Journal of Applied Sciences Research, 3(4): 307-310, 2007
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Biofomigation of Postharvest Fungal Apple Decay with *Muscodor albus* Volatiles

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Abstract: *Muscodor albus*, a fungal bio-fumigant, was tested for control of postharvest fungal diseases on 5 apple cultivars ('Red Delicious', 'Golden Delicious', 'Gala', 'Granny Smith' and 'McIntosh'). Surface-clean fruit were inoculated with known fungal pathogens *(Botrytis cinerea, Penicillium expansum and Sclerotinia sclerotiorum*) by placing 20 µl drops of spore suspension on marked puncture locations on each fruit. Inoculated fruit were exposed to volatiles produced by *M. albus* mycelium growing on rye seeds in sealed glass 4 L jars with air circulation for 24 h at 20ºC. The amount of dry *M. albus* – rye seed culture varied from 0 (control) to 1 g · L^{-1} of jar volume. Immediately after bio-fumigation, the fruit were removed, aerated and placed at 20°C until decay occurred. Fumigation of apples for only 24 h with 0.5 g · L^{-1} with culture of *M. albus* gave complete control of blue mould (*P. expansum*), grey mould (*B. cinerea*) and *S. sclerotiorum* in wound-inoculated fruit. There were no significant changes in fruit quality (i.e., colour values, fruit firmness, total soluble solids and titratable acidity) in treated fruits. However, there were some degrees of lenticels darkening in cultivar of Golden Delicious with using 1 g.L^{-1} of dry *M. albus*-rey seed.

Key words: biofumigation, apple, postharvest decay, *M. albus*

INTRODUCTION

There is increasing concern about the environmental effects and safety of chemical pesticides and fungicides all over the world. Regulatory agencies have reacted to public pressure and introduced comprehensive legislation to reduce pesticide use. Some of fungicides registered for postharvest use, particularly benzimidazol, are becoming ineffective due to the development of fungicide-resistant strains of postharvest pathogens[4]. World trends are moving toward reduced chemicals use. Biocontrol agents possess a number of important advantages over traditional chemical pesticides which make their commercial outlook particularly promising, as in general, they are considered non-hazardous to humans and animals; biodegradable and environmentally friendly; attack specific target organisms, leaving other beneficial organisms unaffected[1].

Biological control occurs when the number and activity of a pathogen or insect is controlled by another member of the community other than man. Several components of the normal microflora living on plants serve naturally to regulate the activities of some pathogens and such naturally occurring control can be enhanced by manipulation. Base on these points, a lot of pre-and postharvest methods have been employed in recent times to manipulate the natural living community in a given space or surface[9].

Fresh fruits and vegetables are often washed and sanitized immediately after harvest and handled under low temperatures in controlled or modified atmosphere. This contributes to the low incidence of pathogen attacks. However, microbial pathogens are more likely to constitute a major problem in stored vegetables and fruits.

An interesting candidate for biological control is *Muscodor albus* isolated 620[13] an endophytic fungus isolated from a cinnamon tree[3,12]. *Muscodor albus* inhibits and/or kills microorganisms by production of a number of volatiles, mainly alcohols, acids and esters. Stinson *et al.*[11] reported that sugar beet stand establishment increased and disease severity decreased in sterile soil artificially infested with *Rhizoctonia* (Kuhn), *Verticillium dohlae* Kleb. and *Fusarium oxysporum* Schlech. In vitro exposure of a wide range of fungal and bacterial microorganisms to volatile compounds released from *M. albus* kills or inhibits spore germination and mycelial or colony growth, without physical contact, including *Sclerotinia*

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sclerotiorum, Botrytis cinerea and Penicillium expansum, all major postharvest pathogens[10]. Mercier and Jimenez[6] have reported that biofumigation for 24 h with a culture of *M. albus* grown on rye grain completely controls blue and gray mold of apple, as well as brown rot of peaches in wound-inoculated fruit. Biofumigation with *M. albus* also controls sour rot and green mold of lemons[7] as well as grey mold of grapes[8].

