Efficacy of the Biofumigant Fungus *Muscodor albus* (Ascomycota: Xylariales) for Control of Codling Moth (Lepidoptera: Tortricidae) in Simulated Storage Conditions

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ABSTRACT Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a serious pest of pome fruit, is a threat to exportation of apples (*Malus* spp.) because of the possibility of shipping infested fruit. The need for alternatives to fumigants such as methyl bromide for quarantine security of exported fruit has encouraged the development of effective fumigants with reduced side effects. The endophytic fungus *Muscodor albus* Worapong, Strobel and Hess (Ascomycota: Xylariales) produces volatile compounds that are biocidal for several pest organisms, including plant pathogens and insect pests. The objectives of our research were to determine the effects of *M. albus* volatile organic compounds (VOCs) on codling moth adults, neonate larvae, larvae in infested apples, and diapausing cocooned larvae in simulated storage conditions. Fumigation of adult codling moth with VOCs produced by *M. albus* for 3 d and incubating in fresh air for 24 h at 25°C resulted in 81% corrected mortality. Four- and 5-d exposures resulted in higher mortality (84 and 100%, respectively), but control mortality was also high due to the short life span of the moths. Exposure of neonate larvae to VOCs for 3 d on apples and incubating for 7 d resulted in 86% corrected mortality. Treated larvae were predominantly first instars, whereas 85% of control larvae developed to second and third instars. Exposure of apples that had been infested for 5 d, fumigated with *M. albus* VOCs for 3 d, and incubated as described above resulted in 71% corrected larval mortality. Exposure of diapausing cocooned codling moth larvae to VOCs for 7 or 14 d resulted in 31 and 100% mortality, respectively, with negligible control mortality. Our data on treatment of several stages of codling moth with *M. albus* VOCs indicate that the fungus could provide an alternative to broad spectrum chemical fumigants for codling moth control in storage and contribute to the systems approach to achieve quarantine security of exported apples.

KEY WORDS microbial control, apple exportation, postharvest, quarantine treatments

Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), is the key insect pest of apple (*Malus* spp.) in most regions of the world where apple and other pome fruits are grown (Barnes 1991). The following life cycle of codling moth is summarized from Barnes (1991) and Beers et al. (1993). Diapausing larvae overwinter in cocoons in cryptic habitats (under loose bark, leaf liter at the base of trees, in nearby woodpiles and fruit bins). In the spring, they pupate and emerge as adults soon afterward. Eggs are deposited on foliage or fruit, and neonate larvae bore into fruit and remain there until fully grown fifth instars exit and search for cocooning sites. In summer generations, larvae pupate soon after constructing cocoons and emerge as second generation adults. A small percentage of first generation larvae may enter diapause, but diapause usually begins in subsequent generations in late summer or early fall. There are one to four generations per year depending on climatic conditions.

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Insecticide resistance in codling moth is one of the factors responsible for survival of larvae within and emerging from fruit (Reyes et al. 2007). Exportation of apples and other fruit from the United States has been threatened by the presence and possibility of infested fruit and diapausing larvae in cartons and shipping containers (NWHC 2008). Fumigation with broad-spectrum chemicals has been the principal method for protection of fruit exported to certain countries (Follett and Neven 2006). Although efficacious, some of these fumigants, such as methyl bromide, have serious environmental consequences (Yagi et al. 1995, Thomas 1996, UNEP 2008). The need for alternatives to these chemicals has encouraged the search for and development of effective fumigants with reduced side effects.

The fungus *Muscodor albus* Worapong, Strobel and Hess (Ascomycota: Xylariales) is an endophyte of cinnamon, *Cinnamomum zeylanicum* Breyne (Lauraceae), with biofumigant activity (Strobel et al. 2001, Worapong et al. 2001). The biocidal properties of the

fungus are due to the production of a mixture of volatile organic compounds (VOCs) (alcohols, esters, ketones, acids, and lipids) (Strobel et al. 2001). These VOCs are lethal for a variety of plant pathogens, rot-causing organisms, plant parasitic nematodes, and insects (Strobel et al. 2001, Mercier and Jiménez 2004, Mercier and Smilanick 2005, Strobel 2006, Lacey et al. 2008, Riga et al. 2008). An artificial mixture of VOCs that mimics those naturally produced by *M. albus* is comparable to that produced by the fungus for control of plant and human pathogenic fungi and bacteria (Strobel et al. 2001, Strobel 2006, Grimme et al. 2007). However, the individual VOCs have variable activity against different organisms (Strobel et al. 2001, Ramin et al. 2005). The specific compounds that are responsible for insecticidal activity are not yet known. Therefore, our tests were conducted with the naturally produced VOCs of *M. albus.*

Control of codling moth adults and larvae with VOCs in cartons and shipping containers would reduce the threat of accidental exportation of codling moth. The objectives of this study were to determine the effects of *M. albus* VOCs on codling moth adults, neonate larvae on apples, early instars within apples, and diapausing cocooned larvae in simulated storage conditions.

