DEVELOPMENT OF MYCOFUMIGATION FOR CONTROL OF SOIL-BORNE PLANT PATHOGENS

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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 presented in 2001 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.* In 2002 we presented data on winter survival, 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, and testing of mycofumigation for de-infestation of seed. During late 2002 and 2003, substrate effects on gas production and mycofumigation efficacy have been quantified, effect of mycofumigation on a number of pathogens buried at different depths have been studied, and field research on potato has been repeated and expanded.

To determine effect of substrate on production of volatile organic compounds by *M. albus*, five different synthetic growth media were tested. Each medium was selected on the basis of its nutrient content especially with regard to carbohydrate and nitrogen sources. The media used included water agar, water agar with sucrose, water agar with sucrose and yeast extract, potato dextrose agar, and potato sucrose agar natural. A sucrose enriched medium primarily yielded methyl isobutylketone and acetic acid, butyl ester as the primary volatiles, yet neither of these compounds appeared in any other medium. More enriched media were more effective in inhibiting a suite of plant pathogens used as test microbes with the sucrose and yeast extract amended water agar resulting in suppression of all of the test organisms (Table 1). A mixture of propanoic acid, 2-methyl-, and several related esters were associated with the elevated bioactivity of these fungal support media. An artificial mixture of the volatile compounds emitted by the *M. albus* on the various growth media mimicked the effects of the natural volatiles in the bioassay test system. Both IC_{50} and IC_{100} values, for each test organism were generally the lowest for the artificial test mixture representing the atmosphere of the fungus grown on PDA.

A field experiment was performed testing the effect of mycofumigation on the soilborne pathogens *Pythium ultimum, Aphanomyces cochlioides,* and *Rhizoctonia solani*. 200 g soil infested with individual pathogens was placed in nylon bags and buried at 0, 10, 20 and 30 cm. There were 5 replicate samples for each treatment. *M. albus* formulated as ground infested barley was applied to the soil

surface using a fertilizer spreader (61 cm wide swath) at a rate of 870lbs/treated acre. Black plastic mulch was applied with a mechanical bedder/layer and irrigation was applied through drip tape one day after the mycofumigant was applied to the soil surface. Mulch was removed after one week, and infested soil samples were removed and taken to the greenhouse where 25 sugarbeet seeds were planted in pots containing individual samples. Seedling establishment was recorded 2 weeks later. In mycofumigated soil, seedling establishment was significantly higher than for non-mycofumigated soil when averaged over all depths for *Aphanomyces* and *Pythium* (Table 2). In this experiment, *Rhizoctonia* inoculum levels were too high and mycofumigation did not result in enhanced seedling establishment at any of the depths. This experiment is currently being repeated and additional data will be presented.

In 2003, a field experiment was performed on potato to test the potential of mycofumigation to reduce disease caused by *Rhizoctonia solani, Verticillium sp albo-atru* and *dahliae. M. albus* formulated as infested ground barley was applied either as seed/dust treatment or and in-furrow application of 400lb/acre. Mid-season ratings for Rhizoctonia stolon canker indicate that the 400lb rate significantly reduced disease from 19.0% in the untreated control to 7.5% while the seed dust treatment resulted in 11.8% disease. Verticillium wilt was assessed by assaying potato stem pieces for growth of verticilliate structures and indicated that the 400 lb rate reduced infection incidence from 68% in the untreated control to 40% (Table 3) For both diseases, the mycofumigant performed as well as the standard chemical controls for these pathogens.

Conclusions: Multiple experiments in the greenhouse and field demonstrate the potential for mycofumigation to reduce disease caused by a broad spectrum of soil-borne pathogens. As we expand our understanding of the metabolism and biochemistry of this fungus, the potential for development of an efficacious and reliable mycofumigant product should be enhanced.

