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Short communication

The potential of the fungus, *Muscodor albus*, as a microbial control agent of potato tuber moth (Lepidoptera: Gelechiidae) in stored potatoes

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Abstract

Potato tuber moth (PTM), *Phthorimaea operculella*, is a serious pest of stored potato in most countries where potatoes are grown. Entomopathogens offer promise as alternatives to broad spectrum insecticides for management of this pest. The fungus *Muscodor albus*, which produces a mixture of antimicrobial volatile organic chemicals, was tested for its insecticidal activity against PTM. Adults and neonate larvae were exposed to volatiles generated by 15 or 30 g of *M. albus* rye grain culture plus water for 72 h in hermetically sealed 28.3 L chambers at 24 °C. Mean percent mortalities in adult moths exposed to 0, 15, and 30 g of fungal formulation were 0.9, 84.6, and 90.6%, respectively. Development to the pupal stage of PTM that were exposed as neonate larvae to 15 or 30 of *M. albus* culture was reduced by 61.8 and 72.8%, respectively, relative to controls.
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Keywords: *Muscodor albus*; *Phthorimaea operculella*; Potato tuber moth; Biofumigant; Microbial control

1. Introduction

Potato tuber moth, *Phthorimaea operculella*, is a widespread pest of potato plants and tubers throughout the tropics and subtropics in most countries where potatoes are grown, including the United States. Recently, *P. operculella* has become established in potato growing areas of the temperate Pacific Northwest of the United States (Eastern Washington and Oregon) where it is regarded as a major pest of potato (Jensen et al., 2005). Current means of control prior to harvest comprise several broad spectrum insecticides and cultural methods. The preharvest interval of most chemical insecticides does not permit treatment of tubers just prior to storage. The bacterium, *Bacillus thuringiensis*, and the granulovirus of *P. operculella* have been used in certain countries for protection of tubers in storage (Das et al., 1992; Hamilton and Macdonald, 1990; Kroschel et al., 1996; Zeddam et al., 2003). Treatment of tubers with fumigants for control of *P. operculella* has received limited attention. Effective chemical fumigants such as methyl bromide pose human safety and environmental risks.

A biologically generated fumigant could provide a sustainable alternative method for protecting tubers in storage without some of the risk associated with chemical fumigants and pesticides. Biological fumigation with the volatile-producing fungus *Muscodor albus* was shown to control postharvest decay in a number of commodities such as apples, lemons, and peaches (Mercier and Jiménez, 2004; Mercier and Smilanick, 2005). *M. albus* is a recently described endophytic fungus isolated from the cinnamon tree, *Cinnamomum zeylanicum*, that produces a mixture of volatile organic compounds (alcohols, esters, ketones, acids, and lipids) and kills a broad range of plant and human pathogenic fungi and bacteria (Strobel et al., 2001; Worapong et al., 2001). In addition to postharvest diseases, biofumigation with *M. albus* was shown to control smut on

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grain (Strobel et al., 2001), as well as soil-borne diseases (Mercier and Manker, 2005; Stinson et al., 2003b). However, the insecticidal activity of this fungus has not yet been evaluated and could be interesting for various postharvest or soil uses. Our objectives in this study were to assess the insecticidal properties of *M. albus* against *P. operculella* adults and larvae, in and on potato tubers.

2. Materials and methods

2.1. Source and activation of fungus

Samples of desiccated rye grain culture of *M. albus* (Mercier and Jiménez, 2004) were obtained from Julien Mercier, AgraQuest (Davis, CA) and stored at 8 °C until used. Just prior to testing, aliquots of 15 and 30 g of the formulation were weighed and placed in separate 0.47 L plastic containers and activated by rehydration. Fifteen and thirty milliliters of deionized water were added to containers with 15 and 30 g of formulated fungus, respectively. The containers were then sealed with a lid and set aside for 30 min prior to use for fumigation.

2.2. Source and production of P. operculella

Larvae and adults of *P. operculella* were originally collected from potato plots at the Oregon State University Agricultural Research and Extension Center, Hermiston, Oregon in September 2004 and reared on Russet Burbank tubers at the Yakima Agricultural Research Laboratory (YARL) in Wapato, Washington according to procedures described the International Potato Center Training Bulletin (CIP, 1992). Placing infested tubers over fine sand in $25 \times 35 \times 13$ cm ventilated plastic storage containers (Rubbermaid, Fairlawn, OH) facilitated ease of harvesting pupae which were used for experiments with adults. Full grown larvae exit the tubers and spin their cocoons in the sand. Filter paper disks (Whatman No. 1, 9.0 cm diam.) placed on top of organdy covered 0.47 L plastic cups containing approximately 100 adults served as oviposition substrates. Eggs that were collected on filter paper from the oviposition cups on a daily basis provided hatching larvae that were used for direct exposure to *M. albus*.

2.3. Experimental procedures

Fiberglass chambers (28.3 L, Labconco vacuum desiccators, model 5530, Kansas City, MO) were used for exposing *P. operculella* larvae and adults to volatiles of *M. albus*. Each chamber was hermetically sealed and equipped with a continuously running circulating fan. Ten such fumigation chambers were located in a walk-in incubator at YARL maintained at 24 ± 0.2 °C. For each replicated test, adult moths (50:50 mixture of males and females) were prepared for testing by placing 20 pupae in each of fifteen 0.47 L plastic food containers. A 6.4 cm diameter hole cut in the lid and covered with polyester mesh provided ventilation. Within 24–48 h of emergence, five containers with adults were placed in each chamber used for controls and low and high concentrations of *M. albus* and were provided with honey water (15% honey) on the surface of the organdy screen in each cup. A sixth container with either 15 or 30 g of formulated *M. albus* that was prepared as described above was placed in each of the treatment chambers under the circulation fan immediately after removing the lids. Containers with 30 ml of water were placed in control chambers. The chambers were then hermetically sealed for 72 h. After exposure, the containers with adults were removed from the chambers and the number of surviving moths was counted. Substantial growth of *M. albus* on the rye seeds was observed after the 72 h exposure period. The test was replicated on five separate dates.