Materials and Methods

Source of Adult and Larval Codling Moth. Codling moth adults, eggs, and diapause-destined cocooned larvae were obtained from a colony maintained at the Yakima Agricultural Research Laboratory by using the rearing system and artificial medium described by Toba and Howell (1991) and Hansen and Anderson (2006).

Origin and Activation of *M. albus*. Samples of a desiccated *M. albus* mycelium on rye (*Secale* spp.) grain were obtained from AgraQuest (Davis, CA) and stored at 4°C until used. Before testing, aliquots of 30 g of the formulation were placed in 0.47-liter plastic cups and activated by adding 30 ml of sterile deionized water. The cups were then sealed with a lid and placed in an incubator at 25°C for 24 h before use. Cups with 30 g of autoclaved rye grain and 30 ml of sterile deionized water were prepared in the same manner and used for control chambers.

Bioassay Procedure. After incubation of *M. albus* and control cups as described above, exposures of codling moth were made in 28.3-liter hermetically sealed fiberglass chambers. For each assay, a container with hydrated *M. albus* was uncovered and immediately placed in treatment chambers. Control chambers received hydrated rye grain. Each chamber was equipped with a continuously running circulation fan. The chambers are housed in a walk-in incubator maintained at 25 ± 0.5°C. More detailed description of the chambers is provided by Lacey et al. (2008).

During each of the tests, temperature and percentage relative humidity (%RH) were monitored with a Hobo H8 Pro Series data logger (Onset Computer Corp., Pocasset, MA). Mean temperatures in the exposure chambers ranged between 25.4 and 25.8°C for all tests. Mean %RH for all tests rose to 97.5–103.2% soon after closing the chambers. The light, temperature, and %RH in the walk-in incubator used for holding adult moths and infested apples after exposure to *M. albus* was 16:8 (L:D) h, 25 ± 0.5°C, and 45.8%RH, respectively. After each exposure period, oxygen (O_2) and carbon dioxide (CO_2) were measured with an O_2/CO_2 meter (Pacific CA System Techni-Systems, Chelan WA). A 180-cm clear reinforced PVC tube was connected to the exhaust outlet of the chambers and to the intake of the meter. The contents of the chamber were drawn through the meter for 5 min until readings had stabilized. Mean ambient air was 20.6% O_2 and 0.3% CO_2.

Adult Mortality. Moths used for experiments had emerged within 24 h before testing. For each replicated test, ≈20 moths (50:50 mixture of males and females) were placed in each of thirty 0.47-liter cups. A 6.4-cm diameter hole cut in the lid and covered with polyester mesh (Econet-T) provided ventilation. Five containers with moths were placed in each of three chambers used for controls and three for *M. albus* treatments. Each cup was provisioned with 15% honey water in 2 ml of Micro-Eppendorf tubes with the lids removed and a 3.8-cm piece of cotton inserted into the tube. The tubes were taped to the side of each cup. Just before the chambers were sealed, a cup with 30 g of hydrated rye grain was placed in each of the three controls. A cup containing 30 g of hydrated *M. albus* mycelium on rye grain was placed in the remaining three chambers. Moths were left in the chambers for 3, 4, or 5 d. After the desired exposure period, mortality was assessed at 0, 24, and 48 h postexposure. Moths were kept in a walk-in incubator at 25°C during the postexposure intervals. Substantial growth of *M. albus* on the rye grain was observed after the exposure periods. Five replicated tests were conducted for each exposure period on five separate dates.

Neonate Larval Mortality, Apple Damage, and Instar Distribution. Neonate codling moth larvae were exposed to *M. albus* VOCs on apples in 0.47 plastic cups in the chambers described above. Ten neonate codling moth <4 h old were placed on an unwaxed Fuji apple (≈7.4 cm in diameter and 187.6 g per apple) in each of 10 ventilated cups. Five cups were placed in each chamber along with a sixth container with either 30 g of hydrated rye grain or 30 g of *M. albus* mycelium on rye grain that had been prepared as described above. The chambers were then hermetically sealed for 72 h. After exposure, the cups were removed from the chambers and incubated for 7 d at 25.8 ± 1.4°C after which the apples were dissected to determine the number of entries, living and dead larvae, and age distribution of larvae. Entries were categorized as deep or shallow. Larvae in shallow entries were found just below the surface of the apples with production of a minimum amount of frass. Deep entries produced considerably more frass with larvae penetrating deeper into the fruit. Five replicate tests were conducted on separate dates. During dissections there were usually fewer larvae than there were entries into the apples. Missing larvae were not counted as dead in either controls or treated apples. We used the total

number of larvae found, dead or alive, upon which to make our mortality calculations.

