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Postharvest treatment research at USDA-ARS: stored product fumigation

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Abstract

The overall goal of this USDA-ARS research is to ensure the protection and quality of stored product foodstuffs. The results of this research directly enhance production, distribution, and safety of foodstuffs, promote and retain access of United States-grown crops to domestic and foreign markets, and protect the United States and trading partners from the agricultural, ecological and economic threat posed by quarantine and invasive pests. In general, USDA-ARS research related to the fumigation of stored products focuses on the development of techniques to rapidly disinfest raw products of field pests, control pests in processed products amenable to re-infestation and microbial infection, and reduce reliance on fumigation as a stand-alone measure for postharvest disinfestations and disinfections. Specific research objectives include: comparative evaluation of alternative fumigants to methyl bromide in postharvest applications, development of novel technologies to reduce and eliminate atmospheric emissions from chambers used in postharvest fumigation, and design production strategies that allow for a more strategic postharvest use of methyl bromide and alternative fumigants. Recent research findings will be presented and discussed, including: exposure requirements of phosphine on key stored product pests (as related to resistance management), the establishment of efficacy and experimental criterion for quarantine applications, and the development of models to quantitatively understand the underpinnings of fumigations and related phytosanitary treatments.

Keywords: food security, food safety, quarantine treatments, postharvest methyl bromide

1. Introduction

The use of postharvest phosphine fumigation as a quarantine phytosanitary requirement is increasing coincident with the globalization of agriculture. However, operational and regulatory framework for implementing and certifying efficacious treatments have not bee firmly established. In this work we describe a postharvest fumigation with phosphine to control Warehouse beetle, *Trogoderma variable* (Ballion) (Coleoptera, Dermestidae), a pest of concern to certain countries that import Dried Distillers Grains (DDGs) from USA. A series of laboratory-scale exploratory fumigations with phosphine at 10.0 ± 0.3 ºC ($\bar{x} \pm 2s$) were conducted to evaluate the postharvest control of

eggs as well as diapausing larvae of *T. variable*, the most phosphine-tolerant life stages of this pest. Models of the duration-mortality response predicted >99% mortality when headspace concentrations of phosphine, [PH3], are maintained at levels ≥ 0.8 mgL^{-1} (500 ppmv) and ≤ 1.5 mgL^{-1} (1000 ppmv) for ≥ 120 h, as estimated by the lower boundary limit of the 95% confidence interval. A fumigation schedule is proposed based on the results of this research and the seminal studies of Vincent and Lindgren (1975) as well as Banks and Cavanaugh (1985). Data is presented and discussed in the context of controlling *Trogoderma variable* following commercial fumigations for export of DDGs.

2. Materials and Methods

Insects and egg collection

Specimens were cultured in the insectary at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), San Joaquin Valley Agricultural Sciences Center, Parlier, CA (USDA, 2012). Cultures were housed in an 15.2-m^3 rearing unit maintained at 27 ± 1°C ($\bar{X} \pm s$) and 60 ± 5% RH ($\bar{X} \pm s$) with a photoperiod of 16:8 (L:D) h, unless otherwise noted. Rearing procedures and diets, briefly mentioned below, were as reported in the Crop Protection and Quality Research Unit electronic rearing manual (USDA, 2012). Methods for collection of eggs and diapausing larvae are detailed below.

Warehouse beetle (WHB), *Trogoderma variable* (Ballion) (Coleoptera, Dermestidae), was originally collected in 1967 from whereabouts unknown in Fresno County, California USA. WHB adults (200 to 300) were transferred to a 946-mL glass jar filled with 20 to 25 g of a dried dog food substrate. The jar was sealed with filter paper (Whatman® #1, 90-mm diameter) followed by a wire screen (U.S. #40 mesh, 90-mm diameter) and both were secured a top the jar with a threaded metal ring. The jar was transferred to the rearing unit for a 72-h ovipositional period, after which, eggs were separated from the adults and flour using a stack of sieves (Seedburo Equipment Company, Des Plaines, IL). The contents of the jar were poured into the top sieve (U.S. #25, 0.71 mm^2 openings) and shaken vigorously for a few minutes. The eggs were retrieved from the underlying sieve (U.S. #60, 0.25 mm^2 openings) by decanting them into a glass Petri dish (100 mm diameter x 15 mm h). Counting eggs required for treatments was performed under the microscope by transferring small amount of eggs into a trough made out of black construction paper. Using a small, horsehair brush, ca. 100 eggs were transferred onto black velour paper that lined the inside of a 35-mm diameter Petri dish (Falcon, Oxnard, CA). Wheat bran diet (5 g), prepared as described in the rearing manual (USDA, 2007), was placed in each of several 10-cm diameter plastic Petri-dish cages. The diet was spread concentrically to the outer edge of each cage bottom and a single 35-mm Petri dish, containing the < 72-h old eggs, was placed in the center of the void. After fumigation treatment, or concomitant use as non-treated control specimens, the Petri-dish cages were lidded.