For exposure of neonate larvae, individual Russet Burbank tubers (ca. 100 g each) were placed in 0.47 L plastic food containers and infested with 20 neonate *P. operculella* larvae <4 h old. A 6.4 cm diameter hole cut in the lid and covered with polyester mesh provided ventilation. Five such containers were placed in each chamber along with a sixth container with either water only or 15 or 30 g of formulated *M. albus* that was prepared as described above. The chambers were then hermetically sealed for 72 h. Following exposure, the potatoes were removed from the chambers, placed on sand in the same containers and incubated for 16 days at 25.8 ± 1.4 °C until emergence of larvae and pupation of survivors. Potatoes from three of the test dates were dissected to determine if any larvae or pupae remained in the tubers after 16 days. The test was replicated on five separate dates.

2.4. Statistical analyses

Mortality data were transformed using the arcsine of the square root transformation prior to analysis of variance. ANOVA was performed using SAS (v. 8.02) (SAS, 2004) for tests on the effect of fungus concentration adult and larval mortality. Means were separated using Duncan's new multiple range test (MRT).

3. Results and discussion

In addition to its antimicrobial activity, the results of our studies indicate an additional benefit of the use of *M. albus* as a biofumigant agent for the control of storage insects. Adult *P. operculella* that were exposed to 15 or 30 g of fungal formulation in fumigation chambers responded with a highly significant level of mortality (Table 1) ($F_{2,11} = 140.93$, $P < 0.0001$). There was no significant difference in adult mortality produced by the two dosages of fungal formulation. Neonate larvae were also significantly affected by exposure to volatiles produced by *M. albus* ($F_{2,12} = 8.03$, $P = 0.0061$), but were apparently less susceptible than adults (Table 2). Development to the pupal stage of PTM that were exposed as neonate larvae to 15 or 30 g of formulated *M. albus* mycelia was reduced by 61.8 and 72.8%,

Table 1
Mortality in adult potato tuber moth, *Phthorimaea operculella*, after 72 h exposure to volatiles generated by two dosages of the fungus *Muscodor albus* in 28.3 L fumigation chambers at 24 °C

Dosage of fungus (g)/0.028 m^3	Mean % mortality ± SEM[a]
0	0.9 ± 0.68 a
15	84.6 ± 2.21 b
30	90.6 ± 4.65 b

[a] Means followed by the same letter are not significantly different as determined using Duncan's New Multiple Range test.

Table 2
Completion of larval development after exposure of *P. operculella* neonate larvae to volatiles generated by *M. albus* in 28.3 L fumigation chambers at 24 °C

Dosage (g/0.028 m^3)	Mean % ± SEM completing larval development[a]
0	86.5 ± 4.34 a
15	32.9 ± 8.31 b
30	23.5 ± 6.82 b

[a] Means followed by the same letter are not significantly different as determined using Duncan's New Multiple Range test.

respectively, relative to controls (corrected using Abbott's formula). There was no significant difference in larval development and mortality produced by the two dosages of fungal formulation. It is likely that larvae within tubers are less exposed to *M. albus* volatiles than are adults. Within a few hours after placing neonate larvae on potatoes, the majority burrowed into the surface of the tubers. Control tubers from three of the replicates that were dissected after 16 days of incubation revealed very few live larvae and live pupae inside of the potatoes, each representing only 1% of the original number of neonates used in the tests. No dead larvae or pupae were found in control tubers. In tubers treated with 15 or 30 g of fungus, live larvae representing 3 and 1.3% of the original number of neonates, respectively, were still inside of the potatoes. Similarly, live pupae representing 1.3 and 2.3% of original numbers of neonates, respectively, were still inside of the potatoes. Only three dead individuals of 600 treated larvae were found in the potatoes. The low number of cadavers found provides some indication that mortality occurred in small larvae that were imperceptible after 16 days of incubation within the tubers.

Strobel et al. (2001) observed that the most effective class of inhibitory compounds for bacteria and fungi was the esters, of which 1-butanol,3-methyl-acetate was the most biologically active. Jiménez and Mercier (2005) reported isobutyric acid as the main indicator of antifungal activity. The specific group or mixture of volatile organic compounds responsible for mortality in PTM has yet to be determined.

There is a need to consider other volatile-producing fungi for their activity against arthropod pests. Three species of *Muscodor* and one *Gliocladium* sp. that produce volatile organic compounds with biocidal activity have been isolated from several host plants in geographically diverse areas (Daisy et al., 2002; Ezra et al., 2004; Stinson et al., 2003a,b; Strobel et al., 2001; Worapong et al., 2001, 2002). Daisy et al. (2002) showed that naphthalene, an insect repellent, is produced by a related fungus, *Muscodor vitigenus*. Stinson et al. (2003a) demonstrated that *Gliocladium* sp. produces a mixture of volatile organic compounds that are lethal to plant pathogenic fungi. Their report showed that the production of selective volatile antibiotics by endophytic fungi is not exclusively confined to the *Muscodor* spp. The opportunity to isolate additional species, especially from habitats that are becoming increasingly endangered, warrants attention and effort.

Several avenues of research for development of *M. albus* as an insecticidal biofumigant remain to be investigated with *P. operculella* and other insect pests of stored products. Longer exposures and or higher dosages of fungus may result in high mortality of larvae within tubers. The practicality of using *M. albus* for control of *P. operculella* under potato storage conditions will be the subject of additional studies at YARL in the near future.

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