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Postharvest chamber fumigation with cylinderized phosphine to control key insect pests of fresh citrus

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ABSTRACT

Each year, the central valley of California exports fresh citrus valued at >200 million USD. The goal of this research was to provide this sector with a commercially viable postharvest methyl bromide alternative that is effective against insect pests that serve, or have a potential to serve, as trade barriers to export. We discuss the progression of this research from initial toxicological investigations, through laboratory-scale optimization, to commercial-scale confirmatory testing. We report how to modulate the fumigation parameters to ensure control of key insect pests (e.g., Fuller's rose beetle, bean thrips, California red scale, etc.) across a variety of citrus types, including: navel oranges, Valencia oragnes, lemons, and mandarins. Quantifying the residues and off-gassing potential were critical steps in assessing commercial viability, as any proposed use must result in residues compliant with domestic (United States) food tolerances, international maximum residue level (MRL) regulations, and worker exposure regulations. Detailed below is efficacy data related to the control of bean thrips, *Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae).

Key words: Citrus, Ecofume, Quarantine, Vaporphos

Bean thrips (BT), *Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae), is a pest of concern to certain countries that import fresh citrus fruit from California, USA. A series of laboratory-scale exploratory fumigations with phosphine at 4.9 ± 0.3ºC ($\bar{x} \pm 2s$) were conducted to evaluate the postharvest control of adult BT. Confirmatory fumigations were then conducted using infested navel oranges at pulp temperature ≤5°C with three formulations of cylinderized phosphine (1.6% (v/v) balanced in nitrogen, VAPORPH$_3$OS®, and ECOFUME®. Data are discussed in the context of quarantine control of BT following commercial fumigation of fresh citrus exports to Australia.

MATERIALS AND METHODS

Insects, rearing and infestation: BT were captured from an alfalfa, *Medicago sativa,* planting near Parlier, California approximately 40 km southeast of Fresno, California. Plants were uprooted, transferred to a 0.0283 m^3- fine mesh (U.S. #40 mesh) enclosure, and delivered to the USDA-ARS-SJVASC. Enclosures were fumigated with ca. 70,000 ppmv carbon dioxide for ~45 s to anaesthetize the captured specimens. Immobilized adult BT specimens were transferred with a fine brush (Daler Rowney, Script/Liner), damped with Ringer's solution, from leaves or stems onto a glass microscope slide. Slides were viewed using a dissection microscope and species identification was based on the presence of white banding on the legs and a transverse white band on the front wings as described in (UC ANR, 2015). Species were cataloged and are available for independent species confirmation. Following species confirmation, adult BT specimens were transferred to lima bean plants (*Phaseolous lunatus*) housed in a ca. 1m^3 rearing enclosure covered with fine mesh located in a shaded greenhouse at the USDA-ARS-SJVASC maintained at 20° to 30°C and 60 ± 5% r.h.($\bar{x} \pm s$). The BT colony was reared on lima bean plants in the enclosure described above. Approximately twice each fall, BT were captured, identified to species, and introduced into the SJVASC colony.

Adult specimens were collected from the enclosure using a mouth aspirator. To obtain an aliquot of adult

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BT for exploratory and confirmatory fumigations, 10 specimens were consecutively aspirated into a 10 mL stainless-steel mesh cage using a customized arrangement of the aspirator and cage. The cages were sealed with rubber stoppers. Five BT-containing vials were placed in each chamber for the exploratory fumigations (vide infra). For the confirmatory fumigations involving infested fruit, fresh navel oranges commensurate with postharvest commercial distribution from California USA, and particularly export to Australia, were obtained from Bee Sweet Citrus (Fowler, CA). Prior to use, fruit was refrigerated at 0.9 ± 0.7°C ($\bar{x} \pm s$) in a 21.9 m^3 cold-storage unit (Super Insulated Structures, Imperial Manufacturing, Portland, OR). Infestation was based on modification of methods described in Leesch et al. (2004, 2008) and Harman et al. (2007). Preceding infestation, each fruit was warmed to 25°C overnight (ca. 24 h), inspected and those exhibiting fungus, damage, rot, or bruising were discarded. Specimens (10) were then anaesthetized by fumigating the vials with carbon dioxide as above. All ten immobilized specimens were then gently tapped out of the vial peripherally to the navel of a navel orange, which had a ring of "sticky-tac" mounting putty (Menco, Inc. Avon, OH) molded concentric to the navel opening. A nylon mesh disc was then anchored to the putty to contain the BT. To drive the BT into the navel, the infested oranges were then cooled to 5°C at ca. 2°C h^{-1} over the course of 10 h in a Binder MK 53 Freezer Chamber.

