved formulations were used.

Influence of mycofumigation on seedling diseases. In *R. solani*-infested soil, mycofumigation with *M. roseus* formulated as agar strips, ground barley, or stabilize formulations resulted in stand establishment greater than the pathogen control, and only the ground barley formulation was similar to that observed in the autoclaved soil (Table 1). The pesta and alginate formulations were less effective and were dropped from further consideration after initial experiments.

In *P. ultimum*-infested soil, none of the mycofumigant treatments resulted in stand establishment similar to that in the autoclaved soil. Mycofumigation with *M. roseus* formulated as stabilize, ground barley, and agar strips, however, resulted in greater stand establishment than that in nonmycofumigated *P. ultimum*-infested soil (Table 1). Mycofumigation with *M. roseus* formulated as pesta granules was not effective and was dropped from consideration after initial experiments.

In soil infested with *A. cochlioides*, none of the mycofumigant formulation treatments resulted in stand establishment similar to that in the autoclaved soil. Mycofumigation with *M. roseus* formulated as stabilize and ground barley, however, resulted in greater stand establishment than that in nonmycofumigated *Aphanomyces* spp.-infested soil. The stabilize formulation resulted in greater stand establishment than the ground barley formulation (Table 1).

These experiments showed that the *M. roseus* stabilize formulation resulted in the best control of *A. cochlioides*, and was equal to mycofumigation with *M. roseus* ground barley formulation in *R. solani*- and *P. ultimum*-infested soil; therefore, additional experiments were designed for the stabilize formulation. Although emergence was similar, plants grown in soil mycofumigated using the stabilize formulation were smaller (*data not shown*).

Efficacy of *M. roseus* and *M. albus* stabilize formulation for mycofumigation. In soil infested with *R. solani*, mycofumigation with *M. albus* and *M. roseus* resulted in lower ($P < 0.05$) stand establishment than that in autoclaved soil. Mycofumigation with *M. albus* and *M. roseus*, however, did result in greater stand estab-

lishment than that in *R. solani*-infested soil. Mycofumigation with *M. albus* resulted in significantly greater stand establishment than mycofumigation with *M. roseus*. Seedlings grown in *R. solani*-infested soil mycofumigated with *M. albus* showed less disease than those grown in soil mycofumigated with *M. roseus*. Neither mycofumigation treatment resulted in a DI similar to the DI of seedlings grown in autoclaved soil (Table 2).

In soil infested with *P. ultimum*, mycofumigation with *M. albus* resulted in stand establishment similar to that in the autoclaved soil (Table 2). Mycofumigation of *P. ultimum*-infested soil with *M. roseus* did not result in stand establishment similar to that in the autoclaved soil; however, stand establishment was greater than that in nonmycofumigated *P. ultimum*-infested soil. Mycofumigation with *M. roseus* resulted in stand establishment similar to that in soil mycofumigated with *M. albus* (Table 2). Seedlings in soil mycofumigated with *M. albus* and *M. roseus* had similar DIs, which were lower than the DI of seedlings grown in nonmycofumigated *P. ultimum*-infested soil. Neither mycofumigation treatment resulted in a DI similar to the DI of seedlings grown in autoclaved soil (Table 2).

Mycofumigation of *A. cochlioides*-infested soil with *M. albus* and *M. roseus* resulted in stand establishment lower than that in the autoclaved soil; however, myco-

fumigation with *M. albus* resulted in a greater stand establishment than that in nonmycofumigated *A. cochlioides*-infested soil or infested soil mycofumigated with *M. roseus*. (Table 2). Seedlings grown in soil mycofumigated with *M. albus* had a lower DI when compared with the DI of seedlings grown in nonmycofumigated *A. cochlioides*-infested soil. Seedlings grown in *A. cochlioides*-infested soil mycofumigated with *M. roseus* had a DI similar to that of seedlings grown in *M. albus*-mycofumigated soil (Table 2).

Mycofumigation of *F. oxysporum* f. sp. *betae*-infested soil for 1 week with *M. albus* and *M. roseus* resulted in 292 and 116 CFU, respectively, of *F. oxysporum* f. sp. *betae* per gram of soil, compared with nonfumigated soil with 120 CFU/g of soil. These levels were not significantly different at $P = 0.1$. Disease severity indexes were 56.6 and 61.9 for *M. albus* and *M. roseus*, respectively, and 66.2 for nonmycofumigated infested soil. These differences were not significantly different at $P = 0.1$.