Inspite of these efforts, demand for further research seems to remain high as only a small number of commercial biological control products are available on the market shelf. In this paper we investigate the biofumigation of five apple cultivars with *M. albus* to control major postharvest fungi (*B. cinerea, P. expansum and S. sclerotiorum*).

MATERIALS AND METHODS

Experiments were conducted with commercially grown apples, cvs. Gala, Golden Delicious, Red Delicious, Granny Smith and McIntosh from cold storage at Kentville, Nova Scotia, (Canada). Fruits were selected for freedom of injuries and infections for treatments. *Muscodor albus* strain 620-colonized rye seed (from J. Mercier, AgraQuest Inc., Davis, Calif.) was stored at 4°C and warmed to room temperature for 2 h before being used in experiment. Inhibition of *S. sclerotiorum* growth by *M. albus* was tested on mycelium. Each fruit was wounded on one location at the equator with a 4 mm cork borer to make a 2 mm hole. A disk (4mm) of agar containing mycelium was cut with a flam sterilized cork borer from the margin of a 2-d-old colony and then put it on the hole.

Inhibition of *P. exponsum and B. cinerea* was tested on spore germination. *P.* exponsum inoculum's was prepared by cutting an 8-mm diameter disk from a sporulating culture and transferring it to 10 mL of SDW in a 15 mL Falcon centrifuge tube. The tube was capped tightly and shaken vigorously to dislodge and break up the chains of spores. *B. cinerea* spores were collected by flooding a sporulating culture with 10 mL of SDW and dislodging the spores by rubbing the culture with a bent glass rod. Spour suspension of *P. expansum* (and *B. cinerea* were (20 µl) was pipette into each wound fruit. The effect of fumigation with M. albus was tested by adding a measured amount of grain colonized with *M. albus* in a plastic cap to each box. To assist the passage of volatiles over the surface of the fruits within the sealed jar a 12V DC fan measuring 40x40x20 mm with a 5.2 cubic foot per min capacity was attached to the inside of each lid. The control consisted of inoculated fruits in jars with no colonized grain. The exposure period was 24h at ambient air temperature (20°C). After the prescribed incubation period the jars were returned to a vented laminar flow hood and the dishes which contained the *M. albus* were removed and ventilated with 2 min of compressed air to remove treatment volatiles. The infection was measured after 7 and 14 days by counting fruit with developing lesions. Fruit quality such as soluble solid content (SSC), titrable acidity, pH, color value and lenticels breakdown were measured after 7 days treatment of fruit[5]. The experiment was conduct in a completely randomized design with three replication jars with 10 fruit each.

RESULTS AND DISCUSSIONS

Muscodor albus volatiles significant inhibited the growth and survival of the all postharvest pathogens apple cultivars tested in this study (Table 1). The results clearly show that increasing the weight of *M. albus*-colonized grain from 0.25 to 1 $g.L^{-1}$ had a significant effect ($P<0.05$) on the ability of *M. albus* volatiles to inhibit spore germination of *B. cinera* and *P. expansum* and colony diameter increase of *S. sclerotiorum*. While in Gala and McIntosh, spore

Table 1: Effect of *M. Albus* on growth of postharvest fungi of apple cultivar.

