EFFICACY OF PROPYLENE OXIDE IN COMBINATION WITH CARBON DIOXIDE AGAINST EGGS OF POSTHARVEST INSECT PESTS AT NORMAL ATMOSPHERIC PRESSURE

S. Walse[1], S. G. Gautam[2], G. P. Opit[2] , J. S. Tebbets[1]

[1]USDA ARS San Joaquin Valley Agricultural Sciences Center, Parlier, CA, 93648, U.S.A.

[2]Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK, 74078, U.S.A.

Abstract

Postharvest chamber fumigation is a critical element of the California specialty crop industry to guarantee pest-free security and food safety of agricultural products. After the regulated phaseout of methyl bromide (MeBr), sulfuryl fluoride (SF) is now considered an important postharvest alternative when control is required within hours or days of harvesting. In fact, SF has already filled some of the void created by the regulatory phaseout of MeBr for the dried fruit and nut industries. However, species-specific ovicidal deficiencies of SF at the label dose are evident and some key California pests may require several days of exposure for effective control. Given the rapid disinfestation requirements and constraints imposed by SF label dose, this work was conducted in the context of overcoming ovicidal deficiencies of SF by blending it with propylene oxide (PPO). Dose-response relationships of *Carpophilus hemipterus* (L.), *Tribolium castaneum* (Herbst), *Lasioderma serricorne* (F.), *Amyelois transitella* (Walker), *Ephestia elutella* (Hübner), and *Plodia interpunctella* (Hübner) eggs to PPO in combination with carbon dioxide (PPO:CO_2 – 8:92) were established at normal atmospheric pressure (NAP). Fumigations were conducted at normal atmospheric pressure and 25°C for 24 h. LD_{99} values (concentration x time, mgh/liter[1]) for *C. hemipterus*, *T. castaneum*, *L. serricorne*, *A. transitella*, *E. elutella*, and *P. interpunctella* were determined as 414.8, 348.6, 113.1, 133.1, 104.7, and 95.3 mgh/liter, respectively. Corresponding LC_{99} (mg/liter) were 17.3, 14.5, 4.7, 5.6, 4.4, and 4.0 mg/liter, respectively. These toxicity data represent a critical initial step in formulating a SF:PPO blend to meet postharvest disinfestation requirements of the California dried fruit and tree nut industry. Future research will quantify the sorption, residue levels, and field testing of the blend.

Keywords: Methyl bromide, sulfuryl fluoride, rapid disinfestation, propylene oxide, postharvest

1. Introduction

Postharvest chamber fumigation is a critical element of the ≈$18 billion/yr. California specialty crop industry. The Central Valley of California accounts for nearly all the dried fruits and nuts produced in the United States totaling >2,000,000 metric tons of commodities (USDA ERS, 2013). However, several postharvest and microbiological pests can seriously affect production, food safety, and subsequently profits of these high value commodities. Therefore, California's dried fruits and nuts industries implement commodity specific postharvest pest management strategies to prevent crop loss and to provide pest free security (Schneider et al., 2003 and Johnson et al., 2012). This work was conducted in the context of overcoming ovicidal deficiencies of postharvest fumigants in general and particularly for those in the California walnut industry that use sulfuryl fluoride (SF) for postharvest disinfestation at normal atmospheric pressure (NAP). California is home to nearly all US-produced walnuts and walnut production in 2012 was valued at $1.34 billion out of which $1.1 billion of this comprised exports. In-shell and shelled walnuts exported during this period were valued at $466 and 646 million, respectively (NASS, 2013 and USDA ERS, 2013). Several insect pests infest walnuts in the field and in storage, among which *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) and *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) are of key concern because they are quarantine pests in several countries that import in-shell walnuts. These field pests infest in the field and are brought to storage where they continue to feed and cause additional damage (Johnson et al., 2009 and Burks and Johnson 2012). *Carpophilus hemipterus* (L.) (Coleoptera: Nitidulidae) is another key field pest of dried fruits and nuts. Pests of concern during storage are *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), and *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) (Johnson et al., 2009 and Burks and Johnson, 2012). Whereas field pests can be controlled by initial disinfestation following harvest, stored-product insect pests may require repeated disinfestations or long-term preventive control measures because of their ability to cause repeated infestations (Johnson et al., 2009).

