PROGRESS IN THE DEVELOPMENT OF MYCOFUMIGATION FOR CONTROL OF SOIL-BORNE PLANT DISEASES

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In 2001, we presented mycofumigation as a new alternative to methyl bromide fumigation for control of soil-borne plant diseases. Mycofumigation is the use of gas-producing fungi (*Muscodor albus* and *M. roseus*) to kill other microorganisms via production of volatile microbiocidal compounds. Data from greenhouse experiments demonstrated disease reducing efficacy against *Rhizoctonia, Pythium, Aphanomyces,* and *Verticillium* by both species of *Muscodor.* In late 2001 we obtained a permit from APHIS for field release of *M. albus* and have performed a winter survival study, completed gas chromatography/mass spectroscopy (GC/MS) analyses of compounds produced by both *Muscodor species*, disease control assays on *Verticillium dahliae* on eggplant, disease control studies on black scurf, scab and Verticillium wilt of potato in the field, testing of mycofumigation for de-infestation of seed, and a survival study of *M. albus* in soil using various mulches.

A winter survival study on *M. albus* was initiated in the fall of 2001. Four 100g samples of soil were inoculated with 1g of *M. albus* formulation, placed in mesh bags, and buried in field soil at 0, 15, 30, and 45 cm. The bags were removed in April and assayed for viable *M. albus.* None of the soil samples had viable *M. albus* after overwintering in field soil. Due to the fact that *M. albus* is an introduced/exotic organism, its inability to persist in the environment over the long term is a desirable trait. Experiments are currently underway testing the ability of *M. albus* to survive in field soil (short term) under a variety of plastic mulches. *M. albus* was buried at two depths under plastic mulches including black, white, clear, red and IRT. Results will be reported in November.

GC/MS analysis of the volatiles produced by these fungi include alcohols, acids, esters, ketones, and lipids. Mixtures of individual classes of compounds were prepared and a range of plant pathogenic fungi were exposed to the volatile mixtures for 2 days. The mixture with the highest activity was the esters followed by the alcohols (Table 1). While all classes of volatiles had some impact on the test fungi, normal growth resumed when the gasses were removed from the cultures. This is in contrast to previous experiments where fungi were exposed to a synthetic mixture of the entire group of volatiles. In these tests, most of the fungi were killed after 2 days exposure. A synthetic mixture "cocktail" of all of the commercially available components has been tested in greenhouse pot assays. 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*, *Pythium*, and *Aphanomyces*, and all treatments except the pathogen controls resulted in seedling establishment statistically similar to the untreated control (Table 2). These experimental results show that the development of a biorational fumigant based on gases produced by *Muscodor* sp. may have potential.

An eggplant/*Verticillium dahliae* pathosystem has been used to test efficacy of mycofumigation for control of a pathogen which produces microsclerotia. Pasteurized potting mix was infested with *Verticillium* ground barley inoculum. Treatments included an untreated control, *Verticillium* alone control, and mycofumigation for 1 week with *Muscodor albus* and *M. roseus* after which eggplant seedlings were transplanted into the treated potting mix. Four weeks after treatment, eggplants in the *Verticillium* alone treatments were severely diseased and showing signs of wilt. A few plants in mycofumigated soil (both *Muscodor* sp.) showed only very minor symptoms and the overall disease rating for *M. albus* was similar to the untreated control (Table 3).

During the summer of 2002, field experiments were performed testing *M. albus* isolate 620 for control of *Verticillium dahliae, Rhizoctonia solani* (black scurf) and *Streptomyces scabies* (scab) of potato. Stolon disease ratings indicate that 620 reduces cankers caused by *R. solani* comparable to many registered fungicides. Plants in the *Verticillium* test are currently being assayed for percent infection. Tuber ratings for both black scurf and scab, and yield data will be collected at harvest.