Mortality of Larvae within Apples, Apple Damage, and Instar Distribution. Methods used to assess how *M. albus* affected larvae within infested apples were identical to the neonate tests, except that after placing neonate larvae on apples in the cups they were incubated for 5 d at 25.8 ± 1.4°C before a 3-d exposure to *M. albus.* We estimated that the 5-d-old larvae were predominantly first instars at the time exposures started. Assessment of mortality, apple damage, and instar distribution was done as described above. Five replicate tests were conducted on separate dates.

Mortality of Diapausing Larvae. Cardboard strips (double-faced, B flute, Weyerhauser, Tacoma, WA) were placed on larval rearing trays before emergence of diapause-destined fifth-instar larvae. After larvae exited the medium and constructed cocoons within the strips, they were stored at 2°C until used. Sections of strips (8 by 2 cm) containing ≈20–25 cocooned larvae were then cut from the larger strips. One strip was placed in each of five cups for each treatment and control chamber for each exposure period for each replicate test. A cup of hydrated *M. albus* on rye grain or hydrated rye grain only were added to each treatment or control chamber, respectively. Due to their lower respiration relative to young larvae and adults, diapausing larvae were exposed to *M. albus* VOCs for either 7 or 14 d. Mortality was assessed immediately after the larvae were removed from the chambers. The strips were moistened to facilitate separation of larvae from cocoons by pulling the sides of the cardboard apart. Larvae that did not respond to probing were considered dead. Five replicate tests were conducted on separate dates.

Statistical Analysis. All data analyses were done in SAS (SAS Institute 2002). The data often failed to meet assumptions of normality and homogeneity of variances required by analysis of variance (ANOVA); thus, we used either nonparametric tests or analyses that modeled the underlying distribution of the data. Effects of *M. albus* on mortality of neonate or 5-d-old codling moth larvae, depth of entry holes, and CO_2 production were examined using Wilcoxon tests in PROC NPAR1WAY. The effects of treatment (control versus *M. albus*), exposure duration (3, 4, or 5 d), and time after exposure (0, 24, or 48 h) on percentage mortality of adult codling moth were assessed using a three-way ANOVA. In some treatment combinations, mortality was either 0 or 100% in all replicates (i.e., there was no variance), which led to departures from the assumptions of ANOVA that could not be corrected using data transformation. Therefore, we modeled the data as a binomial response (number dead/number monitored) by using PROC GLIMMIX. A small constant (0.5) was added to or subtracted from replications having 0 or 100% mortality, respectively, because the analysis failed to converge without this correction. The ar(1) covariance structure was used to account for correlations among the repeat observations (0, 24, and 48 h postexposure). The ilink option was used to back-transform least squares means and standard errors to the original proportion data; these backtransformed means rather than means from the transformed data are presented. In the event of a significant interaction in the ANOVA between the treatment factor and either of the other two factors (days of exposure, hours postexposure), tests on treatment simple effects were done using the SLICE command. These same methods were used to analyze the effects of treatment and day of exposure on mortality of diapausing codling moth larvae, again due to the occurrence of 0 or 100% mortality in all replicates of some treatment × day combinations.

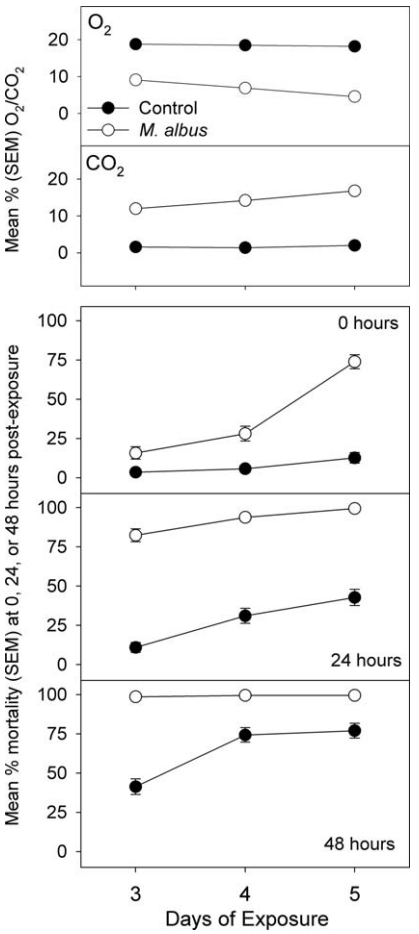

Fig. 1. Effects of fumigation with the fungus *M. albus* on mortality of adult codling moth and levels of CO_2 and O_2. Exposure durations were 3, 4, or 5 d. Assessments of mortality were made immediately after exposure and at 24 and 48 h thereafter.