As described in Banks and Cavanaugh (1985), larvae known to be in diapause were obtained by isolating single larvae taken from stock cultures. Each larvae was introduced, along with 0.5 g of diet, 7-dram clear plastic "snap cap" cages modified with 8-mm diameter stainless-steel 100 wire mesh gas-portals on the bottom, snap cap, and side. The cages were incubated at 30 ± 1°C ($\bar{X} \pm s$) and 60 ± 5% RH ($\bar{X} \pm s$) with a photoperiod of 16:8 (L:D). Larvae that had not metamorphosed after 6 wk were considered to be in induced diapause, as described by Burges (1961, 1965).

Chemical Analysis and calibration of standards

Cytec Canada, Inc. (Niagara Falls, Ontario, Canada) provided the 300-lb cylinders of 1.6 % (v/v) phosphine balanced with nitrogen. A source cylinder (300-lb) of breathing air was obtained from Airgas (Fresno, CA, USA). The 1.6% PH3 mixture was used as the source for gas chromatography calibrations and the exploratory fumigations. [PH3] and steady-state concentrations thereof, $[PH3]_{ss}$, were measured using gas chromatography (GC); retention time (PH3, t_r = 3.2 ± 0.2 min, $\bar{X}$

$\pm s$, n = 10) was used for chemical verification and the integral of peak area, referenced relative to liner least-squares analysis of a 5-point concentration – detector response curve, was used to determine concentration. Detector response was determined by diluting known volumes of gases into volumetric gas vessels. A response curve was generated respective to each sampling interval with each sample referenced to the response. [PH3] levels were reported as average (±) standard deviation ($\overline{X} \pm s$) from duplicate measurements (vide infra). Analyses were with a Varian 3800 and splitless injection (140 °C) using a gas sampling port with a 10 μL-sample loop, a Teflon column (L = 2 m, OD = 2 mm) packed with Porpak N (80/100 mesh) held at 130 °C for 10 min, and a pulsed flame photometric detector (PFPD) detector (13 mL/min H_2, 20 mL/min air, and 10.0 mL/min N_2 make-up) at 250 °C that received only 10% of the 15 ml He/min column flow.

Exploratory fumigations

Laboratory-scale exploratory fumigations were conducted in a matching set of 24 Labonco® 28.32-L vacuum chambers housed in a walk-in environmental room with programmable temperature and humidity (USDA, 2010). Temperature and humidity set-points were 10.0 °C and 80% RH, respectively. A series of exploratory experiments was used to determine the treatment duration, ranging from 24 to 168 h, required to control larvae with applied doses, and subsequently, steady-state concentrations of phosphine in chamber headspace (i.e, $[PH3]_{ss}$) of ca. 250 (0.4), 500 (0.8), 1000 (1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}) phosphine, respectively. Each of five chambers was loaded with an egg dish; four of the chambers were, respectively, subject to the phosphine treatments above and the fifth was not fumigated to yield non-treated control specimens. In addition, the control chamber and the chamber treated with 1000 ppmv (μLL^{-1}) (1.5 mgL^{-1}) $[PH3]_{ss}$ were loaded with 30 caged larvae. Each "block" of five chambers was subject to treatment durations of 24, 48, 72, 96, 120, 144, and 168 h. Each "block" was conducted in triplicate, which yielded a total of ca. 300 eggs at each $[PH3]_{ss}$ and each treatment duration, as well as 90 larval specimens treated with 1000 ppmv (μLL^{-1}) (1.5 mgL^{-1}) $[PH3]_{ss}$ at each treatment duration. This design corresponded to a total of 630 and ca. 2100 control larvae and eggs, respectively.