Methyl bromide (MeBr), which had been a standard fumigant for more than 50 yr. is no longer available for postharvest disinfestation of walnuts, even under critical use exemptions after 2014 (US EPA, 2014). The industry requires high throughput fumigation to accommodate large volumes of walnuts harvested during the peak of harvest when disinfestation needs to be fast. Coincident with the phaseout of MeBr, the walnut industry transitioned to using sulfuryl fluoride (SF) for field disinfestation. Sulfuryl fluoride is considered a replacement for MeBr because of its comparable application costs, penetration ability, low reactivity potential, and non-combustible nature. However, a drawback to using SF at the recommended label rate, i.e., 1,500 mg h/liter at normal atmospheric pressure (NAP) is that it requires much higher concentrations or several days of exposure to kill eggs of some insect pests (UNEP, 2011). Therefore, ways to circumvent ovicidal deficiencies of SF during postharvest fumigations need to be found. One possible solution is blending SF with another fumigant that is highly effective against eggs.

Propylene oxide (PPO) is a logical choice for blending with SF because it is a FDA-approved sterilant for pasteurizing nuts. It has a very low environmental risk compared to MeBr because it does not deplete ozone, is degraded into nontoxic propylene glycol in soil and in the human stomach (Griffith, 1999). Fumigation studies have shown that PPO is an effective ovicide (Isikber et al., 2004, Navarro et al., 2004 and Isikber et al., 2006). In fact, a 1:1 combination of PPO and SF has been shown to be effective against all life stages of *T. castaneum* (Muhareb et al., 2009). Its major disadvantage is flammability, which can be

reduced by combining it with CO_2 (8:92 – PPO:CO_2) at NAP or fumigating at low pressure (Navarro et al. 2004). Evaluating efficacy of PPO to establish its toxicity data against eggs of different walnut pests at NAP is a first step in formulating a blend of SF:PPO for postharvest disinfestation at NAP. Therefore, the objective of this study was to establish the response of *C. hemipterus*, *T. castaneum*, *L. serricorne*, *E. elutella*, *A. transitella*, *P. interpunctella*, *C. pomonella*, and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) eggs to PPO in combination with CO_2 at NAP.

2. Materials and Methods

2.1.Insects

Eggs of eight insect pest species, namely, *C. hemipterus*, *T. castaneum*, *L. serricorne*, *P. interpunctella*, *E. elutella*, *A. transitella*, *C. pomonella*, and *E. ceratoniae* were used for the experiment. All eggs required for the experiment were obtained from an insectary at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), San Joaquin Valley Agricultural Sciences Center (SJVASC), Parlier, CA. Rearing conditions for all species were 27 ± 1°C, 60 ± 5% RH, and 16:8 (L:D) h.

Freshly laid *C. hemipterus*, *A. transitella*, *E. elutella*, and *P. interpunctella* eggs used for fumigation were collected using the procedure described by Gautam et al. (2014). Eggs of *T. castaneum*, *L. serricorne*, *E. ceratoniae*, and *C. pomonella* were obtained using the procedure described by Gautam (2013). All eggs used for fumigation except those of *C. hemipterus* were 0 to 72 h old. Because the incubation period of *C. hemipterus* eggs is <48 h at 25°C (James and Vogele, 2003), we used 0- to 18-h-old eggs for fumigation. The setup containing eggs for fumigation for each species were prepared using the procedure described by Gautam (2013). For all insect species, each setup had at least 200-300 eggs.

2.2.Fumigation Chambers

Chambers used for fumigation were modified 28.3-liter Labconco® vacuum desiccators (Labconco® # 5530000) (referred to as chambers hereafter). These were part of a multi-chamber manifold system that can support up to twelve 28.3-liter fiberglass chambers (Tebbets, 2009). The entire system was housed inside a stainless steel room (17 x 17 x 17 m) with temperature and relative humidity controls (SJVASC fumigation facility; Tebbets 2009).

2.3.Fumigant

Propylene Oxide. Pure liquid propylene oxide (≥ 99.5%; #82320 Aldrich, Sigma-Aldrich Co. St. Louis, MO) was drawn from a 50-ml conical flask with a glass stopper under a certified fume hood using a 0.1-ml gas syringe (Hamilton, Foxboro/Analabs, North Haven, CT) or 1-, 2-, or 5-ml gas syringes (Precision syringe, Dynatech Precision Sampling, Baton Rouge, LA) befitting the applied dosages. The syringes were equipped with a small bore 5-cm long hypothermic needle. Carbon dioxide was drawn from a cylinder (13.4 x 45.7 cm) containing compressed CO_2 using 500-, 1,000-, or 1,500-ml gas syringes. The required volume of CO_2 was obtained from the cylinder through a regulator that controlled flow of gas to a tube that was connected to a syringe of appropriate size.