Results

Mortality of Adult Codling Moths. Mortality rates in moths increased with days of exposure in both control and *M. albus* treatments (days of exposure: $F_{2, 24} = 9.3$; $P = 0.001$ and treatment × days of exposure interaction was nonsignificant, $F_{2, 24} = 0.5$; $P = 0.62$) (Fig. 1. Averaged over days of exposure and hours postexpo-

Table 1. Effect of a 3-d exposure of neonate codling moth larvae placed on apples to volatiles produced by *M. albus*[a]

Treatment[b]	% mortality ± SE	% entries		% CO_2	% O_2	% instar distribution	
		Shallow	Deep				
M. albus	87.2 ± 5.0a	92.7 ± 4.2a	6.7 ± 4.4a	16.2 ± 0.4a	4.7 ± 0.2a	L1	91.7
						L2	8.3
Control	9.0 ± 3.7b	16.2 ± 3.6b	83.8 ± 3.6b	8.0 ± 1.1b	12.6 ± 0.8b	L1	13.5
						L2	54.1
						L3	30.5
						L4	1.9

[a] Assessments of mortality, depth of entries, and instar distribution were made after 7 d of incubation at 25°C after exposure to *M. albus*. CO_2 and O_2 concentrations were measured immediately following the 3-d exposures.
[b] Means in the same column followed by a different letter are significantly different (Wilcoxon P values ≤ 0.012).

sure, mortality was higher in the *M. albus* treatment than the control treatment (92.6 versus 26.4%) ($F_{1, 24} = 80.6$; $P < 0.0001$). The interaction between treatment and h postexposure was also significant ($F_{2, 47} = 8.7$; $P = 0.0006$) (Fig. 1); thus, treatment effects should be examined separately at each level of the hour factor. In all three comparisons, mortality rates in the *M. albus* treatment were higher than in the control treatment: 0 h postexposure, 6.5% mortality in control treatment versus 37.2% in *M. albus* treatment (data averaged over days of exposure; $F_{1, 47} = 52.4$; $P < 0.0001$); 24 h postexposure (25.8 versus 95.9%; $F_{1, 47} = 67.8$; $P < 0.0001$), and 48 h postexposure (65.5 versus 99.3%; $F_{1, 47} = 30.8$; $P < 0.0001$). Despite the low rates of mortality in moths from the 3-d exposure to *M. albus* and 0-h assessment, the moths were noticeably less active than those in the control treatment.

For CO_2 levels (Fig. 1, top), the treatment × days of exposure interaction was significant ($F_{2, 24} = 16.2$; $P < 0.0001$). Simple effects comparisons showed that CO_2 levels were higher in the *M. albus* treatment than the control treatment for each of the three levels of days of exposure ($F_{1, 24} > 300.0$ and $P < 0.0001$ for all three comparisons). CO_2 levels increased significantly with increasing days of exposure in the *M. albus* treatment ($F_{2, 24} = 38.5$; $P < 0.0001$) due to the additional growth of the fungus, but not in the control treatment ($F_{2, 24} = 0.6$; $P = 0.55$). Conversely, O_2 levels were significantly lower in the *M. albus* treatment than in controls (Fig. 1, top), at each level of exposure duration ($F_{1, 24} > 600$ and $P < 0.0001$ for all three tests). Levels of O_2 decreased with increasing days of exposure in the *M. albus* treatment ($F_{2, 24} = 107.7$, $P < 0.0001$) but not in the control treatment ($F_{2, 24} = 1.8$; $P = 0.19$).

Neonate Larval Mortality, Apple Damage, and Instar Distribution. In all categories that were measured (percentage mortality, number of shallow versus deep entries, and CO_2 levels) treated larvae differed significantly from controls (Table 1). Larvae found in the treated apples were predominantly first instars (91.7%); hence, the significantly higher number of shallow entries and very few deep entries. The majority of control larvae (84.6%) had developed to second and third instars accounting for the higher percentage of deep entries. CO_2 levels were significantly higher and O_2 levels were significantly lower in the treated chambers than the control chambers.

Mortality of Larvae within Apples, Apple Damage, and Instar Distribution. Results similar to those in the tests with neonate larvae were obtained with 5-d-old larvae within apples. All treated larvae differed significantly from controls in every category that was measured (Table 2). However, mortality was lower for the 5-d-old larvae than that observed for neonates. The age structure of treated 5-d-old larvae differed from controls, with 93.4% in first and second instars and only 5.3% progressing to the third instar. Control larvae were considerably older than treated larvae, with >90% ranging from second to fifth instars and a significantly greater percentage of deep entries. As in the other 3-d exposures, CO_2 levels were significantly higher, and O_2 levels were significantly lower in the treated chambers than the control chambers.

