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Postharvest Fumigation of Fresh Citrus with Cylinderized Phosphine to Control Bean Thrips (Thysanoptera: Thripidae)

Spencer S. Walse * and Leonel R. Jimenez

United States Department of Agriculture, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA 93648-9757, USA; ljimenez@horn-technologies.com
* Correspondence: spencer.walse@usda.gov; Tel.: +1-559-779-8750

Abstract: Bean thrips (BT), *Caliothrips fasciatus* (Pergande), 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 ± 0.3 °C (mean ± 2 SD; $\bar{x} \pm 2s$) were conducted to evaluate the postharvest control of adult BT. Models of the duration–mortality response predicted ca. 99% mortality of BT populations when headspace concentrations of phosphine, [PH_3], are maintained at levels ≥0.4 g m^{-3} (250 ppmv (μL L^{-1})) and ≤1.5 g m^{-3} (1000 ppmv (μL L^{-1})) for 12 h, with the duration representing the lower bound of the 95% confidence level (CL). Confirmatory fumigations, each lasting 12 h, were then conducted using BT-infested sweet oranges, *Citrus sinensis* (L.), at pulp temperature (T) ≤ 5 °C to corroborate the exploratory results. Three formulations of cylinderized phosphine were used: 1.6% phosphine by volume in nitrogen, VAPORPH3OS®, and ECOFUME®, all applied at two levels, ca. 1.5 g m^{-3} (1000 ppmv (μL L^{-1})), as well as 0.5 g m^{-3} (300 ppmv (μL L^{-1})). Collectively, across the formulations, an applied dose of ca. 1.5 g m^{-3} (1000 ppmv (μL L^{-1})) resulted in 0 survivors from 38,993 (probit 8.60, 95% CL; probit 9, 72% CL) treated BT, while an applied dose of 0.5 g m^{-3} (300 ppmv (μL L^{-1})) resulted in 0 survivors from 31,204 (probit 8.56, 95% CL; probit 9, 70% CL) treated BT. Results were discussed in the context of commercial and operational features of quarantine and pre-shipment (QPS) uses of phosphine to treat fresh fruit and, specifically, the control of BT in fresh citrus exported from California, USA, to Australia.

Keywords: quarantine and pre-shipment (QPS); cylinderized phosphine; postharvest citrus

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1. Introduction

To satisfy the ever-increasing demand for food quality, safety, and security in domestic and international markets, horticultural producers must find the safest and most economical ways to control insect pests. Relative to treatments applied during production, postharvest opportunities typically allow for greater synchronization of the treatment with the logistical, infrastructural, and regulatory constraints of marketing. While efforts continue across the gamut of postharvest strategies (e.g., cold-treatments, heat-treatments, irradiation, controlled-atmosphere, fogging, etc.), fumigation remains an invaluable option for insect pest control [1]. One fumigant, methyl bromide, has dominated the postharvest treatment of horticultural crops over the last four decades, being the primary option for quarantine and pre-shipment (QPS) disinfestations, where pest-free security must be "guaranteed". This use has resulted in a global horticultural industry, producers, and port facilities alike, with logistics and infrastructure largely geared to conduct QPS methyl bromide fumigations.

From a regulatory perspective, many dynamics surround the continued use of postharvest methyl bromide for QPS treatments [2]. While exempted from the global phase-out prescribed in the Montreal Protocol, the use of QPS methyl bromide is ultimately at the discretion of the importing country [3], leaving the exporter with uncertainty regarding market retention. Foremost, any alternative fumigants must have a labeled use, and cor-

responding residue tolerance, in the country of application. Additionally, the alternative fumigant must have a Maximum Residue Level (MRL) in the destination market.

Other than methyl bromide, only a single postharvest fumigant, phosphine, is currently approved to treat citrus in the USA. Owing to the pioneering work of Dr. Fransiskus Horn [4] in the late 1990s, cylinderized phosphine is now used across the globe to treat numerous types of horticultural crops, typically at the optimal cold-storage temperature, and MRLs of 10 μg kg^{-1} (ppb) have been established in key markets, including those relevant to citrus exports from California, USA [5]. However, postharvest fumigations with cylinderized phosphine have not yet been used on citrus from California, let alone on exports requiring a QPS treatment. Logistical, infrastructural, and/or regulatory limitations have curtailed use by industry and regulators, but recent efforts are making the QPS use of phosphine a reality. For example, the research reported below supports the use of cylinderized phosphine to control bean thrips in 'Navel' orange, *Citrus sinensis* (L.), and was used by the California citrus industry to gain access for exports into Australia [5]. To facilitate the potential for adoption of phosphine as a QPS treatment for this scenario and beyond, we discuss the how schedules for phosphine fumigation best conform with toxicological efficacy, as well as operational feasibility.