The effect of mycofumigation with *M. albus* and *M. roseus* on inoculum of *V. dahliae* was highly variable (coefficient of variation 310.4). In soil initially infested with *V. dahliae* at 1.64×10^4 CFU/g of soil, mycofumigation with *M. albus* resulted in a population statistically equal to autoclaved soil. Mycofumigation with *M.*

Table 1. Percent survival of sugar beet 21 days after planting into autoclaved soil infested with *Rhizoctonia solani* AG 2-2, *Pythium ultimum*, or *Aphanomyces cochlioides*, compared with infested soil mycofumigated for 7 days with ground barley, stabilize, and agar strip formulations of *Muscodor roseus*

Treatment	Pathogen used to infest soil ^z		
	<i>R. solani</i>	<i>P. ultimum</i>	<i>A. cochlioides</i>
Noninfested autoclaved control	90.0 a	81.8 a	86.8 a
Pathogen-infested control	40.4 d	20.8 c	10.7 d
<i>M. roseus</i> ground barley	80.4 ab	61.3 b	20.4 c
<i>M. roseus</i> stabilize	75.0 b	60.0 b	30.4 b
<i>M. roseus</i> agar strips	73.6 b	49.2 b	ND

^z Soil was infested as follows: *R. solani*, 5 g of dry ground barley inoculum/kg of soil; *P. ultimum* and *A. cochlioides*, one homogenized, completely colonized 10-cm petri plate/6.4 kg of soil. Means followed by the same letter are not significantly different at $P < 0.05$. Each value is the mean of three experiments with five replicates in each experiment. There was no treatment-experiment interaction. ND = not done.

Table 2. Percent seedling survival and disease index (DI) of sugar beet 21 days after planting into nonmycofumigated sterilized soil infested with *Rhizoctonia solani* AG 2-2, *Pythium ultimum*, and *Aphanomyces cochlioides*, compared with infested soil mycofumigated for 7 days with *Muscodor albus* or *M. roseus* in the stabilize formulation^z

Treatment	<i>R. solani</i>		<i>P. ultimum</i>		<i>A. cochlioides</i>	
	Stand (%)	DI	Stand (%)	DI	Stand (%)	DI
Noninfested control	96 a	0 d	88.8 a	0 c	78.4 a	0 c
Pathogen infested control	8.8 d	68.3 a	14.4 c	76.4 a	13.6 c	82.6 a
<i>M. roseus</i> stabilize	31.2 c	47.7 b	73.6 b	24.1 b	29.6 bc	69.1 ab
<i>M. albus</i> stabilize	63.2 b	28.5 c	76.8 ab	26.8 b	45.6 b	55.1 b

^z Means followed by the same letter are not significantly different at $P < 0.05$. Each value is the mean of five replicates from two experiments. There was no treatment-experiment interaction. DI was based on a scale of 0 to 3, where 0 = healthy, 1 = root or hypocotyls discolored, 2 = root or hypocotyls rotted but seedling still green, and 3 = seedling dead. DI was calculated using the following formula: $DI = [\sum(\text{number of plants in each severity class} \times \text{class number})] \times 100 / (\text{mean number of plants grown in autoclaved soil} \times \text{number of disease classes})$.

roseus resulted in populations statistically similar to nonmycofumigated *V. dahliae*-infested soil (Table 3). However, the DI was significantly lower for eggplant growing in mycofumigated soil compared with eggplant growing in nonmycofumigated *V. dahliae*-infested soil after 4 and 5 weeks (Table 3). The DI of the eggplant grown in soil mycofumigated with *M. albus* and *M. roseus* was statistically similar to the DI of eggplant growing in autoclaved soil after 4 weeks. At 5 weeks, only plants grown in *M. albus*-mycofumigated, *V. dahliae*-infested soil had a DI similar to that of plants grown in autoclaved soil (Table 3; Fig. 1). After 5 weeks, the DI of eggplant growing in soil mycofumigated with *M. roseus* was statistically equal to that of *M. albus*-mycofumigated plants but higher than that of plants grown in autoclaved soil.