Table 2. Effect of a 3-d exposure of 5-d-old larvae within apples to volatiles produced by *M. albus* (see footnotes in Table 1)

Treatment	% mortality ± SE	% entries		% CO_2	% O_2	% instar distribution	
		Shallow	Deep				
M. albus	71.2 ± 5.0a	58.7 ± 5.0a	39.5 ± 3.6a	14.4 ± 0.7a	6.1 ± 0.9a	L1	50.8
						L2	42.6
						L3	5.3
						L4	0
						L5	1.3
Control	8.0 ± 3.0b	8.4 ± 3.5b	91.6 ± 3.5b	5.0 ± 0.8b	14.6 ± 0.7b	L1	8.6
						L2	35.9
						L3	25.5
						L4	10.7
						L5	19.3

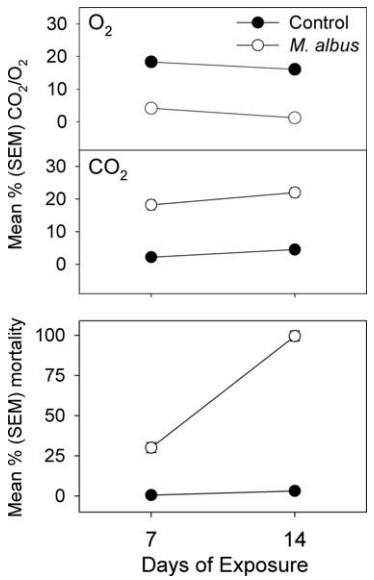

Fig. 2. Effects of fumigation with the fungus *M. albus* on mortality of diapausing codling moth larvae and levels of CO_2 and O_2. Exposure durations were seven and 14 d. Assessments of mortality were made immediately after exposure.

Mortality of Diapausing Larvae. For diapausing codling moth, the treatment × exposure duration interaction was highly significant ($F_{1,\ 16} = 11.2$; $P = 0.004$), due to the jump in mortality associated with increasing duration of exposure in the *M. albus* treatment but not in the control treatment (Fig. 2). The two treatments were compared at the two exposures separately. Mortality rates were higher than that of controls in the *M. albus* treatment for both exposures ($P < 0.005$ for both contrasts). Carbon dioxide levels increased with increasing exposure duration ($F_{1,\ 14} = 6.7$; $P = 0.02$) (Fig. 2, top). Levels of CO_2 were significantly higher in the *M. albus* treatment than the control treatment ($F_{1,\ 14} = 201.6$; $P < 0.0001$). The CO_2 concentration in the 7- and 14-d exposures was considerably higher than the 3-d exposures due to the increased growth of *M. albus.* The corresponding O_2 levels were significantly lower in the *M. albus* treatment than in the controls ($F_{1,\ 14} = 142.1$; $P < 0.0001$). Although control mortality was minimal, the cardboard strips in which larvae were held supported substantial growth of fungus (*Penicillium* sp.) after 14 d in the chambers. No fungal growth was seen in treated chambers.

Discussion

Exposure to *M. albus* volatiles for as few as 3 d followed by 24–48-h posttreatment incubation produced mortality in codling moth adults and neonate larvae that was comparable to that reported by Lacey and Neven (2006) and Lacey et al. (2008) for potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). Fumigation with *M. albus* during the initial few days of storage at relatively warm temperatures (24°C) followed by good ventilation may be adequate to control adults and neonate larvae, but longer periods of exposure would be required for infested apples and diapausing larvae.

Although 4- and 5-d exposures of adult codling moth were highly efficacious, the short life span and stress associated with handling and confinement also resulted in high mortality in controls. At the time postexposure incubation had concluded for the 3-, 4-, and 5-d exposures, the adults were 5, 6, and 7 d old, respectively. Even under optimal conditions, codling moth adults do not have an especially long life span. Untreated moths that were held in ventilated plastic cups provisioned with honey water in a walk-in incubator without prior confinement in the exposure chambers lived slightly longer than control moths in our study (L.A.L. et al. unpublished data). Howell (1981) reported longer life spans than those presented here when adults were kept under optimal conditions and provided with sucrose solution.

Fumigation of neonate larvae that were placed on apples and 5-d-old larvae within apples resulted in significantly higher mortality compared with control larvae. Considering the number of larvae per apple and incubation for 10–15 d, respectively, cannibalism could be responsible for the discrepancy between the number of entries into apples and the number of larvae found, especially in the controls with a high proportion of older instars. Hence, the missing larvae were eliminated from both treatments and controls for calculation of mortality. Ferro and Harwood (1973) concluded that the carrying capacity for 4-cm-diameter apples is ≈3 larvae per apple.