Advantages of implementation of mycofumigation:

- **Biological alternative to methyl bromide**
- **Broad spectrum control of multiple plant pathogens**
- **Does not require mulch covering for fumigation**
- **Well suited to pot culture**

Potential problems with implementation of mycofumigation:

- **Need approval by EPA**
- **Retaining viability of the formulated mycofumigant**
- **May require high application rates**

Table 1. Effects of *M. albus,* supported on different media, expressed as percentage of the growth of a control, on test fungi after 3 and 6 days exposure to the fungal atmosphere.

Test microbe	Growth after 3 d exposure to *M. albus* on various media (% growth vs control on the same medium)*					Growth after 6 d exposure to *M. albus* on various media (%growth vs control on the same medium)*				
	HA*	HS	HSY	PDA	PSAN	HA	HS	HSY	PDA	PSAN
Fusarium solani	17.3±11.9	0±0	0±0	0±0	0±0	45.1±2.5	14.8±11.2	0±0	0±0	22±2.5
Sclerotinia sclerotiorum	66.6±0	66.6±0	0±0	0.8±1.6	2.6±4.2	66.6±0	66.6±0	0±0	0±0	8.3±16.6
Rhizoctonia solani	27.8±2.3	9.4±5.8	0±0	2.3±1.1	2.7±3.8	18.3±1.9	9.8±2.1	0±0	2.3±1.3	2.8±4.2
Aspergillus ochraceus	10±8.3	0±0	0±0	0±0	0±0	35.1±6	0±0	0±0	0±0	0±0
Pythium ultimum	41.6±0	24.6±19.9	0±0	0±0	0±0	41.6±0	32.1±19.1	0±0	0±0	0±0
Muscodor albus	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0

*HA = water Agar, HS = water agar + sucrose, HSY = water agar + sucrose + yeast extract, PDA = potato dextrose agar, PSAN = potato sucrose agar natural

Table 2. Sugarbeet seedling establishment in pathogen infested field soil , buried from 0 to 30 cm, then mycofumigated with *Muscodor albus* for 1 week.

	% Seedling Establishment at Four Depths				
Treatment	All Depths	0 cm	10 cm	20 cm	30 cm
UTC	81a	79a	77ab	82a	87a
UTC + MF	78a	43bc	86a	91a	91a
Aphanomyces	26cd	64ab	29c	8c	5c
Aphanomyces + MF	46b	44bc	60b	36b	44b
Pythium	16de	24cd	20c	14c	15bc
Pythium + MF	33bc	45bc	11c	34b	34bc
Rhizoctonia	13de	9d	10c	11c	21bc
Rhizoctonia + MF	8e	3d	18c	7c	6c

Table 3. Effect of mycofumigation by *Muscodor albus* on Rhizoctonia stolon canker and Verticillium wilt of potato in the field

	Disease Ratings	
Treatment	Rhizoctonia Stolon Canker[1]	Verticillium Wilt[2]
Untreated Control	19.9a	68a
Tops MZ (12oz/cwt)	12.5ab	44ab
Maxim MZ (12oz/cwt)	6.6bc	44 ab
Maxim MZ (8oz/cwt)	6.6bc	68a
Quadris (0.15oz/1000ft)	2.5c	48 ab
Moncoat MZ @12oz/cwt	4.8bc	48ab
Blocker (5pt/A) in furrow	7.7bc	52ab
Blocker (10pt/A) + Quadris (0.15oz ai/1000ft) in furrow	6.6bc	32b
Muscodor albus (seed treatment)	11.8ab	56ab
Muscodor albus (400lb/A) in furrow	7.5bc	40b
$LSD_{(0.05)}$	8.5	24

[1]Rhizoctonia stolon canker disease indexing performed using a rating scale of 0-4, 0=no disease, 1=few scattered cankers, 2=cankers coalescing, 3= cankers girdling stem and stolons, 4 = dead plant. Converted to disease index using the following formula: $= \frac{\Sigma(\text{Number of stolons in each severity class x class number})}{(\text{Mean number of stolons sampled x number of disease classes})} \times 100$

[2]Percent Verticillium wilt determined by taking 5 stem samples per plot and plating 4 sections/stem on water agar. Visual observation for verticilliate growth was performed between 4 and 7 days and scored as +/- for each stem sample.