RESULTS AND DISCUSSION

Exploratory fumigations: The average air temperature ($\bar{x}$), 4.9 ºC, 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 ºC, the 95% confidence interval (Quinn, 1983). Respective duration-mortality regressions for (applied doses and) $[PH3]_{ss}$ of 250 ppmv (μLL^{-1}) (0.4 mgL^{-1}), 500 ppmv (0.8 mgL^{-1}), and 1000 ppmv (1.5 mgL^{-1}) were modeled using Polo Plus (LeOra Software, 2002-2007). The number of adult BT specimens treated (250 ppmv: 1644 subjects and1648 controls; 500 ppmv: 1644 subjects

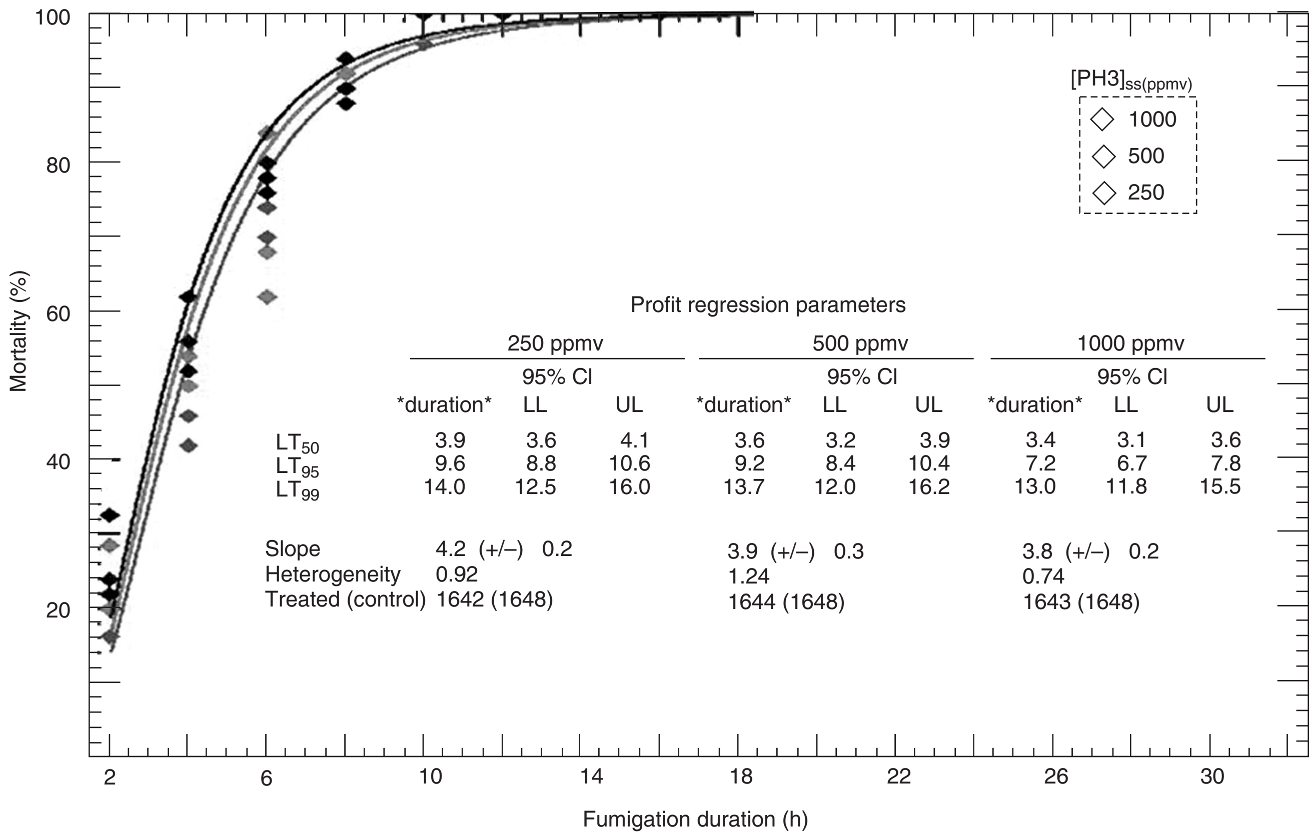

Fig.1. Mortality of bean thrips adults following phosphine fumigation at 4.9 ± 0.3 ºC ($\bar{x} \pm 2s$) and probit regression analyses (Polo Plus, LeOra Software, 2002-2007) of the duration-mortality response respective to applied doses and steady state headspace concentrations, $[PH3]_{ss}$ of 250, 500, and 1000 ppmv, showing the number of specimens treated, non-fumigated control specimens, 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 upper (UL) and lower limits (LL) at the 95% confidence interval (CI).

