

# Postharvest treatment of California apples with cylinderized phosphine to control Oriental fruit moth (OFM), *Grapholita molesta*

October 28, 2014

Spencer S. Walse and Steven Tebbets  
USDA-ARS-SJVASC  
Parlier, CA 93648

CONFIDENTIALITY NOTICE: This report (including any attachments) contains confidential information and is for the sole use of the intended recipient(s) and its stated purpose and is protected by law. Any disclosure, copying, or distribution of this letter and its contents, or the taking of any action based on it, is strictly prohibited.

## Executive Summary.

A new postharvest treatment option to control OFM has been developed for California apple growers/packers. Packed-boxes can be fumigated at cold-storage temperature for 48 h. A report can now be drafted and presented to industry (and thereafter APHIS) for consideration. Currently, market options include those countries willing to fumigate with phosphine on arrival (e.g., Chile, Australia). ARS is working with industry and USEPA to gain registration for PH3 so that fumigations can be done at the packinghouse.

## Abstract.

Oriental fruit moth (OFM), *Grapholita molesta* (Busck) is a pest of concern to countries that import apples from California. Fruit were infested with OFM larvae (97% 5<sup>th</sup> instar), buried amongst uninfested fruit in export cartons, and then the cartons were fumigated with PH3 at  $1.7 \pm 0.5$  °C ( $\bar{x} \pm s$ ). Fumigations resulted in 0 survivors from 9,965 (*n*) treated OFM larvae (probit 8.43, 95% level of confidence) when headspace concentrations were maintained at levels  $\geq 1.5$  mgL<sup>-1</sup> (1000ppmv) phosphine (PH3) for 48 h. Data is discussed in the context of quarantine control of OFM following cylinderized PH3 fumigation of commercial apple exports.

## Materials and Methods.

***Insects and infestation.*** OFM colonies originated from wild specimens captured in Fresno County, California USA. OFM was cultured as described in Yokoyama et al. (1987) and USDA (2010). Larvae were extracted for fruit infestation 14-15 days after neonates were placed on diet contents in rearing cups. Fourth (0.425-0.600mm) and fifth (0.725-0.825 mm) instar head capsule widths, were typically extracted from the respective colonies for fumigation. To simulate naturally occurring OFM infestation, apples were cored with a #4 cork borer at 6 equidistant points, equatorially around the fruit, and predominantly 5<sup>th</sup> instar specimens (97%) were placed at the center, near the core, of each cavity. Larvae were sealed into the fruit by inserting a fruit plug, created with a #5 cork borer, until flush with the fruit skin.

**Confirmatory export fumigations.** To simulate a commercial scenario, fumigations were conducted using 241.9-L steel chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2010). The chamber was first loaded with six 0.5 ft<sup>3</sup> sand bags each wrapped in plastic packaging that displaced ~84.9 L total of chamber volume. On the same day that they were packaged for export, two volume bushels (17.2 kg/carton, ct 113) of tray-packed “Granny Smith” apples (50.8 x 32.5 x 30.5 cm, 50.4 L each) were obtained from commercial wholesale sources in California. Fruit (~75) were removed from each of two cartons, infested as described above, transferred back into the respective cartons, and the cartons were loaded into a chamber. The chamber load was estimated as a fractional percentage,  $64.2 \pm 0.8\%$  ( $\bar{x} \pm s$ ), of the volume occupied by the load relative to the chamber volume (i.e.,  $V_L (V_{chamber})^{-1} \times 100$ ) (Monro, 1969).

Chambers loaded with test specimens and uninfested fruit as well as control specimens were acclimated to fumigation temperature of ~1.7 °C (~35.1°F) for 12 h prior to treatment (i.e., tempered) within the incubator described above. Fruit pulp temperature was confirmed prior to fumigation by each of three probes (YSI scanning tele-thermometer) that recorded the respective pulp temperature in three uninfested fruit distributed at different locations within the load of the fruit undergoing treatment. Temperature probes were then removed and chamber lids clamp-sealed in preparation for treatment. The chamber ventilation valve was opened and chambers were filled with a volume of fumigant from a cylinder of 1.6 % (v/v) PH<sub>3</sub> balanced with nitrogen (Cytec Canada, Inc., Niagara Falls, Ontario, Canada) to achieve the requisite dose of 2.2 mgL<sup>-1</sup> (1500 ppmv) as predetermined in preliminary calibration studies. The valve was then closed which marked the beginning of the exposure period. Gas samples (40 mL) were taken from the chamber headspace through a LuerLok® valve using a B-D® 100 mL gas-tight syringe and quantitatively analyzed for PH<sub>3</sub> with GC-PFPD at standard intervals corresponding to 5 (initial), 60, 480, 1440 (1-d end), or 2880 (2-d end) min. Fumigant exposures were expressed as concentration × time cross products, “CTs”, and calculated by the method of Monro (1969).