		Diseases growth (mm)		
Cultivars	*M. Albus* ($g.L^{-1}$)	*B. Cinerea*	*P. expansum*	*S.sclerotorium*
Golden	0	84.7	48.5	85.7
Delicious	0.25	58.4	45.7	66.1
	0.5	49.6	38.2	39.7
	1	44.6	34.9	31.7
	LSD (P<0.05)	7.8	4.4	8.4
Gala	0	45.8	30.4	59.5
	0.25	2.3	4.3	0
	0.5	0	0	0
	1	0	0	0
	LSD (P<0.05)	1.6	2.07	0.38
Red	0	33.6	44.2	57.2
Delicious	0.25	16.4	17.07	43.09
	0.5	10.2	12.3	5.4
	1	4.6	6.17	2.7
	LSD (P<0.05)	2.57	3.29	2.24
McIntosh	0	34.6	16.97	48.9
	0.25	2.47	12.37	1.37
	0.5	0	0	0
	1	0	0	0
	LSD (P<0.05)	1.46	0.89	2.28
Granny	0	34.97	39.9	71.5
Smith	0.25	8.73	4.1	4.7
	0.5	1.5	0.9	1.4
	1	0	0	0.37
	LSD (P<0.05)	2.19	2.19	1.07

Table 2: Effect of *M. Albus* on fruit quality of apple cultivar.

Cultivars	*M. Albus* ($g.L^{-1}$)	Fruit quality		
		Firmness(Kg)	TSS(%)	Acid (mg/100cc)
Golden Delicious	0	11.97	13.2	335
	0.25	12.1	13.1	322.33
	0.5	11.55	13.15	326.17
	1	11.51	13.45	368.67
	LSD (P<0.05)	0.42	0.16	4.79
Gala	0	11.48	12.82	246
	0.25	11.61	12.73	241
	0.5	11.16	12.9	233
	1	11.35	13	260
	LSD (P<0.05)	0.57	0.13	3
Red Delicious	0	14.44	14	338
	0.25	14.4	13.9	336
	0.5	14.52	13.59	328
	1	14.02	13.71	325
	LSD (P<0.05)	0.65	0.28	6.8
McIntosh	0	9.8	11.65	509.8
	0.25	9.83	11.59	507.5
	0.5	9.64	11.59	501.3
	1	9.26	11.41	496.5
	LSD (P<0.05)	0.35	0.06	2.85
Granny Smith	0	16.94	12.9	579.1
	0.25	16.76	12.81	571
	0.5	17.14	12.76	551.8
	1	16.48	12.99	545.1
	LSD (P<0.05)	1.2	0.22	10.86

Table 3: Effect of *M. Albus* volatiles on color values of apple cultivar.

Cultivars	*M. Albus* ($g.L^{-1}$)	Color values				
		a*	b*	L	C	H*
Golden Delicious	0	-0.73	48.46	73.99	50.13	91.07
	0.25	-0.73	50.27	73.97	50.57	93.2
	0.5	-0.13	48.92	71.97	49.53	86.37
	1	-0.03	50.32	72.73	49.03	90.33
	LSD (P<0.05)	-0.4	1.46	1.31	0.92	2.87
Gala	0	26.2	25.5	53.8	39.5	41.54
	0.25	19.5	28.8	55.4	40.28	51
	0.5	21.1	28	54.4	41	50
	1	26.9	22.5	49.1	28.86	44
	LSD (P<0.05)	5.4	10.8	11.1	8.43	7.94
Red Delicious	0	26.91	6.85	36.14	27.72	13.64
	0.25	26.75	7.27	35.55	28.53	14.59
	0.5	25.28	5.31	34.95	26.23	12.01
	1	24.93	4.66	33.69	26.01	10.25
	LSD (P<0.05)	2.47	3.31	1.65	3.11	5.57
McIntosh	0	14.81	12.46	41.35	23.59	34.4
	0.25	14.92	15.5	42.6	27.86	40.1
	0.5	11.67	17.46	45.51	27.71	47.3
	1	11.64	17.84	45.22	28.67	47.2
	LSD (P<0.05)	7.02	3.15	9.82	2.86	9.86
Granny Smith	0	-17.92	36.92	62.26	40.92	115.85
	0.25	-17.62	36.52	61.68	40.82	115.87
	0.5	-17.14	35.74	61.99	39.66	115.36
	1	-17.18	36.13	63.1	40.02	115.63
	LSD (P<0.05)	0.54	0.73	1.6	0.97	0.83