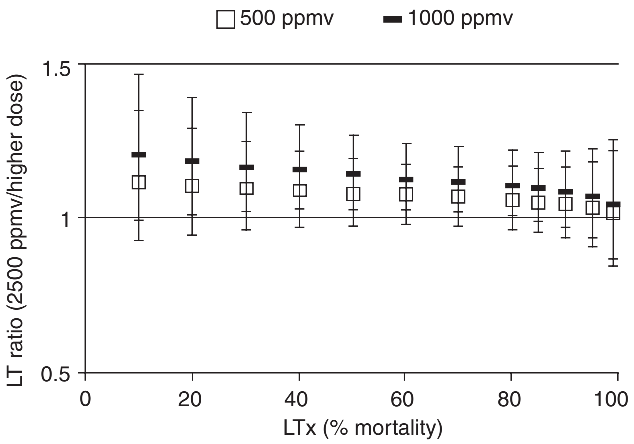

Fig. 2. Lethal time ratios (LTRs) associated with steady-state headspace concentrations, $[PH3]_{ss}$, of 250, 500, and 1000 ppmv were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of adult bean thrips. LTRs respective to durations predicted to yield >85% mortality all overlapped a value of 1 (unity), indicating that maintaining a $[PH3]_{ss}$, of 250, was no more efficacious than maintaining $[PH3]_{ss}$, at 500 or 1,000 ppmv levels

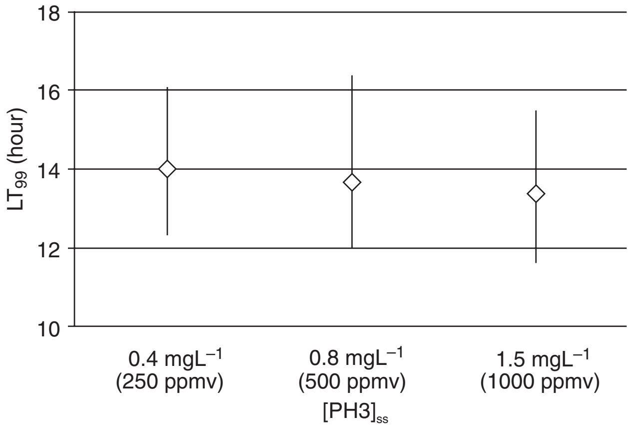

Fig. 3. The projected durations to cause 99% mortality in the treated population (LT99) of bean thrips adults did not vary as a function of steady-state headspace concentrations, $[PH3]_{ss}$, over the range 250 to1,000 ppmv, indicating that variability in phosphine levels within that range will not change the efficacy of fumigation. Error bars are the estimates of the upper (UL) and lower limits (LL) at the 95% confidence limit (see Fig. 1)

and 1,648 controls; 1,000 ppmv: 1,643 subjects and 1,648 controls), 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 upper (UL) and lower limits (LL) of the 95% confidence level (CL) are shown in Fig.1 (Finney, 1944, 1971). Likelihood ratio-based hypothesis testing of equality was not rejected (P = 0.118, $\chi^2 = 7.36$, $df = 4$), indicating that the slopes as well as the intercepts of the regressions respective to $[PH3]_{ss}$ were not significantly different. Likelihood ratio-based hypothesis testing of parallelism was not rejected ($P = 0.660$, $\chi^2 = 0.83$, $df = 2$), indicating that the slopes of the regressions respective to $[PH3]_{ss}$ were not significantly different.