2. Materials and Methods

2.1. Insects, Rearing, and Infestation

Bean thrips (BT), *Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae), infest sweet oranges, *Citrus sinensis* (L.), in California, USA, groves, notably 'Navel' varieties during the winter (harvest) months when, presumably, the navel provides refuge from the cold [6]. BT adults were captured from an alfalfa, *Medicago sativa,* planting near Parlier, California, USA (36.6116° N, 119.5271° W), approximately 40 km southeast of Fresno, California, USA, during spring and early summer. Plants were uprooted, transferred to a 0.0283-m^3 fine mesh (U.S. #40 mesh) enclosure, and delivered to the United States Department of Agriculture, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center (USDA-ARS-SJVASC). Enclosures were fumigated with ca. 200 g m^{-3} (170,000 ppmv (μL L^{-1})) carbon dioxide for ~45 s to anaesthetize the captured specimens. Immobilized adult BT specimens were transferred with a fine brush (Daler Rowney, Script/Liner, Lake Mary, FL, USA) 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 previously [7]. 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. 1-m^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% relative humidity (unitless) ($\bar{x} \pm s$). The BT colony was reared on lima bean plants in the enclosure described above. Approximately twice each fall, BT were captured over the course of ca. 2 h in the morning, identified to species, and introduced into the USDA-ARS-SJVASC colony.

Adult specimens were collected from the enclosure using a mouth aspirator. To obtain an aliquot of adult 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 (Supplementary Material Figure S1). 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, USA). Prior to use, oranges were 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, USA). Infestation was based on modification of methods described in Leesch et al. [8,9] and Harman et al. [10]. Preceding infestation, each fruit was warmed to 25 °C overnight

(ca. 24 h) and inspected. Fruit exhibiting fungus, damage, rot, or bruising was discarded. BT 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 and into the navel of a navel orange, which had a ring of "sticky-tac" mounting putty (Menco, Inc. Avon, OH, USA) molded concentrically to the navel opening (Supplementary Material Figure S2). A nylon mesh disc was then anchored to the putty to contain the adult BT. To localize the BT within 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 MK 53 Freezer Chamber (Binder GmbH, Tuttlingen, Germany).

2.2. Exploratory Fumigations

Laboratory-scale exploratory fumigations were conducted in a matching set of 24 modified Labonco® (Kansas City, MO, USA) 28.32-L vacuum chambers housed in a walk-in environmental room with programmable temperature and humidity [11]. Temperature and humidity set-points were 5 °C and 80% relative humidity, respectively. This series of exploratory experiments was used to determine the treatment duration, ranging from 2 to 32 h, required to control adults with phosphine applied at steady-state concentrations in chamber headspace (i.e., $[PH_3]_{ss}$) of ca. 0.4 g m^{-3} (250 ppmv (μL L^{-1})), 0.8 g m^{-3} (500 ppmv (μL L^{-1})), or 1.5 g m^{-3} (1000 ppmv (μL L^{-1})), respectively. Each of the four chambers was loaded with five stainless-steel vials containing the BT; three of the chambers were, respectively, subject to the phosphine treatments above, and the fourth was not fumigated in order to yield non-treated control specimens. Each "block" of four chambers was subject to treatment durations of 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, and 32 h. Each "block" respective to each duration was conducted in triplicate, which yielded a total of ca. 150 treated specimens at each $[PH_3]_{ss}$ and each of the 11 treatment durations.

Loaded chambers, a 136.1 kg (300-lb) source cylinder of breathing air (Airgas, Fresno, CA, USA), a 136.1 kg (300-lb) source cylinder of 1.6% phosphine by volume in nitrogen, and gas-tight syringes were acclimated to fumigation temperature (i.e., tempered) within the walk-in environmental incubator for at least 24 h prior to treatment. Air temperature in the walk-in incubator was confirmed prior to fumigation by a HOBO data logger (HOBOware version 2.7, Onset Computer Corporation, Bourne, MA, USA). Chamber lids were then clamp-sealed in preparation for treatment. A slight vacuum of approximately 10.1–13.3 kPa was established in each chamber. Gas-tight super-syringes (500, 1000, or 1500 mL, Hamilton Company, Reno, NV, USA) were filled with a volume of phosphine from the 136.1 kg (300-lb) source cylinder of 1.6% phosphine by volume in nitrogen 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 and 1.6% phosphine by volume in nitrogen were metered, respectively, into each of the three gas blending manifolds (Aalborg Model G gas proportioner meter, Brooks Instruments, Hatfield, PA, USA) that allowed for tunable $[PH_3]_{ss}$ to exit the manifold, and ultimately enter a chamber. Exit flow from the manifold, which totaled 25 mL min^{-1}, regardless of $[PH_3]_{ss}$ (i.e., breathing air was the make-up gas), was directed to the input port/valve on the chamber; 6.35-mm ($\frac{1}{4}$-inch) diameter Teflon® tubing was used for all plumbing and all connections were with standard Swedgelock fittings, unless otherwise noted. Flow exiting the chambers was directed through a LuerLok® sampling port into a centralized ventilation system (USDA, 2010). $[PH_3]_{ss}$ and air inputs were tuned to the desired level in preliminary calibration studies, prior to the introduction of any test specimens. While not commercially available, 1.6% phosphine by volume in nitrogen was sourced to facilitate relatively small volume laboratory-scale experiments not amenable to VAPORPH3OS® and ECOFUME®, the commercially available formulations intended for larger enclosure volumes (vide infra).