2.4.Fumigation

In the determination of dose-response of *C. hemipterus*, *T. castaneum*, *L. serricorne*, *A. transitella*, *E. elutella*, and *P. interpunctella* eggs to PPO, the range of PPO concentrations tested were 6-20, 4-18, 2.75-6.0, 1.5-6.0, 1.5-6.0, and 1.5-6.0 mg/liter, respectively. Responses of *C. pomonella* and *E. ceratoniae* eggs to PPO were determined using 7.94 and 11.18 mg/liter of PPO. Preliminary range finder experiments had determined these values as LC_{50} and LC_{90}, respectively, for *T. castaneum* eggs. There were no survivors at 7.94 mg/liter; therefore, further tests were not conducted for these two species. Conditions to simulate 8:92 mixture of PPO:CO_2 were created by injecting a calculated volume of CO_2 equivalent to that

which would have been administered into each chamber in a scenario where the gas introduced was premixed PPO:CO_2 (8:92 mixture). Prior to injecting liquid PPO into the chambers, a pressure of 250 mmHg was created. This ensured space for CO_2 and for PPO volatilization while preventing the development of positive pressure in the entire chamber. Subsequently, pre-calculated volumes of liquid PPO were then injected into the chambers, through rubber septa, using syringes of different sizes, to attain desired concentrations. Liquid PPO was injected onto a filter paper on an open 10-cm glass Petri dish that sat on top of an inverted glass jar that volatilized within 2-5 min. After the PPO volatilized, the pressure inside the chamber was adjusted to normal by permitting air in from the stopcock. This marked the start of the 24-h fumigation exposure period.

The concentrations of PPO inside each chamber were determined after the pressure was normalized at the start of each fumigation (0 h) and after 2 and 24 h. Gas samples were taken using a 100-ml gas syringe (Becton, Dickinson and Co., Franklin Lakes, NJ) by withdrawing 40 ml of gas through the stopcock. Concentrations of PPO in fumigation chambers were quantitatively monitored and analyzed using a gas chromatograph (GC) (Model 3800, Varian Inc., Walnut Creek, CA). Concentrations of CO_2 were monitored after 0, 2, and 24 h of fumigation using an oxygen/carbon dioxide analyzer (Model 902D, Quantek Instruments, Inc., Grafton, MA). Doses of PPO, expressed as concentration x time (CT) product (mg h/liter; hereafter referred to as lethal doses or LDs) were calculated by the method of Bond (1984). All tests were conducted at 25°C. Immediately after the 24-h gas sampling, chambers were aerated for 20-30 min. Egg setups were then transferred to a chamber maintained at 27 ± 1°C and 65 ± 5% RH and incubated.

2.5.Data Analysis

The experimental design was a randomized complete block design with three temporal replications. Responses of eggs of the respective species tested to PPO:CO_2 were subjected to probit analysis using PoloPlus (Leora Software, Petaluma, CA) to determine the LD_{50}, LD_{95}, and LD_{99} values and their 95% confidence intervals (CIs). Differences in toxicity were considered significant when 95% CIs did not overlap. A ratio test to compare LDs was also conducted (Robertson et al., 2007). LD_{50}, LD_{95}, and LD_{99} values of *C. hemipterus*, *T. castaneum*, *L. serricorne*, *E. elutella*, and *A. transitella* eggs were compared with those of *P. interpunctella* eggs that were consistently the lowest. Because *C. pomonella* and *E. ceratoniae* eggs did not survive PPO concentrations of 7.94 and 11.18 mg/liter, data analyses were not conducted.

3. Results and Discussion

Propylene oxide in combination with CO_2 was toxic to eggs of all the species tested. Probit mortality data for PPO:CO_2 at NAP against eggs of *C. hemipterus*, *T. castaneum*, *L. serricorne*, *E. elutella*, *A. transitella*, and *P. interpunctella* are presented in Table 1. LD_{99} toxicities for eggs of the six species ranged from 95.3-414.8 mg h/liter (Table 1). In general, lower doses of PPO were required to attain the same level of mortality for lepidopteran eggs compared to coleopteran eggs; *L. serricorne* was the exception. LD_{99} values for *P. interpunctella*, *E. elutella*, and *A. transitella* eggs were 95.3, 104.7, and 133.1 mg h/liter, respectively; these values were lower compared with that required for *T. castaneum* and *C. hemipterus* eggs, which were 348.6 and 414.8 mg h/liter, respectively (Table 1).