germination of *P. expansum* and *B. cinera* and colony growth of *S. sclerotiorum* were completely inhibited in the presence of 0.5 gL^{-1} *M. albus*-colonized grain with air circulation in the jars. The growth and spore germination of three test fungi of Golden delicious and Red Delicious were significantly reduced to one third of the control non-treatment at 0.5 $g.L^{-1}$. None of the germinated spores or mycelium colonies exposed to M. albus volatiles continued to growth except for Golden and Red Delicious at I $g.L^{-1}$ *M. albus* –colonized grain. Meanwhile, the control non-treatment had higher pathogen infection than apple treated with *M. albus* in all apple cultivars. The results of the placement in ambient air temperature are slightly different than the germination and growth results measured one week after removal (Data not presented).

The effects of *M. albus* concentration on fruit firmness, total soluble solid (TSS) and titarable acid (malic) are presented in Table 2. A little change in fruit firmness was appeared with increasing rate of *M. albus* in the jars, but the difference was slightly significant. However, the differences between control and 0.5 $g.L^{-1}$ of *M. albus* were not significant. Similar

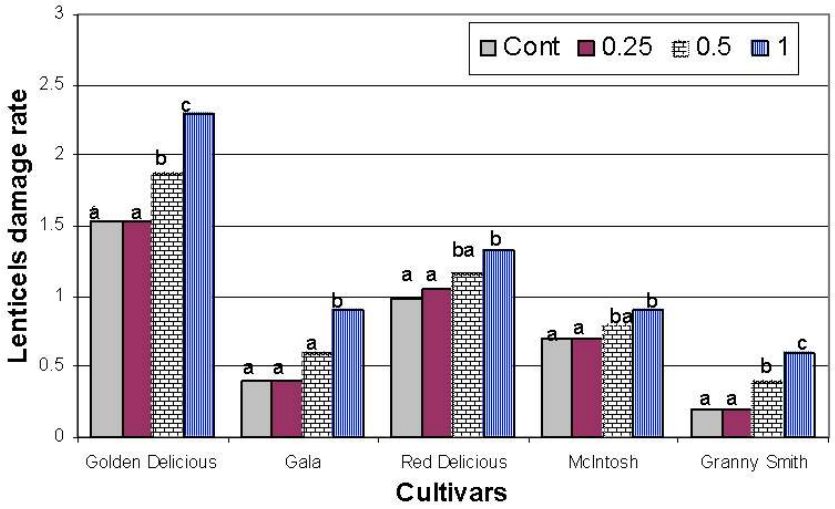

Fig. 1: Effect of *M. Albus* concentration on lenticel breakdown of apple cultivars.

changes in TSS and acid content was observed after 14 days removal of fruits from the jars at room temperatures (20°C).

Lenticels breakdown (LB) appears on fruit flowing *M. albus* treatment (Figure 1). Higher concentration of *M. albus* (1 $g.L^{-1}$) has more LB on fruit especially in cv. Golden Delicius. However, in other cultivars, the severity of damage was less and fruits had acceptable quality after 14 days storing fruit at the room temperatures.

Discussion: Mycofumigation of apple cultivars with *Muscodor albus* was effective in control or reducing disease severity associated with the postharvest plant pathogen: *Botrytis cinerea, penicillium expansum* and *Sclerotinia sclerotiorun.* (Table, 1). There was considerable germination and radial mycelia growth of three postharvst pathogen on control non-treated apple cultivars. Previously, it was shown that volatiles released from in vitro would kill spores of pathogenic fungi and inhibited mycelia growth on the surface of the media[10,2]. *M. albus* was also demonstrated to have a potential practical use in the mycofumigation of seeds in closed chambers[2]. Previously, it had been established that the main volatile compounds responsible for the inhibitory activity of *M. albus* against microorganisms were esters, alcohols and acids[3,12]. In several studies, artificial combinations of a few to 20 of the identified volatiles have been tested for antimicrobial activity comparable to *M. albus* [3,6,10]. *M. albus,* acting through volatile antibiotics, may be more compatible with existing postharvest handling system than current biological or chemical fungicides requiring spraying or drenching as application methods. As a biolfumigant, *M albus* could be useful for minimizing handling of treated commodities and allow treatment of fruit species too fragile for regular fungicide treatment. Previous experiments indicated 1to 13 $g.L^{-1}$ of *Muscodor albus*-colonized grain controlled blue and gray mold of apple cv. Gala[6]. However, our finding that only 0.5 $g.L^{-1}$ with atmosphere circulation was adequate to control fungal pathogens *in vivo* suggests that using lower quantities of *M. albus*-colonized grain may be feasible.