Table 3. *Verticillium dahliae* soil population (CFU/g of dry soil) after infested soil was mycofumigated for 7 days by either *Muscodor albus* or *M. roseus* using the stabilize formulation, compared with nonmycofumigated soil^a

Treatment	Population (CFU/g)	DI (4 weeks)	DI (5 weeks)
Autoclaved, noninfested control	0 b	0.0 b	0.0 c
<i>V. dahliae</i>	66,840 a	55.0 a	60.0 a
<i>V. dahliae</i> + <i>M. roseus</i>	21,438 ab	17.5 b	32.5 b
<i>V. dahliae</i> + <i>M. albus</i>	1,257 b	10.0 b	20.0 bc

^a Initial population of *V. dahliae* was 1.64×10^4 CFU/g of dry soil. Means followed by the same letter are not significantly different at $P < 0.05$. Each value is the mean of 15 replications from two experiments. Disease index (DI) was based on a scale of 0 to 3, where 0 = symptoms absent; 1 = symptoms slight, may have small areas of chlorosis, transplant not stunted; 2 = symptoms moderate, leaves yellowing, partial wilt of a couple of leaves, slightly stunted; and 3 = complete wilt, all leaves affected, transplant severely stunted. DI was calculated using the following formula: $DI = [\Sigma(\text{number of plants in each severity class} \times \text{class number})] \times 100 / (\text{mean number of plants grown in autoclaved soil} \times \text{number of disease classes})$.

DISCUSSION

Mycofumigation with *M. albus* and *M. roseus* was effective in reducing disease severity associated with the soilborne plant pathogens: *R. solani*, *P. ultimum*, *A. cochlidioides*, and *V. dahliae*. The percent stand establishment of sugar beet seedlings in treatments using *M. albus* was consistently greater and the DI was lower relative to the nonmycofumigated pathogen-infested soil. Neither *M. roseus* nor *M. albus* reduced soil populations of *F. oxysporum* f. sp. *betae* or disease severity caused by this fungus. This was consistent with in vitro tests, which showed only inhibitory but nonlethal effects of the mycofumigant gas on *F. oxysporum* f. sp. *betae* (21).

In the initial experiments with *R. solani* and *M. roseus* (Table 1), stand establishment in the *R. solani*-infested soil treatment at 3 weeks after planting the seed was

38.4%; whereas, in the experiment reported in Table 2, stand establishment was reduced to 8.8%. These results suggest that, when disease pressure is very high, mycofumigation with *M. roseus* may be unsatisfactory with the formulations studied here. A similar trend was observed in the *Aphanomyces* experiments. At 14 days, mycofumigation with *M. roseus* stabilize formulation stand establishment was 53.6% but, after 21 days, stand establishment dropped to 30.4% and the ground barley formulation dropped from 34.4 to 20.4%. In a different set of experiments comparing the stabilize formulation of *M. roseus* and *M. albus*, the percent emergence in the *Aphanomyces* treatment at 21 days was 13.6%, the percent emergence in the *M. roseus* treatment was 29.6% (statistically equivalent), and the *M. albus* treatment was statistically higher at 45.6%. These data suggest that *M. albus* mycofumigation is more effective on *A. cochlidioides* and that *M. roseus* mycofumigation may leave enough oospores unaffected to produce sufficient inoculum after 3 weeks to cause severe disease. Experiments designed to test efficacy of *M. roseus* in relation to inoculum level could prove beneficial in explaining the inconsistencies in results reported here.

In experiments not reported here, it has been shown that neither *M. albus* nor *M. roseus* are pathogenic or phytotoxic to wheat, barley, chickpea, spotted knapweed, sugar beet, mint, potato, eggplant, or tomato (25,26). Both endophytes are described as deuteromycetous (mycelia ster-

Fig. 1. Eggplant grown for 8 weeks in *Verticillium dahliae*-infested soil that had been mycofumigated for 1 week with *Muscodor albus* or *M. roseus* before planting compared with nonmycofumigated, *V. dahliae*-infested soil.

ilia) (23,24). The fact that neither fungus produces spores may limit unintended dispersal in the natural environment. It should be noted that mycofumigation with both fungi in the stabilize formulation did result in stunting of sugar beet seedlings and eggplant transplants. Mycofumigation with both fungi in the barley formulation did not result in growth differences, indicating that the reduced growth was a result of the stabilize formulation itself and not the fungi. However, the formulation alone was not tested. Perhaps the sucrose available in the stabilize formulation provided a substrate for bacteria or other microorganisms that acted like deleterious rhizobacteria that damage plants by producing hydrogen cyanide or other products deleterious to plant growth (10).