Loaded chambers, 300-lb source cylinders of breathing air (Airgas, Fresno, CA, USA) as well as 1.6 % (v/v) phosphine balanced with nitrogen, and gas-tight syringes were acclimated to fumigation temperature (i.e., tempered) within the walk-in environmental room for at least 24 h prior to treatment. Air temperature in the walk-in room was confirmed prior to fumigation by a HOBO data logger (HOBOware version 2.7). Chamber lids were then clamp-sealed in preparation for treatment. A slight vacuum of approximately 76-127 mmHg was established in each chamber. Gas-tight super-syringes (Hamilton ® 500, 1000, or 1500 mL) were filled with a volume of phosphine from the 300-lb source cylinder of 1.6 % (v/v) phosphine to achieve the requisite dose as predetermined in preliminary calibration studies. The syringe was fitted to a LuerLok ® sampling valve, which was subsequently opened so that fumigant was steadily drawn into the chamber. The syringe was then removed and normal atmospheric pressure (NAP) was reestablished; this marked the beginning of the exposure period.

Flow from 300-lb source cylinders of breathing air (Airgas, Fresno, CA, USA) and 1.6 % (v/v) phosphine were metered, respectively, into each of four gas blending manifolds (Aalborg Model G gas proportioner meter) that allowed for tunable $[PH3]_{ss}$ to exit the manifold, and ultimately enter a respective chamber. Exit flow from the manifold, which totaled 25 $mLmin^{-1}$ regardless of $[PH3]_{ss}$ (i.e. breathing air was the make-up gas), was directed to the input port/valve on the chamber; ¼'-diameter Teflon tubing was used for all plumbing and all connections were with standard stainless-steel Swedgelock fittings, unless otherwise noted. Flow exiting the chambers was directed through a LuerLok® sampling port into a centralized ventilation system (USDA, 2010). $[PH3]_{ss}$ and air inputs were tuned to the desired level in preliminary calibration studies, prior to the introduction of any test specimens into the chamber.

A gas sample of the chamber headspace was acquired using the LuerLok® sampling valve, which accessed the effluent of the respective chambers. A B-D® 100-mL gas-tight syringe was allowed to slowly fill to ~ 40 mL with the chamber effluent. Contents of the syringe were quantitatively analyzed with gas chromatography (GC) as described below. In the exploratory fumigations, the standard sampling interval for measurement of $[PH3]_{ss}$ was at 0.12 h (initial) and every 12 h thereafter through the duration of the treatment. Carbon dioxide and oxygen concentration were measured with a gas sampling pump connected in series between a port accessing chamber effluent and an atmospheric gas analyzer (GFC-7000E, Teledyne Instruments, City of Industry, CA), which recorded at standard temporal intervals over the duration of treatment.

After the final sampling of $[PH3]_{ss}$, cylinder valve-stems were shut, thereafter inputs of breathing air and phosphine ceased, chamber valves were opened to atmosphere, and a 30-min aeration period was initiated. Chamber lids were then opened and the treated as well as non-treated specimens were retrieved and transferred to an incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\overline{X} \pm s$) in prelude to mortality evaluation (vide infra).

Mortality evaluation

Mortality of diapausing larvae was diagnosed visually by discoloration, while survivability was diagnosed by locomotion or by prodding-induced motion 14 to 21 d post treatment. Ultimately, however, evidence of pupation served as diagnostic of survival. Egg mortality assessments were conducted using a dissecting microscope (8 to 10 x magnification) 14 d after treatment, as the 5 to 7 days typically required for hatching was delayed due to physiological suppression at the 10°C treatment temperature.

Mortality was calculated as a percentage of the response per treatment. Mortality of control specimens was assumed to be equal to that in fumigation trials, per the method of Abbott (1925), and was included as a natural response in modeling the efficacy results from exploratory trials. The total number of specimens that were treated for each exploratory-trial was estimated by summing the numbers treated, while the total number of specimens treated (*n*) across exploratory-trials was estimated by summing the numbers from each respective trial. Mortality was analyzed via probit analysis of Finney (1944 & 1977) at the 95% confidence level, as further derived in Couey and Chew (1986) as well as Liquido and Griffin (2010).