Interestingly, the LD_{99} for eggs of *L. serricorne*, a coleopteran species, was relatively low (113.1 mg h/liter) and not different from LD_{99} values of the lepidopteran species (Table 1). Based on LD_{50} or LD_{99} comparisons, eggs of *C. hemipterus* and *T. castaneum* required a significantly higher PPO dose to kill than eggs of other species. LD_{50} values of *L. serricorne*

were higher than those required for *A. transitella*, *E. elutella*, and *P. interpunctella*. But LD_{99} values of *L. serricorne* were not different than LD_{99} values for *E. elutella* and *A. transitella*. Based on LD_{50} values from this study, the tolerance of species tested to PPO in decreasing order was *C. hemipterus* ≥ *T. castaneum* > *L. serricorne* > *A. transitella* ≥ *E. elutella* ≥ *P. interpunctella* (Table 1). LD_{50}, LD_{95}, and LD_{99} values of all other species were compared with those of *P. interpunctella* to determine the degree (level) of their tolerance to PPO relative to those of the most susceptible species (Table 2).

Propylene oxide LC_{99} values for the most tolerant *C. hemipterus* eggs and most susceptible *P. interpunctella* eggs were 17.3 and 4.0 mg/liter, respectively. These LC_{99} values are much lower than the FDA recommended PPO concentration for pasteurization of walnuts. For pasteurization of walnuts, 840 mg/liter of PPO applied for a 4-h period, at temperatures of 41-45.6°C is recommended (California Walnut Board, 2012). Because PPO LC_{99} values that are effective against all the species tested are much lower than that recommended for walnut pasteurization implies that the PPO and CO_2 (8:92) mixture has potential to be used as a fumigant to blend with SF in order to achieve high egg mortality during rapid field disinfestations of walnuts at NAP. The SF:PPO blend to be developed should be effective against eggs of all field and stored-product pests. Although statistically similar, concentrations required to kill *C. hemipterus* eggs were numerically higher than those required for *T. castaneum*. LC_{99} values for *C. hemipterus* and *T. castaneum* were 14.5 and 17.3 mg/liter, respectively. Based on these data, investigating the effectiveness of ≈18 mg/liter of PPO, in a PPO and CO_2 (8:92) mixture, applied at NAP for 24 h, against postembryonic stages of eight species evaluated in this study is recommended. This would establish the feasibility of blending 18 mg/liter of PPO with SF for the control of all stages of the aforementioned eight postharvest pests at NAP. In addition, further studies on in-shell walnuts to determine PPO sorption based on fumigation using 18 mg/liter for 24 h at NAP are recommended. This study will determine the compensatory increase in PPO as a result of sorption to maintain a headspace concentration of 18 mg/liter PPO during fumigation. Prior studies have shown that walnuts absorb up to 97% of PPO during 48-h fumigation using mixture of PPO and CO_2 (Zettler et al., 2002). Consequently levels of potential residues, namely, PPO and propylene chlorohydrins (PCH) and propylene bromohydrins (PBH) need to be determined for commercial use of PPO for postharvest disinfestations of insects. Furthermore, sorption and residue data for shelled walnuts would be needed to formulate a blend of SF:PPO to be used for fumigating shelled walnuts in storage.

SF dose response studies at NAP have shown that ≈ 8 mg/liter of this fumigant applied for 24 h will result in ≥ 99% mortality of all postembryonic stages of *C. hemipterus*, *T. castaneum*, *P. interpunctella*, *E. elutella*, *L. serricorne*, and *A. transitella* including diapausing larvae of *C. pomonella* (Walse, 2012; Su and Scheffrahn, 1990; Leesch and Zettler, 2000; Zettler and Arthur, 2000; Baltaci et al., 2009). Therefore, it seems logical that a SF:PPO blend composed of 8 mg/liter of SF and 18 mg/liter of PPO, in a PPO and CO_2 (8:92) mixture, applied for 24 h at NAP needs to be tested for its efficacy against all stages of *C. hemipterus*, *T. castaneum*, *L. serricorne*, *A. transitella*, *E. elutella*, *C. pomonella*, *E. ceratoniae*, and *P. interpunctella*. Given that these insect species infest other dried fruits and nuts besides walnuts, the PPO efficacy data this study has generated could be used to explore ways to disinfest these commodities as well. For example, the date industry uses MeBr under CUE for field disinfestations of freshly harvested dates that need to be shipped to markets promptly. This is because dates have to be shipped to markets within 3 d of harvest in order to fetch a premium price and because SF cannot kill eggs of *C. hemipterus* and *E. ceratoniae* at the label rate. Based on results from the current study, the concentration of PPO recommended for the SF:PPO blend at NAP can control eggs of *C. hemipterus* and *E. ceratoniae*, two key field