The primary cause of codling moth mortality, especially with the 3-d exposures of adults and neonate larvae with minimal buildup of CO_2, is clearly due to *M. albus.* However, the contributing effect of CO_2 cannot be ruled out for longer exposures. In contrast, the highest levels of CO_2 in our studies are still far below those used by other researchers for longer exposures to kill codling moth (Soderstrom et al. 1990, Cossentine et al. 2004). The specific VOCs or mixtures of volatiles responsible for insecticidal activity have not yet been identified, but the use of one or more of the most active moieties for insects would enable control of codling moth without the generation of CO_2.

In addition to the biocidal activity of the natural mixture of VOCs produced by *M. albus*, studies on the effect of individual *M. albus* VOCs and mixtures of volatiles on plant pathogens also have been conducted. Strobel et al. (2001) reported that the most effective class of inhibitory compounds was the esters, of which 1-butanol, 3-methyl-, acetate was the most active biologically. Ramin et al. (2005) observed the highest fungicidal and bactericidal activity was due to isobutyric acid and 2-methyl-1-butanol. Ezra et al. (2004) used proton transfer reaction-mass spectrometry to qualitatively and quantitatively measure a wide range of volatile emissions from *M. albus*, thus providing identification of more specific components to test against pest organisms including codling moth.

The results of fumigating diapausing codling moth larvae with *M. albus* VOCs were especially encourag-

ing due to their lower respiration relative to adults and active larvae. Diapausing larvae in fruit bins can be a problematic source of codling moth in storage and for introduction of codling moth into orchards (Higbee et al. 2001). One of the most effective methods for controlling codling moth larvae in cartons is fumigation with methyl bromide (Hansen et al. 2002), but in addition to the environmental and safety issues mentioned above, phytotoxicity also can be a concern (Drake et al. 1988). As alternatives to fumigation with methyl bromide and other broad-spectrum chemicals, less deleterious methods also have been investigated for treatment of fruit bins. Fumigation with CO_2 has been used to kill codling moth larvae, but very high concentrations and longer exposures are required to produce high levels of mortality. Soderstrom et al. (1990) observed 95% mortality in diapausing codling moth larvae after exposure to 60% CO_2 for 13–24 d, and Cossentine et al. (2004) reported >80% mortality when codling moth larvae were exposed to 40–60% CO_2 for >8 d. Neven and Rehfield (1995), Higbee et al. (2001) and Hansen et al. (2006) reported the effects of heat on diapausing codling moth survival. Lacey and Chauvin (1999), Cossentine et al. (2002), and Lacey et al. (2005) provided information on the efficacy of entomopathogenic nematodes for codling moth control in bins. *M. albus* would offer an additional alternative approach for control of diapausing codling moth larvae in fruit cartons and bins.

If *M. albus* is to be used as for codling moth control in stored or packed fruit, additional research to assess the effects of prolonged exposure at colder temperatures on efficacy of the fumigant is required. Lacey et al. (2008) observed decreased effectiveness of *M. albus* against potato tuberworm at 10 and 15°C. Research on the effect of temperature and dosage on the efficacy of *M. albus* for control of gray mold (*Botrytis cinerea*) on table grapes at 5 and 20°C was reported by Mlikota Gabler et al. (2006). The biofumigant was more active at the higher temperature, but higher dosages of the fungus and longer periods of exposure at 5°C still provided control. Schnabel and Mercier (2006) demonstrated protection of peaches in shipping cartons from brown rot caused by *Monilinia fructicola* in cold storage (1–2°C) by using an *M. albus* pad delivery system in bagged shipping cartons. They reported the importance of containment of *M. albus* VOCs for effective control under cold conditions. In addition to lower temperatures, the effect of fumigation in fruit cartons with *M. albus* for longer periods also warrants further research with codling moth.

Fumigation of apples for successful control of plant pathogenic fungi was reported by Ramin et al. (2007) and Schotsmans et al. (2008). Ramin et al. (2007) observed that a 24-h exposure to 0.5 g of *M. albus* culture per liter at 20°C controlled *B. cinerea*, *Penicillium expansum*, and *Sclerotinia sclerotiorum* and had no effect on fruit quality. However, the concentration of CO_2 generated by *M. albus* was not reported by Ramin et al. (2007) or Schotsmans et al. (2008). Other authors have observed deleterious effects of exposure to CO_2 depending on apple variety and storage conditions (Argenta et al. 2002, Fawbush et al. 2008). However, treatment of fruit with diphenylamine, an antioxidant used routinely to prevent superficial scald disorder, markedly reduced or eliminated fruit damage due to CO_2 (Fawbush et al. 2008). The effect of prolonged exposure to high concentrations of CO_2 and *M. albus* VOCs on apple quality in sealed cartons in shipping temperatures (2–7°C) has yet to be determined and warrants further investigation.