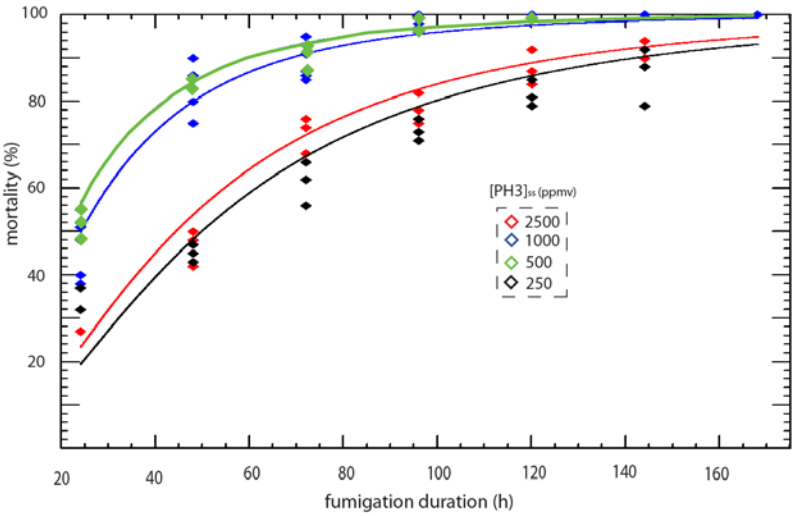
3. Results

Exploratory fumigations

The average air temperature ($\overline{X}$), 10 °C, was calculated across all trials. Deviation in temperature was assumed to follow a normal distribution with the estimated margin of error reported as ± 2*s*, 0.3 °C, the 95% confidence interval (Quinn, 1983). Of the 630 untreated diapausing larvae, only 24 expired with no more than 3 deaths per 30-specimen control grouping. As for mortality in 2,106 untreated eggs, 45 expired with no more than 5% mortality in each control grouping of ca. 100. While the control mortality of the diapausing larvae was consistent with previous reports (Banks and Cavanaugh, 1985), control mortality of eggs was ~15 % less than observed by Vincent and Lindgren (1975).

Respective duration-mortality regressions for (applied doses and) $[PH3]_{ss}$ of 250 (0.4), 500 (0.8), 1000 (1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}) were modeled using Polo Plus (LeOra Software, 2002-2007) with the mortality of control specimens included as a natural response. The number of egg specimens treated (250 ppmv: 2105 subjects; 500 ppmv: 2112 subjects; 1000 ppmv: 2109 subjects, 2500 ppmv: 2093 subjects), the regression heterogeneity (H), the projected durations to cause 50, 95, and 99% mortality in the treated population (respectively LT_{50}, LT_{90}, and LT_{99}), and the bounds (upper (UL) and lower (LL) limits) at the 95 % confidence level (CL) are shown in Figure 1. Likelihood ratio-based hypothesis testing of equality was rejected ($P < 0.05$, $\chi^2 = 621$, $df = 6$), indicating that the

slopes as well as the intercepts of the regressions respective to $[PH3]_{ss}$ were significantly different. Likelihood ratio-based hypothesis testing of parallelism was rejected ($P < 0.05$, $\chi^2 = 44.7$, $df = 3$), indicating that the slopes of the regressions respective to $[PH3]_{ss}$ were significantly different.



Probit Regression Parameters

	250 ppmv			500 ppmv			1000 ppmv			2500 ppmv		
	"duration"$_x$	95% CL LL	UL	"duration"$_x$	95% CL LL	UL	"duration"$_x$	95% CL LL	UL	"duration"$_x$	95% CL LL	UL
LT_{50}	46.8	38.7	54.4	24.4	21.6	26.9	28.2	24.5	31.6	43.9	38.3	49.0
LT_{95}	241.6	189.8	342.6	73.9	67.4	82.7	83.5	74.4	96.4	174.0	149.3	212.5
LT_{99}	475.0	335.6	802.8	117.2	102.5	138.6	130.9	111.3	161.9	308.0	247.1	414.1
Slope	2.3 (+/-) 0.2			3.4 (+/-) 0.2			3.85 (+/-) 0.2			2.7 (+/-) 0.1		
Heterogeneity	3.95			1.30			2.29			2.64		
Treated	2105			2112			2109			2093		

Fig. 1 Mortality of warehouse beetle, *Trogoderma variable* (Ballion), eggs following phosphine fumigation at 10.0 ± 0.3 ºC ($\bar{x} \pm 2s$) and probit regression analyses (Polo Plus, LeOra Software, 2002-2007) of the duration-mortality response respective to applied doses and steady state headspace concentrations, $[PH3]_{ss}$ of ca. 250 (0.4), 500 (0.8), 1000(1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}), showing the number of specimens treated, the regression heterogeneity (H), the projected durations to cause 50, 95, and 99% mortality in the treated population (respectively LT_{50}, LT_{90}, and LT_{99}), and the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95 % confidence level (CL).