Lethal effect of *M. albus* volatiles on storage pathogen (Table 1) as well as other microorganisms suggests that fumigation with *M. albus* could have widespread applications in controlling microbial spoilage of fresh produce, as well as other commodities, such as grains and nut[12].

REFERENCES

1. Adaskaveg, J.E., H. Forester and N.F. Sommer, 2002. Principles of postharvest pathology and management of decays of edible horticultural crops, p. 163-195: In A.A. Kader (Ed). Postharvest technology of horticultural crops. 3rd ed. University Calif. Agr. Natural Resources Pub. 3311.
2. Ezra, D. and G.A. Stroble, 2003. Effect of substrate on the bioactivity of volatile antimicrobials produced by *Muscodor albus.* Plant Science, 165: 1229-1238.
3. Ezra, D., W.M. Hess and G.A. Strobel, 2004. New endophytic isolates of *Muscodor albus,* a volatile-antibiotic-producing fungus. Microbiology 150: 4023-4031.
4. Irtwange, S.V., 2006. Application of biological control agents in pre-and postharvest operations. Agricultural Engineering International, No. 3 Vol. VIII.
5. Kupferman, E., 2004. Lenticel breakdown in Gala apples. Washington State University article, Postharvest Information Network.
6. Mercier, J. and J.I. Jimenez, 2004. Control of fungi decay of apples and peaches by the biofumigation fungus *Muscodor albus.*Postharvest Biol. Technol., 31: 1-8.
7. Mercier, J. and J.L. Smilanick, 2005 Control of green mold and sour rot of stored lemon by biofumigation with *Mscodor albus.* Biol. Control., 32: 401-407.
8. Mlikota Gabler, F., F.R. Fassel, J. Mercier and J.L. Smilanik, 2005. Influence of packaging, temperature and inoculation timing on the effectiveness of biofumigation with *Muscodor albus* to control postharvest gray mold on table grapes. Amer. Phytopathol. Soc. Ann. Mtg. (abstr).
9. Prange, K.R., Ramin, A. Ali, B.J. Daniels-Lake, J.M. DeLong and P.G. Braun, 2006. Perspectives on postharvest biopesticides and storage technology for organic produce. HortScience, 41: 301-303.
10. Ramin, Ali A., P.G. Braun, R.K. Prange and J.M. DeLong, 2005. In vitro effects of *Muscodor albus* and three volatile components on growth of selected postharvest microorganisms. HortScience, 40: 2109-2114.
11. Stinson, A.M., N.K. Zidack, G.A. Strobel and B.J. Jacobsen, 2003. Mycofumigation with *Muscodor albus* and *muscodor roseus* for control of seedling diseases of sugar beet and Verticillium wilt of eggplant. Plant Dis., 87: 1349-1354.
12. Strobel, G.A., E. Drike, J. Sears and Markworth, 2001. Volatile antimicrobials from *Muscodor albus,* a novel endophytic fungus. Microbiology, 147: 2943-2950.
13. Worapong, J., G.A. Strobel, E.J. Ford, J.V. Li, G. Baird and W.M. Hess, 2001. *Muscodor albus* anam gen. et. sp. Nov., an endophyte from *Cinnamomum zeylanicum.* Mycotaxon, 9: 77-79.