The systems approach to achieve quarantine security of exported apples and other fruit capitalizes on cumulative pest mortality from multiple control components (Jang and Moffitt 1994, Follett and Neven 2006). Fumigation with *M. albus* could provide an additional component in this strategy to prevent live codling moth from being exported. In addition to codling moth control, *M. albus* VOCs could contribute to the control of any plant pathogenic organisms of apple that happen to be present (Ramin et al. 2007, Schotsmans et al. 2008).

The primary objective for investigating *M. albus* as a biofumigant in our study was to determine its potential for preventing the shipment of infested fruit in sealed cartons. Compared with methyl bromide and other broad-spectrum fumigants, *M. albus* is relatively safe to use. Nevertheless, determination of nontarget and environmental effects of the biofumigant warrants investigation.

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References Cited

Argenta, L. C., X. T. Fan, and J. P. Mattheis. 2002. Responses of 'Fuji' apples to short and long duration exposure to elevated CO_2 concentration. Postharvest Biol. Technol. 24: 13–24.

Barnes, M. M. 1991. Tortricids in pome and stone fruits, codling moth occurrence, host race formation and damage, pp. 313–327. *In* L.P.S. van der Geest and H. H. Evenhuis [eds.], Tortricid pests: their biology, natural enemies, and control. Elsevier, Amsterdam, The Netherlands.

Beers, E. H., J. F. Brunner, M. J. Willett, and G. M. Warner. 1993. Orchard pest management: a resource book for the Pacific Northwest: Good Fruit Grower, Yakima, WA.

Cossentine, J. E., L. B. Jensen, and L. Moyls. 2002. Fruit bins washed with *Steinernema carpocapsae* (Rhabditida: Steinernematidae) to control *Cydia pomonella* (Lepidoptera: Tortricidae). Biocontrol Sci. Technol. 12: 251–258.

Cossentine, J. E., P. L. Sholberg, L.B.J. Jensen, K. E. Bedford, and T. C. Shephard. 2004. Fumigation of empty fruit bins with carbon dioxide to control diapausing codling moth larvae and *Penicillium expansum* Link. ex Thom spores. HortScience 39: 429–432.

Drake, S. R., H. R. Moffitt, J. K. Fellman, and C. R. Sell. 1988. Apple quality as influenced by fumigation with methyl bromide. J. Food Sci. 53: 1710–1712.

Ezra, D., J. Jasper, T. Rogers, B. Knighton, E. Grimsrud, and G. Strobel. 2004. Proton transfer reaction-mass spectrometry as a technique to measure volatile emissions of *Muscodor albus.* Plant Sci. 166: 1471–1477.

Fawbush, F., J. F. Nock, and C. B. Watkins. 2008. External carbon dioxide injury and 1-methylcyclopropene (1-MCP) in the 'Empire' apple. Postharvest Biol. Technol. 48: 92–98.

Ferro, D. N., and R. F. Harwood. 1973. Intraspecific larval competition by the codling moth, *Laspeyresia pomonella.* Environ. Entomol. 2:783–789.

Follett, P., and L. G. Neven. 2006. Current trends in quarantine entomology. Annu. Rev. Entomol. 51: 359–385.

Grimme, E., N. K. Zidack, R. A. Sikora, G. A. Strobel, and B. J. Jacobsen. 2007. Comparison of *Muscodor albus* volatiles with a biorational mixture for control of seedling diseases of sugarbeet and root-knot nematode on tomato. Plant Dis. 91: 220–225.

Hansen, J. D., D. J. Albano, and M. L. Heidt. 2002. Efficacy of using in-carton fumigation with the quarantine treatment against codling moth on apples intended for export to Japan. HortTechnology 12: 441–443.

Hansen, J. D., and P. A. Anderson. 2006. Mass rearing codling moths: improvements and modifications. J. Entomol. Soc. Br. Columbia 103: 33–36.

Hansen, J. D., M. L. Heidt, and P. A. Anderson. 2006. Bin sterilization to prevent reintroduction of codling moth. J. Agric. Urban Entomol. 23: 17–26.

Higbee, B., C. Calkins, and C. Temple. 2001. Overwintering of codling moth (Lepidoptera: Tortricidae) larvae in apple harvest bins and subsequent moth emergence. J. Econ. Entomol. 94: 1511–1517.

Howell, J. F. 1981. Codling moth: the effect of adult diet on longevity, fecundity, fertility, and mating. J. Econ. Entomol. 74: 13–18.

Jang, E. B., and H. R. Moffitt. 1994. Systems approaches to achieving quarantine security, pp. 225–237. *In* J. L. Sharp and G. J. Hallman [eds.], Quarantine treatments for pests and food plants. Westview Press, Boulder, CO.

Lacey, L. A., and R. L. Chauvin. 1999. Entomopathogenic nematodes for control of codling moth in fruit bins. J. Econ. Entomol. 92: 104–109.