Lethal time ratios (LTRs) were calculated with (+/-) 95 % confidence intervals (CI) across the durations projected to cause 10 to 99% mortality in the treated population. Figure 2 shows that fumigation with $[PH3]_{ss}$ of 250 or 2500 ppmv required longer treatment durations, relative to 1000 ppmv, to yield the same egg mortality response as noted by respective LTRs < 1 (unity) (Fig. 2). On the other hand, the LTRs for $[PH3]_{ss}$ of 500 ppmv overlapped or superseded a value of 1 (unity) respective to all projected durations > LT_{10}, indicating that time required for a particular percentage of egg control is equivalent when $[PH3]_{ss}$ is 500 or 1000 ppmv.

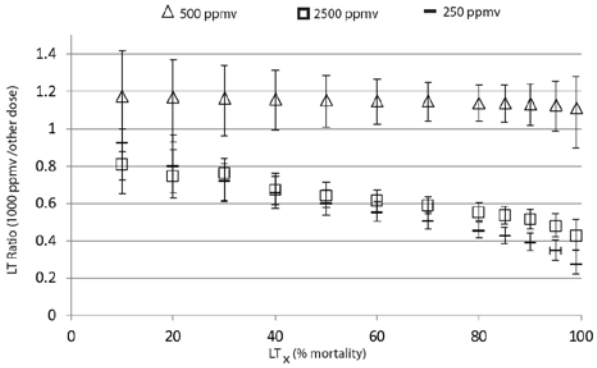


Fig. 2 Lethal time ratios (LTRs) associated with steady-state headspace concentrations, $[PH3]_{ss}$, of ca. 250 (0.4), 500 (0.8), 1000 (1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}) were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of warehouse beetle, *Trogoderma variable* (Ballion), eggs. LTRs respective to durations predicted to yield >10% mortality overlapped a value of 1 (unity) for 500 ppmv, indicating that maintaining $[PH3]_{ss}$ at 500 ppmv was no more efficacious than maintaining $[PH3]_{ss}$ at 1000 ppmv levels. However, LTRs were less than a value of 1 (unity) for 250 and 2500 ppmv, indicating that these treatments required significantly longer durations to evoke an equivalent response in the treated populations, relative to treatments with $500 \leq [PH3]_{ss} \leq 1000$ ppmv.

Additionally for a $[PH3]_{ss}$ of 1000 ppmv, LTRs were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of eggs and diapausing larvae. LTRs respective to durations predicted to yield >10% mortality overlapped a value of 1 (unity), indicating that equivalent treatment durations resulted in equivalent response of eggs relative to diapausing larvae (Fig. 3). This finding is consistent with that of Banks and Cavanaugh (1985) in that neither study establishes diapausing larvae as being more phosphine-tolerant than eggs, which are clearly more phosphine tolerant than all other life stages of *T. variable* (Vincent and Lindgren, 1975).

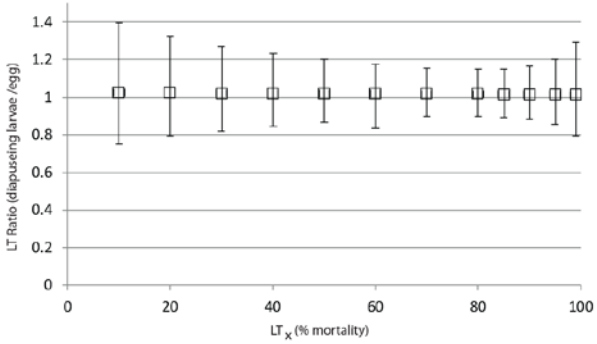


Fig. 3 Lethal time ratios (LTRs) associated with steady-state headspace concentrations, $[PH3]_{ss}$, of 1000 ppmv (μLL^{-1}) (1.5 mgL^{-1}) were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of warehouse beetle, *Trogoderma variable* (Ballion), eggs and diapausing larvae. LTRs respective to durations predicted to yield >10% mortality overlapped a value of 1 (unity), indicating that equivalent treatment durations resulted in equivalent response of eggs relative to diapausing larvae.

