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Mycofumigation is the use of gas-producing fungi (*Muscodor* sp.) to kill other microorganisms via production of volatile microbiocidal compounds. Data from greenhouse experiments presented in 2001 demonstrated disease reducing efficacy against *Rhizoctonia, Pythium, Aphanomyces,* and *Verticillium* by two species of *Muscodor. M. albus* and *M. roseus.* In late 2001 we obtained a permit from APHIS for field release of *M. albus.* In 2002 we presented data on winter survival, gas chromatography/mass spectroscopy (GC/MS) analyses of compounds produced by *M. albus,* disease control assays on *Verticillium dahliae* on eggplant, disease control studies on black scurf, scab and Verticillium wilt of potato in the field, and testing of mycofumigation for de-infestation of seed. During late 2002 and 2003, substrate effects on gas production and mycofumigation efficacy were quantified, effect of mycofumigation on a number of pathogens buried at different depths was studied, and field research on potato was repeated and expanded. An additional area of study has been on development of a biorational fumigant based on the volatile organic compounds (VOC) produced by *M. albus*. In 2004, we will present new data on using mixtures of VOC's to control soilborne pathogens, and which combinations provide the most activity in *in vitro* assays. Additional information will be presented on mycofumigation for control of soilborne diseases of potato, and nematicidal effects of *Muscodor* sp.

Using a sugarbeet seedling bioassay model, a mixture VOC's was produced based on the full spectrum of gases produced by *M. albus.* In this mixture, highlighted components which were commercially available or easily synthesized were used in the formula (Table 1). Infested soil (100g) was mycofumigated with either formulated *M. albus* or a mixture of the synthetic volatile compounds based on the GC/MS analyses. After one week of mycofumigation, the treated soil was layered over the top of potting mix in a 10 cm^2 pot and sugarbeet seeds were planted and seedling establishment was determined three weeks after planting. The cocktail was as effective as the live fungus at reducing seedling diseases of sugarbeet caused by *Rhizoctonia,* and nearly as effective for control of *Pythium,* and *Aphanomyces.* All treatments resulted in seedling establishment statistically higher than the pathogen infested control (Table 2). These experimental results show that the development of a biorational fumigant based on gases produced by *Muscodor* sp. may have potential.

Additional *in vitro* assays were performed to determine the minimum number of components that could be used to effectively suppress a representative set of soil pathogens. Test pathogens were inoculated onto PDA and aliquots of the most active individual compounds and mixtures were placed in micro-cups in the petri plates. All petri plates were then double wrapped with parafilm. Data was

collected on the minimum amount that was required to cause complete inhibition of growth (MIC 100). The most active individual compounds were propanoic acid and 1-butanol, 3-methyl. Neither of these were active against *Sclerotinia* or *Pythium* when used alone. The most effective mixture had three components and contained 40% methanol, 10% napthalene, and 50% propanoic acid.

Table 1. GC/MS analysis of the volatile compounds produced by *M. albus.* Several minor peaks and the breakthrough peak were omitted from the total analysis since they represent only 1% of the total area. Highlighted compounds represent components of biorational mixture used in sugar beet seedling assays.