Experiments performed on a variety of grains contaminated both naturally and artificially with seed-infesting pathogens indicate broad-spectrum potential for mycofumigation for decontamination of seed. Wheat, chickpea, corn, barley and canola infested with *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and/or *Rhizopus* were placed in closed glass chambers with *M. albus* growing on natural PDA. Three days later 20 seeds were removed, shaken in sterile buffer, and plated onto PDA to determine colony forming units (cfu). For all three samples of wheat and chickpea, mycofumigation with *M.* albus eliminated any detectable fungi and enhanced germination when compared with the non-fumigated control. In the third sample of wheat, some bacterial growth was detected. In corn, only *Rhizopus* grew in the presence of *M.albus.* In the absence of *M.albus, Penicillium*, *Aspergillus* and *Rhizopus* were identified as the main fungi on the seeds. In both samples of barley, the main fungus recovered both in the presence and absence of *M.albus*, was *Rhizopus*. Some *Penicillium* was observed in the second sample and this was the only treatment which did not have enhanced germination after mycofumigation. In canola, only bacteria were recovered from the mycofumigated seeds while *Penicillium* growth on the non-fumigated was too extensive to count (Table 4).

Table 1. The inhibitiory influence of each class of volatile compounds is expressed as the % of the test fungal growth as compared to a control not in the presence of the test compounds. The compounds were tested for a 2 day exposure at the relative concentrations that they occur in *M. albus* at the optimum test concentration 60µl/50 CC air space or 1.2µl /CC.*[1]

Test fungus [1]	Alcohols 0.48 µl/CC % growth of control	Esters 0.53 µl/CC % growth of control	Ketones 0.02 µl/CC % growth of control	Acids 0.09 µl/CC % growth of control	Lipids 0.08µl/CC % growth of control
Pythium ultimum	11.2 ± 4	0	67.5 ± 7	40.9 ± 3	75 ± 0
Rhizoctonia solani	55± 5	0	67.5±7.5	67.5±7.5	40±0
Sclerotinia sclerotiorum	29±3	8.1±1.5	20.6±12	40±0	78±2
Cercospora beticola	58±8	5 ± 5	100±0	83±17	100±0
Fusarium solani	70±10	55± 5	90±10	80±20	80±10

*Mycelial growth measurements compared to the control. [1]None of the microbes was killed after a three day exposure to any of the artificial test mixtures given on this table.

Table 2. Percent emergence of sugarbeet in the greenhouse three weeks after planting into *Rhizoctonia solani* Ag 2-2 infested soil that had been mycofumigated with *Muscodor albus*, *Muscodor roseus*, or a cocktail of volatile compounds based on the profile of compounds produced by *M. albus*.

Treatment	*Rhizoctonia*	*Pythium*	*Aphanomyces*
Uninoculated control	99a[1]	98a	90a
Pathogen control	67b	1b	1b
Muscodor albus	92a	86a	89a
Muscodor roseus	95a	86a	89a
Cocktail	92a	99a	61a
$LSD_{(0.05)}$	2.8	4.4	5.6

[1]Means followed by the same number are not significantly different.

Table 3. Effect of mycofumigation on Verticillium wilt of eggplant in *Verticillium dahliae* infested soil treated with *Muscodor albus* and *Muscodor roseus.*

Treatment	Disease Severity[1]
UTC	0c
Verticillium	55a
Verticillium + M. roseus	17.5b
Verticillium + M. albus	10bc

[1]Disease severity was calculated 4 weeks after transplanting using 4 disease classes from 0-3 with 0 = healthy and 3 = completely wilted. Values were converted to a 100 point scale using the following formula.

$$\text{Disease Severity} = \frac{\Sigma(\text{number of plants in each class} \times \text{class number})}{(\text{mean number of plants} \times \text{number of disease classes})}$$

Table 4: The effect of *M.albus* on populations of seed contaminants and germination rates of a variety of artificially or naturally contaminated seeds

Treatment/ seed type	Mycofumigated		Non-Mycofumigated	
	cfu/20 seed	% Germ	cfu/20 seed	% Germ
Wheat [1]	0	60	$9*10^6$	20
Wheat[2]	0	60	$1.15*10^7$	40
Wheat[3]	a	100	b	80
Chickpea	0	100	1500	80
Corn	$8*10^3$	20	$4.2*10^4$	10
Barley (1)	1*10	80	$2*10^4$	70
Barley (2)	$6*10^4$	40	$2.2*10^5$	60
Canola	a	100	c	95

[1] Artificially contaminated by *Aspergillus ochr.* [2] Naturally contaminated by *Aspergillus,* [3] Naturally contaminated by *Fusarium*

[a]Bacteria only,

[b]Moderate fungal growth

[c]Extreme fungal growth