pests of dates. Therefore, the recommended blend of SF:PPO at NAP has a potential to be a postharvest alternative to MeBr for rapid disinfestations of other dried fruits and nuts commodities as well.

4. Conclusions

This study has provided toxicity data of eggs of six stored-product species, namely, *C. hemipterus*, *T. castaneum*, *L. serricorne*, *A. transitella*, *E. elutella*, and *P. interpunctella* treated with a PPO and CO_2 (8:92) mixture. Based on these data, it is suggested that studies be conducted using a SF:PPO blend composed of 18 mg/liter of PPO (in a PPO and CO_2 (8:92) mixture) and 8 mg/liter of SF applied for 24 h at NAP to determine its efficacy against all life stages of above mentioned insect pests. Future studies will be aimed at collecting data on sorption and levels of residues of PPO, PCH, and PBH by various commodities to develop commodity specific SF:PPO blends for postharvest treatments. The SF:PPO blend recommended for testing based on the results of current study represents an initial but important step in finding an alternative to MeBr for postharvest disinfestation of walnuts at NAP.

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Table 1. Probit analyses of mortality for eggs of *P. interpunctella*, *E. elutella*, *A. transitella*, *L. serricorne*, *T. castaneum*, and *C. hemipterus* after exposure to a mixture of propylene oxide and carbon dioxide (8:92) at 25°C and normal atmospheric pressure for 24 h. Lethal doses are concentration x time (CT) (mg h/liter) products.

Species	Slope ± SE	LD_{50}	LD_{95}	LD_{99}	χ^2
			(95% CI)		
P. interpunctella	7.7 ± 0.3	47.4 (45.1-49.4)c*	77.7 (73.3-83.5)c	95.3 (88.1-105.5)c	19.8
E. elutella	7.2 ± 0.3	49.7 (46.6-52.5)c	84.2 (78.9-91.4)c	104.7 (95.9-117.9)bc	21.6
A. transitella	5.6 ± 0.2	51.2 (47.5-54.6)c	100.6 (95.6-115.9)b	133.1 (115.5-162.1)b	60.4
L. serricorne	11.6 ± 0.3	71.2 (68.9-73.4)b	98.7 (94.6-104.6)b	113.1 (106.5-122.2)b	36.6
T. castaneum	7.8 ± 0.4	174.8 (133.8-195.5)a	284.8 (251.9-389.3)a	348.6 (291.7-581.4)a	60.5
C. hemipterus	7.0 ± 0.2	193.4 (180.6-205.3)a	331.7 (304.3-373.1)a	414.8 (369.4-488.7)a	49.7

*LD values within a column followed by different letters are significantly different based on overlap of 95% CI.

Table 2. Comparisons of lethal doses (concentration x time) required to kill 50, 95, or 99% of *P. interpunctella* eggs* to those required to kill eggs of *E. elutella*, *A. transitella*, *L. serricorne*, *T. castaneum*, and *C. hemipterus*.

		Lethal dose ratios	
Species	LD_{50} (95% CI)	LD_{95} (95% CI)	LD_{99} (99% CI)
E. elutella	1.05 (1.02-1.08)	1.84 (1.04-1.13)	1.09 (1.04-1.17)
A. transitella	1.08 (1.05-1.11)	1.29 (1.24-1.35)	1.39 (1.31-1.49)
L. serricorne	1.50 (1.47-1.54)	1.27 (1.23-1.32)	1.00 (0.92-1.08)
T. castaneum	3.69 (3.55-3.84)	3.67 (3.50-3.85)	3.66 (3.41-3.92)
C. hemipterus	4.09 (3.98-4.19)	4.27 (4.11-4.44)	4.35 (4.13-4.89)

*Because *P. interpunctella* eggs had consistently lower LD values indicating it was the most susceptible, lethal doses of other species were compared to those of *P. interpunctella.*