After completion of the fumigation, chamber valves were opened to atmosphere and vacuum was pulled to aerate the chamber until headspace concentration of the fumigant was below the mandated ventilation requirements of 0.3 ppm (0.45µg/L) phosphine. Chamber lids were opened, the treated and non-treated control specimens were collected, and then transferred to an incubator at  $27.0 \pm 1.0$  °C and  $80 \pm 2\%$  RH ( $\bar{x} \pm s$ ).

**Mortality evaluation.** One day following fumigation, larval specimens were retrieved from treated and untreated controls and placed in a plastic dispo-Petri® dish lined with a filter paper for evaluation. Mortality was diagnosed visually by discoloration, while survivability of larvae was diagnosed by locomotion or by prodding-induced motion. Larvae were categorized as moribund if the survivability was inconclusive. Moribund larva were placed inside a labeled plastic snap-cap cage with fruit plugs to provide substrate and moisture prior to incubation under the conditions above until additional evaluation the following day. For the confirmatory trials, Abbott’s method (1925) described by Finney (1944 and 1971) was used to estimate the percentage mortality of larvae used in Probit calculations, as the mortality of control specimens was assumed to be equal to that in fumigation trials. The total number of specimens that were treated for each exploratory- or confirmatory-trial was estimated by summing the numbers

treated, while the total number of specimens treated ( $n$ ) across confirmatory-trials was estimated by summing the numbers from each respective trial.

***Chemicals and chemical analysis.*** A 300-lb cylinder of 1.6 % (v/v) PH<sub>3</sub> balanced with nitrogen was obtained from Cytec Canada, Inc. (Niagara Falls, Ontario, Canada) and used as the source for gas chromatography calibrations as well as fumigations. PH<sub>3</sub> levels in headspace of fumigation chambers were measured using gas chromatography; retention time (PH<sub>3</sub>,  $t_r = 3.2 \pm 0.2$  min) was used for chemical verification and the integral of peak area, referenced relative to linear least-squares analysis of a concentration – detector response curve, was used to determine concentration (Walse 2012 & 2013). Detector response and retention indices were determined each day in calibration studies by diluting known volumes of known concentrations of PH<sub>3</sub> into volumetric gas vessels. PH<sub>3</sub> analyses were with a Varian 3800 and splitless injection (140 °C) using a gas sampling port with a 10  $\mu$ L-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 PFPD detector (13 mL/min H<sub>2</sub>, 20 mL/min air, and 10.0 mL/min N<sub>2</sub> make-up) at 250 °C that received only 10% of the 15 ml He/min column flow.

## **Results and Discussion.**

***Confirmatory export fumigations.*** Confirmatory PH<sub>3</sub> fumigations of commercially-packaged apples were conducted in the context of verifying control of OFM larvae, the life stage with potential to be in postharvest marketing channels (Yokoyama et al. 1987). PH<sub>3</sub> fumigations at  $1.7 \pm 0.5$  °C ( $\bar{x} \pm s$ ) with headspace concentrations maintained at levels  $\geq 1.5$  mgL<sup>-1</sup> (1000ppmv) for 48 h resulted in  $> 99.969\%$  mortality of OFM larvae (probit 8.43 at 95% level of confidence (LOC), probit 9 at 27% LOC) based on 0 survivors from 9,965 ( $n$ ) treated as calculated by the method of Couey and Chew (1986) and Liquido and Griffin (2010) (Table 1). It is important to note that demonstrating 99.9968% (i.e., Probit 9 at the 95% LOC) mortality of quarantine insect pests is often requested to qualify phytosanitary treatment efficacy, particularly when commodity is moved internationally (Couey and Chew, 1986; Follet and Nevin, 2006).