Figure 4 shows the projected durations to cause 99% mortality in the treated population (LT_{99}) of eggs varies as a function of $[PH3]_{ss}$. To rationalize this result, note the seminal work of Winks on phosphine (1984, 1985, 1986, 1994) as related to Haber's Rule ($C^z t = \omega$), which forms the basis for relating concentration (C) and time (t) to toxicological efficacy (ω), at least with respect to fumigation science (Bliss, 1940; Miller et al., 2000). For phosphine, z, the response evoked by a

specific toxicant in a particular organism, changes with *C*. When considering data on mortality collected at "fixed" concentrations over varying times, such as was done in the exploratory fumigations, the applied dose correlative to the onset of deviation (i.e., change in *n*) is termed the "narcosis threshold", the concentration above which further change in z results in the narcotic effect of phosphine and an increased tolerance. Recently, the work of Walse et al. (2013, 2016, 2017) has expanded on the concept of the "narcosis threshold" in the context of quarantine treatments as well as mitigation strategies for phosphine resistance. The results from the exploratory studies indicates the "narcosis threshold" of *T. variable* eggs spans the range 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv and is centered at 750 ppmv.

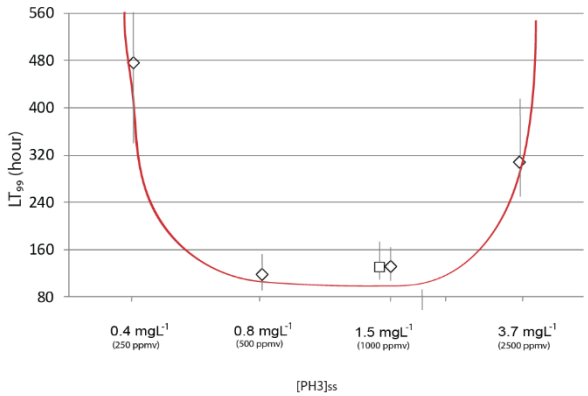


Fig. 4 The projected durations to cause 99% mortality in the treated population (LT_{99}) of warehouse beetle, *Trogoderma variable* (Ballion), eggs (◇) varied as a function of steady-state headspace concentrations, $[PH3]_{ss}$, over the range 250 to 2500 ppmv. However, an equivalent mortality response was observed when at $[PH3]_{ss}$ of 500 and 1000 ppmv, indicating that variability in $[PH3]_{ss}$ within the range 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv, will not change the efficacy of fumigation. It is critical to note that the predicted duration required to control 99% of a treated population of diapausing larvae (□) is equivalent to that required for eggs. Error bars are the estimates of the upper (UL) and lower limits (LL) at the 95% confidence interval (see Fig. 1). The "narcosis threshold" for *T. variable* (Ballion) eggs spans the range 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv as indicated by horizontal portion of the red trace.

The LL (95% CL) of the durations predicted to cause 99% mortality in the treated population (LT_{99}) of eggs and diapausing larvae were ca. 120 h. Moreover, none of the specimens (1,815 eggs & 270 diapausing larvae) survived fumigation with 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv for a duration ≥ 120 h, results that suggest fumigation at ≥ 10.0 ˚C will control *T. variable* infestations if [PH3] is maintained at ≥ 500 and ≤ 1000 ppmv for a duration ≥ 120 h. In general, an increase in treatment temperature is commensurate with an increase of insect metabolism and increase in the efficacy of a fumigant (Monro, 1969). The work of Vincent and Lindgren (1975) supports this conclusion with respect to *T. variable*, as fumigation at 21.1 ºC with 500 or 750 ppmv for 72 h resulted in complete mortality of 1- to 6-d old eggs. Collectively, results provide the technical framework of a fumigation schedule:

Phosphine concentration maintained at 750 ppmv (μLL^{-1}) (1.1 mgL^{-1}) or higher for 72 h or greater at commodity temperature of 20.6 ºC or greater

Phosphine concentration maintained at 750 ppmv (μLL^{-1}) (1.1 mgL^{-1}) or higher for 96 h or greater at commodity temperature of 15.0 ºC but less than 20.6 ºC

Phosphine concentration maintained at 750 ppmv (μLL^{-1}) (1.1 mgL^{-1}) or higher for 120 h or greater at commodity temperature of 10.0 ºC but less than 15.0 ºC

4. Discussion

The use of postharvest phosphine fumigation as a quarantine phytosanitary requirement will only increase in years to come. Here we provided operational and regulatory framework for

implementing and certifying efficacious treatments. Although ISPM 27 ultimately leaves efficacy acceptance criterion to the discretion of the importer, interantional scientific consensus helps guide such reguialtory decisions.

Acknowledgement

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