M. albus and *M. roseus* may offer an attractive alternative to MBC for multiple reasons. In 1998, the United States Department of Agriculture's IR-4 program began to identify chemical alternatives to methyl bromide for preplant soil fumigation in strawberries. Nelson et al. reported on various chemical treatments that gave yields as high or higher than those obtained with methyl bromide (15). However, the use of chemical alternatives still leaves growers vulnerable to future government-mandated reductions in chemical use. Concomitantly, the number of registered pesticides, particularly for minor crops, is decreasing (10). In California, strict guidelines also limit applicability of available chemical alternatives: 1,3 dichloropropene requires a 92-m buffer zone, and limits are placed on the amount that can be used in a township (12). Cost and application procedures also limit the use of chemical alternatives. The cost of dazomet is \$2,793/ha versus \$2,117/ha. for methyl bromide and requires an additional 2-week period between bed preparation and planting. In addition, dazomet must be tilled into the soil before bedding (18). Optimally, formulations of *M. albus* and *M. roseus* could be applied with existing farm equipment. Based on our work, the mycofumigant gases from both *M. albus* and *M. roseus* are not phytotoxic and would not require an interval between application and planting. Conversely, the lack of phytotoxicity also indicates that weed control would not occur. Another limitation is a lack of control of either root knot or sugar beet cyst nematode (B. J. Jacobsen, unpublished data). If commercialized, *M. albus* and *M. roseus* would be less likely to present health hazards to those in its proximity than other fumigant chemicals and would be subject to fewer limitations due to the fact that the volatiles are more selective in their toxicity (21).

Our results indicate that both *M. roseus* and *M. albus* reduce disease severity caused by *V. dahliae* (Table 3). *M. albus* mycofumigation significantly reduced population levels of *V. dahliae*. Because

only 6 to 50 microsclerotia per gram are sufficient to give 100% infection in most susceptible crops (1), this level of control is not acceptable. However, we hypothesize that, if our initial inoculum had been more in the range of 10 to 50 CFU/g, a parallel amount of reduction in inoculum level could result in undetectable levels of the pathogen. Experiments focusing on the mechanism of population reduction and on the effect of inoculum level need to be carried out before it can be speculated as to the efficacy of *M. albus* mycofumigation in the field.

Mycofumigation may present a less weather-dependent and more reliable alternative to solarization. This research shows that effective fumigation of the soil can occur at room temperature in the dark. Mycofumigation appears to be more practical than *Brassica* green manures, rotation crops, or seed meal amendments that have been reported to suppress pest and disease organisms when grown or incorporated in the soil (3,13). Bianco et al. (2) quantified isothiocyanates produced by the freeze-dried roots of Indian mustard (*Brassica juncea*) and a turnip-canola mix (*B. campestris* and *B. napus*) before and after their incorporation into a light clay loam. They demonstrated that isothiocyanates were recovered at lower concentrations from roots incorporated into the soil. These levels were at least 15 times below those required to control most pest and pathogens based on commercial fumigant levels (2). In experiments testing the effects of combining solarization and biofumigation on populations of *Phytophthora*, no additional benefit was observed where cabbage residue was incorporated into the soil (5).

The results presented in this article suggest that formulations differentially influence mycofumigation efficacy; therefore, it is possible to maximize mycofumigation efficacy of both *M. roseus* and *M. albus* in the soil by selecting an appropriate formulation. *M. albus* in the stabilize formulation repeatedly reduced disease severity and populations of *V. dahliae*, indicating that an effective level of volatile antimicrobials is produced consistently in vivo in this formulation. However, the stabilize formulation did cause stunting of plants. Currently, research is underway to determine the optimal formulation of *M. albus* for field trials and to determine whether the effects observed in autoclaved, artificially pathogen-infested soil occur under field conditions. An attractive advantage of mycofumigation with *M. albus* and *M. roseus* is the ability to control disease severity caused by a variety of plant pathogens of various fungal classes. Pathogen inoculum density, however, appears to heavily influence the degree to which the disease is controlled by current mycofumigation formulations and dosages.

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