Lethal time ratios (LTRs) were calculated with with 95% confidence intervals (CI) across the durations projected to cause 10 to 99% mortality in the treated population and used to identify that $[PH3]_{ss}$ of 500 or 1000 ppmv were no more efficacious toward BT adults than an applied dose of 250 ppmv, as LTRs respective to durations > LT_{85} all overlapped or superseded a value of 1 (unity) (Fig. 2). The projected durations (Fig. 3) to cause 99% mortality in the treated population (LT_{99}) of adult BT did not vary as a function of $[PH3]_{ss}$, indicating that variability in [PH3] between 250 and 1000 ppmv did not change the efficacy of fumigation. To rationalize this result, note the seminal work of Winks on phosphine (1984, 1985, 1986, 1994) as related to Haber's Rule (C^zt = ω), 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. The results from the exploratory studies indicated the narcosis threshold for adult BT spans [PH3] ≥ 250 and ≤ 1000 ppmv; future work will explore the minimum and maximum [PH3] associated with the threshold. The LL (95% CL) of the durations predicted to cause 99% mortality in the treated population (LT_{99}) were ca. 12 h. Moreover, none of the 3,134 specimens survived fumigation with [PH3] ≥ 250 ppmv for a duration ≥ 10 h, results that suggest fumigation of fresh citrus at ≥ 5.0ºC will control adult BT infestations if [PH3] is maintained ≥ 250 ppmv for a duration ≥ 12 h. Confirmatory trials were conducted to test this hypothesis, the results of which are presented below.

Commodity fumigations: A series of commodity fumigations were conducted in a scaled-down replicate of commercial fumigation chamber with a load of 48.1% as related to verifying control (i.e., efficacy) of adult BT infesting navel oranges following application

Table 1 Efficacy analysis and parametrics associated with confirmatory fumigations of infested navel oranges having pulp temperature, T, and applied doses of ca. 1.5 mgL^{-1} (1000 ppmv) delivered by three formulations of cylinderized phosphine (1.6% (v/v) balanced in nitrogen- Scheme 1, VAPORPH$_3$OS®- Scheme 2, and ECOFUME®- Scheme 3) with respective carbon dioxide levels ([CO2]) and headspace concentrations of phosphine ([PH_3])

					Applied	2 h	6 h	12 h	Adult BT control		Adult BT treated			
Trial*	PH_3 scheme*	[CO_2] (ppmv)	T (°C)	Load (%)	[PH_3]$_0$	[PH_3]$_t$ (ppmv)	[PH_3]$_t$	[PH_3]$_t$	# obs. : mort	(% mort.)	*obs.:surv.	$p_{(surv.)}$	probit	% mort.$_{(MI)}$
1	1	351±8	4.9±0.2	48.1	958±12	932±7	877±8	842±10	199:13	93.47	1305:0	0.002293	7.835	99.755
2	1	357±5	4.8±0.2	48.1	979±8	967±12	904±6	821±8	200:17	91.50	1301:0	0.002300	7.834	99.749
3	1	368±6	4.7±0.2	48.1	982±10	968±11	931±5	887±9	200:9	95.50	1296:0	0.002309	7.833	99.758
4	1	348±10	4.9±0.2	48.1	974±13	958±12	900±9	856±6	198:18	90.91	1300:0	0.002302	7.834	99.747
5	1	364±7	5.0±0.2	48.1	988±12	961±10	910±12	851±8	201:14	93.03	1304:0	0.002295	7.835	99.753
6	1	376±4	4.9±0.3	48.1	972±9	952±9	904±9	823±12	200:16	92.00	1298:0	0.002305	7.833	99.749
7	1	375±8	4.8±0.2	48.1	986±12	976±12	949±11	937±12	201:11	94.53	1300:0	0.002302	7.834	99.756
8	1	363±5	4.7±0.2	48.1	975±8	962±13	904±12	884±10	200:20	90.00	1302:0	0.002298	7.834	99.745
9	1	371±4	4.9±0.2	48.1	990±12	980±9	967±8	932±9	198:22	88.89	1298:0	0.002305	7.833	99.741
10	1	361±7	5.0±0.2	48.1	977±10	941±12	906±10	849±6	199:15	92.46	1299:0	0.002304	7,833	99.751
11	2	398±2	5.0±0.2	48.1	995±12	967±8	923±8	842±7	202:20	90.10	1300:0	0.002302	7.834	99.745
12	2	401±2	4.8±0.2	48.1	972±11	964±12	917±10	857±14	200:15	92.50	1301:0	0.002300	7.834	99.751
13	2	397±2	4.7±0.2	48.1	969±10	951±11	922±14	877±15	197:8	95.94	1299:0	0.002304	7.833	99.760
14	1	396±2	4.8±0.3	48.1	986±6	971±10	963±16	928±11	200:7	96.50	1296 :0	0.0)2309	7.833	99.761
15	2	400±2	4.9±0.2	48.1	982±11	973±14	954±18	930±8	201:17	91.54	1304:0	0.002295	7.835	99.749
16	2	395±3	4.9±0.2	48.1	991±7	964±9	921±12	867±7	202:14	93.07	1300:0	0.002302	7.834	99.753
17	2	398±2	4.8±0.2	48.1	987±12	973±10	946±14	900±10	200:6	97.00	1300:0	0.002302	7.834	99.763
18	2	397±3	4.7±0.3	48.1	968±9	942±11	892±8	816±12	201:14	93.03	1297:0	0.002307	7.833	99.752
19	2	399±2	4.9±0.2	48.1	979±10	959±13	913±6	854±11	197:20	89.85	1302:0	0.002298	7.834	99.744
20	2	397±2	4.9±0.2	48.1	983±14	972±8	9561±7	924±18	198:5	97.47	1303:0	0.002296	7.834	99.764
21	3	48,756±70	4.8±0.2	48.1	968±12	959±10	932±12	907±11	200:30	85.00	1305:0	0.002293	7.835	99.730
22	3	49,258±72	4.8±0.2	48.1	975±9	942±8	894±7	810±8	200:18	91.00	1304:0	0.002295	7.835	99.748
23	3	48,723±55	4.7±0.3	48.1	988±12	974±5	923±8	865±5	200:5	97.50	1282:0	0.002334	7.829	99.761
24	3	47,967±105	5.0±0.2	48.1	992±11	969±15	916±12	848±9	198:18	90.91	1295:0	0.002311	7.832	99.746
25	3	48,204±95	5.0±0.2	48.1	981±10	972±14	931±10	902 ± 12	202:12	94.06	1301:0	0.002300	7.834	99.755
26	3	48,651±65	4.9±0.2	48.1	99517	967±10	921±11	845±15	205:14	93.17	1302:0	0.002298	7.834	99.753
27	3	49,371±88	4.7±0.3	48.1	968±7	952±15	916±10	876±16	202:12	94.06	1301:0	0.002300	7.834	99.755
28	3	49,004±107	4.7±0.2	48.1	962±10	950±9	903±9	846±11	200:21	89.50	1298:0	0.002305	7,833	99.742
29	3	48,556±91	4.9±0.2	48.1	970±9	953±12	922±14	878±12	200:17	91.50	1300:0	0.002302	7.834	99.748
30	3	48,905±82	4.8±0.2	48.1	968±11	954±11	931±11	90S±10	199:6	96.98	1300:0	0.002302	7.834	99.763
									Σ 4,994:364	92.71	32,492:0	0.000092	8.739	99.990