RT	Total Area (%)	M/z	Possible compound	MW
3:45	0.33	114	Octane	114
4:19	0.93	58	Acetone	58
4:37	0.68	74	Methyl acetate	74
5:56	7.63	88	Ethyl acetate	88
6:51	0.31	102	Propanoic acid, 2-methyl, methyl ester	102
7:16	6.24	*	Ethanol	46
8:03	2.07	116	Propanoic acid, 2-methyl-ethyl ester	116
11:45	0.58	*	Propanoic acid, 2-methyl 2-methylpropyl ester	144
12:05	2.06	74	Isobutyl alcohol	74
12:50	22.24	*	1-butanol, 3-methyl, acetate	130
14:57	1.53	*	Propanoic acid, 2-methyl, 3-methylbutyl ester	158
15:28	22.99	*	1-butanol, 3-methyl-	88
16:08	0.29	138	#Furan, 2-pentyl-	138
18:53	0.29	142	#4-nonanone	142
20:38	0.41	142	2-nonanone	142
21:07	0.30	204	# Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	204
22:54	1.51	204	# Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1.alpha.,4.alpha.,7.alpha.)]	204
23:16	0.94	204	# Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-	204
25:20	3.63	204	# 1H-3a,7-methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8 tetramethyl-, [3R-(3.alpha., 3a.beta.,7.beta.,8a.alpha.)]	204
25:30	6.08	88	Propanoic acid, 2-methyl	88
26:04	0.48	204	Caryophyllene	204
27:55	0.34	204	# Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1R-(1.alpha., 4a.alpha.,8a.alpha.)]	204
28:34	0.36	204	# Spiro[5.5]undec-2-ene,3,7,7-trimethyl-11-methylene-	204
28:50	1.07	204	Azulene, 1,2,3,5,6,7,8, 8a-octahydro-1, 4-dimethyl-7- (1-methylethyenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)] Common Name: Bulnesene	204
28:57	3.24	204	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,[1R-(1.alpha.,7.beta.,8a.alpha.)] Common Name: Valencene	204
31:12	1.74	*	Acetic acid,2-phenylethyl ester	164
33:17	1.06	122	Phenylethyl alcohol	122
39:00	9.76	204	# Unknown	204

* No molecular-ion peak was observed in the spectrum of either the standard compound or the compound undergoing the analysis.
# Denotes that a spectrum and retention time of this component was observed and the substance matched to the most likely compound in the NIST data base, but the data have not been confirmed by use of an appropriate identical standard compound by either retention time or MS. These compounds were not placed in the artifical mixture in the bioassay test.

Table 2: Percent sugar beet seedling survival after 28 days following mycofumigation of *Rhizoctonia solani, Pythium ultimum, or Aphanomyces cochlioides* infested soil using live *Muscodor albus* (stabileze formulation) or biorational mixture of chemicals found in volatiles emitted by *M. albus*.

	Pathogen used to infest soil [1)]		
Treatment	*R. solani*	*P. ultimum*	*A. cochlioides*
Live *M. albus*	94.4 a	92.0 ab	88.8 ab
Biorational chemical mixture	89.2 ab	77.2 b	78.0 b
Pathogen infested control	71.2 c	2.8 c	0.8 c
Non-infested control	98.4 a	97.6 a	93.6 a

[1]) Soil was infested as follows: *R. solani*, 5 g of dry ground barley inoculum / kg 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 two experiments each with five replications. There was no treatment-experiment interaction.

Table 3. MIC 100 values for select individual organic compounds and mixtures based on the most active volatile components produced by *Muscodor albus*

	Individual Volatile Organic Compounds					
Test Fungus	Napthalene	Ethanol[3]	Acetic Acid	1-Butanol, 3-Methyl	Propanoic Acid	Butanol
Sclerotinia	>100[1]	>100NE[2]	>100	>100	>100	>100
Aspergillus	40	>100NE	100	40	40	80
Pythium	>100	>100NE	60	>100	>100	80
Rhizoctonia	80	>100NE	80	60	60	>100

	Mixtures of Volatile Organic Compounds						
	Propanoic Acid + Napthalene	Propanoic Acid + Ethanol	Propanoic Acid + Acetic Acid	Propanoic Acid + 1-Butanol, 3-Methyl	Mixture A[4]	Mixture B[5]	Mixture C[6]
Sclerotinia	>100	>100	>100	100	40	40	20
Aspergillus	40	40	40	60	40	40	40
Pythium	>100	40	>100	80	100	15	100
Rhizoctonia	15	60	80	40	20	15	20

[1]Volume (μl) of compound or mixture that resulted in fungal growth less than 10% of the control. Napthalene was added by weight (mg).
[2]NE = No Effect
[3]Ethanol promoted the growth of many fungi when added in low concentrations.
[4]Mixture A: 33.3% 1-Butanol, 3-methyl, 26.6% Methanol, 6.6% Napthalene, 33.3% Propanoic Acid
[5]Mixture B: 40% Methanol, 10% Napthalene, 50% Propanoic Acid
[6]Mixture C: 45.5% 1-Butanol, 3-methyl, 9% Napthalene, 45.5% Propanoic Acid