Lacey, L. A., and L. G. Neven. 2006. The potential of the fungus, *Muscodor albus* as a microbial control agent of potato tuber moth (Lepidoptera: Gelechiidae) in stored potatoes. J. Invertebr. Pathol. 91: 195–198.

Lacey L. A., L. G. Neven, H. L. Headrick, and R. Fritts, Jr. 2005. Factors affecting entomopathogenic nematodes (Steinernematidae) for control of overwintering codling moth (Lepidoptera: Tortricidae) in fruit bins. J. Econ. Entomol. 98: 1863–1869.

Lacey, L. A., D. R. Horton, and D. C. Jones. 2008. The effect of temperature and duration of exposure of potato tuber moth (Lepidoptera: Gelechiidae) in infested tubers to the biofumigant fungus *Muscodor albus.* J. Invertebr. Pathol. 97: 159–164.

Mercier, J., and J. I. Jiménez. 2004. Control of fungal decay of apples and peaches by the biofumigant fungus *Muscodor albus.* Postharvest Biol. Technol. 31: 1–8.

Mercier, J., and J. L. Smilanick. 2005. Control of green mold and sour rot of stored lemon by biofumigation with *Muscodor albus.* Biol. Control 32: 401–407.

Mlikota Gabler, F., R. Fassel, J. Mercier, and J. L. Smilanick. 2006. Influence of temperature, inoculation interval, and dosage on biofumigation with *Muscodor albus* to control postharvest grey mold on grapes. Plant Dis. 90: 1019–1025.

Neven, L. G., and L. M. Rehfield. 1995. Comparison of prestorage heat treatments on fifth-instar codling moth (Lepidoptera: Tortricidae) mortality. J. Econ. Entomol. 88: 1371–1375.

Northwest Horticultural Council [NWHC]. 2008. Northwest Horticultural Council export manual. (http://www.nwhort.org/).

Ramin, A. 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.

Ramin, A. A., R. K. Prange, P. G. Braun, and J. M. DeLong. 2007. Biofumigation of postharvest fungal apple decay with *Muscodor albus* volatiles. J. Appl. Sci. Res. 3: 307–310.

Reyes, M., P. Franck, P. J. Charmillot, C. Ioriatti, J. Olivares, E. Pasqualini, and B. Sauphanor. 2007. Diversity of insecticide resistance mechanisms and spectrum in European populations of the codling moth, *Cydia pomonella.* Pest Manage. Sci. 63: 890–902.

Riga, K., L. A. Lacey, and N. Guerra. 2008. The potential of the endophytic fungus, *Muscodor albus,* as a biocontrol agent against economically important plant parasitic nematodes of vegetable crops in Washington State. Biol. Control. 45: 380–385.

SAS Institute. 2002. SAS 9.1 for Windows. SAS Institute, Cary, NC.

Schnabel, G., and J. Mercier. 2006. Use of *Muscodor albus* pad delivery system for the management of brown rot of peach in shipping cartons. Postharvest Biol. Technol. 42: 121–123.

Schotsmans, W. C., G. Braun, J. M. DeLong, and R. K. Prange. 2008. Temperature and controlled atmosphere effects on efficacy of *Muscodor albus* as a biofumigant. Biol. Control 44: 101–110.

Soderstrom, E. I., D. G. Brandl, and B. B. Mackey. 1990. Responses of codling moth (Lepidoptera: Tortricidae) life stages to high carbon dioxide or low oxygen atmospheres. J. Econ. Entomol. 83: 472–475.

Strobel, G. 2006. *Muscodor albus* and its biological promise. J. Ind. Microlbiol. Biotechnol. 33: 514–522.

Strobel, G. A., E. Dirkse, J. Sears, and C. Markworth. 2001. Volatile antimicrobials from *Muscodor albus,* a novel endophytic fungus. Microbiol. Read. 147: 2943–2950.

Thomas, W. B. 1996. Methyl bromide: effective pest management tool and environmental threat. Suppl. J. Nematol. 28: 586–589.

Toba, H. H., and J. F. Howell. 1991. An improved system for mass-rearing codling moths. J. Entomol. Soc. Br. Columbia 88: 22–27.

United Nations Environment Programme [UNEP]. 2008. Environmental effects of ozone depletion and its interactions with climate change: progress report, 2007. United Nations Environment Programme, Environmental Effects Assessment Panel. Photochem. Photobiol. Sci. 7: 15–27.

Worapong, J., G. Strobel, E. J. Ford, J. Y. Li, G. Baird, and W. M. 2001. *Muscodor albus* anam. gen. et sp. nov., an endophyte from *Cinnamomum zeylanicum.* Mycotaxon 79: 67–79.

Yagi, K., J. Williams, N.-Y. Wang, and R. J. Cicerone. 1995. Atmospheric methyl bromide (CH_3Br) from agricultural soil fumigations. Science (Wash., D.C.) 267: 1979–1981.

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