of ca. 1,000 ppmv (1.5 mgL^{-1}) for 12 h at pulp temperature (T) ≤ 5.0ºC (Table 1). The average T was calculated over the course of each trial as described above. Collectively, the fumigations resulted in 0 survivors from 32,492 treated BT, 99.990% (corrected) mortality (probit 8.74, with 95% confidence level; probit 9, with 65% confidence level). Demonstrating mortality of quarantine insect pests as a function of probit 9 analyses and associated confidence levels is often requested to qualify phytosanitary treatment efficacy, particularly when commodity is moved internationally (Couey and Chew, 1986; Follet and Neven, 2006; Liquido and Griffin, 2010).

Across all trials, regardless of applied phosphine formulation, [PH3] levels dropped ca. 50 to 160 ppmv over the 12h treatment time course; variation in [PH3] loss is likely due to differential leakage of fumigant from the chamber, as over sorption by (and residue formation within) such similar loads is expected to be nearly identical. Moreover, loads of fresh fruits that vary by amount and type are known to only minimally influence [PH3] levels, particularly with respect to a12 h treatment duration, which is relatively short requirement for treatment efficacy. It is also critical to note that efficacy was not influenced by the three different phosphine formulations (1.6% (v/v) balanced in nitrogen- Scheme 1, VAPORPH$_3$OS®- Scheme 2, and ECOFUME®- Scheme 3), indicating that carbon dioxide levels in chamber headspace, at least over the range ca. 365 to 49,000 ppmv (0.036 to 4.9%), do not influence the treatment efficacy.

In conclusion, results provide evidence to support control, at efficacy levels consistent with international phytosanitary standards (probit 9, with 65% confidence level), of adult BT infesting fresh navel oranges at pulp temperature ≥ 5.0ºC following fumigation with an applied dose of 1,000 ppmv (1.5 mgL^{-1}) phosphine, when headspace levels are maintained ≥ 250 ppmv (0.4 mgL^{-1}) for ≥ 12 h.

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