Headspace concentrations of PH<sub>3</sub> in commercial chamber fumigations of palletized fresh produce at load factors  $\leq 65\%$ , regardless of produce and packaging type, lose  $\sim 200$  ppmv from chamber headspace per day (due to leakage, reactivity, and/or residue formation). Therefore, in the context of commercial considerations, observation of Probit 9-level mortality of OFM larvae in commercial PH<sub>3</sub> fumigations lasting at least 48 h will likely require a single compensatory applied dose  $> 2.2$  mgL<sup>-1</sup> (1500 ppmv), or alternatively, maintenance of steady-state headspace concentrations  $\geq 1.5$  mgL<sup>-1</sup> (1000ppmv) via multiple (daily) applications.

## References.

- Abbott, W. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Couey, M.C., Chew, V., 1986. Confidence limits and sample size in quarantine research. *J. Econ. Entomol.* 79(4), 887-890.
- Finney, D.J., 1944. The application of the probit method to toxicity test data adjusted for mortality in the controls. *Ann. Appl. Biol.* 31, 68-74.
- Finney, D.J., 1971. *Probit Analysis*, third ed. Cambridge University Press, Cambridge.
- Follett, P.A., Neven, L.G., 2006. Current trends in quarantine entomology. *Annu. Rev. Entomol.* 51, 359-385.
- Monro, H.A.U., 1969. *Manual of Fumigation for Insect Control*. FAO Agricultural Studies 79, 381.
- USDA. 2010. Fumigation and Chemistry Group of the Commodity Protection and Quality Research Unit, USDA, Agricultural Research Service, SJVASC, Parlier, CA 93648  
[http://www.ars.usda.gov//Main/site\\_main.htm?docid=18577](http://www.ars.usda.gov//Main/site_main.htm?docid=18577) [Accessed on Mar 15, 2011].
- Walse, S.S; Krugner, R.; Tebbets, J.S. 2012. Postharvest treatment of California USA strawberries with methyl bromide to eliminate the spotted wing drosophila, *Drosophila suzukii*, in exports to Australia. *J. Asian-Pac. Entomol.* 15:451-456.
- Walse, S.S; Liu, Y.B.; Myers, S.W.; Bellamy, D.E., Obenland, D; Simmons, G.S.; Tebbets, J.S. 2013. The treatment of fresh fruit from California with methyl bromide for postharvest control of light brown apple moth, *Epiphyas postvittana* (Walker) *J. Econ. Entomol.* 2013. 106(3), 1155-1163.
- Yokoyama, V. Y.; Miller, G.T.; J& .M. Harvey 1987. Development of oriental fruit moth (Lepidoptera: Tortricidae) on a laboratory diet. *J. Econ. Entomol.* 80, 272-276.

Table 1. Complete control of 9,965 OFM larvae resulted from fumigation of infested apples with  $2.2 \text{ mgL}^{-1}$  (1500ppmv) phosphine for 48 h at  $1.7 \pm 0.5^\circ\text{C}$  ( $\bar{x} \pm s$ ).

Trial	# treated OFM	Applied (mg/L)	[PH3] at 48h (ppmv)	Temp. ( $\pm 0.5^\circ\text{C}$ ) ( $\pm 0.8^\circ\text{F}$ )	survivors	Abott's mort.	Probit (95% LOC)		
1	842	2.2	1500	1020	1.7	35.1	0	1.00	7.69
2	851	2.2	1500	1127	1.7	35.1	0	1.00	7.70
3	904	2.2	1500	1058	1.7	35.1	0	1.00	7.72
4	821	2.2	1500	1073	1.7	35.1	0	1.00	7.68
5	863	2.2	1500	1086	1.7	35.1	0	1.00	7.70
6	874	2.2	1500	1045	1.7	35.1	0	1.00	7.70
7	754	2.2	1500	1196	1.7	35.1	0	1.00	7.66
8	898	2.2	1500	1038	1.7	35.1	0	1.00	7.71
9	752	2.2	1500	1102	1.7	35.1	0	1.00	7.65
10	847	2.2	1500	1053	1.7	35.1	0	1.00	7.69
11	695	2.2	1500	1049	1.7	35.1	0	1.00	7.63
12	864	2.2	1500	1012	1.7	35.1	0	1.00	7.70
$\Sigma$ 9,965 (n)							$\Sigma$ 0	$\Sigma$ 8.43	