A gas sample of the chamber headspace was acquired using the LuerLok® sampling valve, which accessed the chamber effluent. A B-D® 100 mL gas-tight syringe (Hamilton

Company, Reno, NV, USA) was allowed to slowly fill to ~ 40 mL. 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 $[PH_3]_{ss}$ was at 0.12 h (initial) and every 2 h thereafter through the duration of the treatment. Carbon dioxide and oxygen concentrations 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, USA), which recorded at standard temporal intervals over the duration of the treatment.

After the final sampling of $[PH_3]_{ss}$, cylinder valve-stems were shut, thereby stopping inputs of breathing air and 1.6% phosphine by volume in nitrogen. Chamber valves were then opened to atmosphere and a 30-min aeration period was initiated. Chamber lids were opened and the treated as well as non-treated, infested specimens were collected and transferred for subsequent mortality evaluations (vide infra) into respective 0.03-m^3 fine mesh (U.S. #40 mesh) rearing cubicles maintained in an incubator at 27.0 ± 1.0 °C and 80 ± 2% relative humidity (unitless) ($\overline{x} \pm s$). Transfer of infested oranges to the incubator occurred within 15 min following the completion of aeration.

2.3. Confirmatory Fumigations

Results from exploratory fumigations were confirmed in trials that simulated commercial-scale fumigations. A 241.9-L ($V_{chamber}$) steel chamber was housed in the walk-in environmental room with the set points described above; however, the air temperature was set to 4.6 °C (USDA, 2010). Non-infested fruit were removed from each of three 18.1 kg (40-lb) cardboard cartons of fresh packed 'Washington Navel' variety sweet oranges (88 count, 30 l × 46 w × 32 h cm) and replaced with the same number of BT-infested fruit, recently removed from refrigeration at 5 °C (vide supra). The chamber was loaded with a scaled-down replicate of a wooden pallet, as well as two of the orange cartons stacked atop one another. Resulting chamber loads were 48.1 ± 0.8% (mean ± SEM), estimated as a fractional percentage of the volume occupied by the "packaged" load (V_{PL}) relative to the chamber volume ($V_{PL}\ V_{chamber}^{-1} \times 100$), where $V_{PL}\ {V_{chamber}}^{-1}$ = load factor [12]. For each trial, 65 infested fruit were placed into each carton of the two-carton load, while 20 infested fruit were dispersed throughout the single control carton that was not treated (i.e., non-treated).

Loaded chambers, the carton of oranges infested with control specimens, source-gas cylinders, and a gas-tight sampling syringe (vide infra) were acclimated to fumigation temperature, or tempered, in the environmental room for 12 h prior to treatment. Fruit pulp temperature (T) was confirmed to be ≤5 °C prior to fumigation by each of three probes (YSI scanning tele-thermometer) that recorded the respective T of three non-infested oranges distributed at different locations within the load undergoing treatment. Each fruit was speared by a single probe; care was taken to not allow airspace between the probe and the pulp with probe tips terminated in the center of the fruit. In addition, two 5-inch HOBO (U12-015-02) Probes (Onset Computer Corporation, Bourne, MA, USA), inserted into the fruit pulp as above, were used in concert with an Onset HOBO Data Logger to continuously record T with a 30 min scanning/sampling rate. Temperature probes were then removed, except for the HOBO probes, and the chamber lids were clamp-sealed in preparation for treatment. The mean T ($\overline{x}$) and associated standard deviation (s) were calculated based on all probe measurements for a respective trial. Temperature probes were calibrated with the ice-water immersion method, as described in ASTM E563-11, the day before the test.

Three different phosphine formulations were used to achieve the requisite applied dose of ca. 1.5 g m^{-3} (1000 ppmv (μL L^{-1})) or 0.5 g m^{-3} (300 ppmv (μL L^{-1})), as determined in preliminary calibration studies. Each formulation yielded a different carbon dioxide level in the chamber headspace. The first formulation resulted in carbon dioxide at ca. 0.4 g m^{-3} (365 ppmv (μL L^{-1})), just below atmospheric levels, as flow from the 136.1 kg (300-lb) source cylinder of 1.6% phosphine by volume in nitrogen was me-

tered into the inlet valve by a rotameter Hatfield, PA, USA), ultimately displacing 17 to 18 L through the exhaust port of the chamber. A typical atmospheric level of carbon dioxide (ca. 0.5 g m^{-3} = 400 ppmv (μL L^{-1})) was afforded with the second formulation; VAPORPH3OS® was dispensed with a Horn Diluphos System (HDS) HDS-80® (Fosfoquim, Santiago, Chile), which diluted 0.6 g of phosphine in air at 4 g min^{-1}. The third delivery scheme, which resulted in a supra-atmospheric carbon dioxide level (ca. 63 g m^{-3} = 49,000 ppmv (μL L^{-1}) = 5% carbon dioxide by volume in air) involved the application of ECOFUME®, 2.0% phosphine by volume in carbon dioxide. Flow from the source cylinder was metered into the inlet valve by a rotameter (#10410-R5, Brooks Instruments, Hatfield, PA, USA) as described above. Note that the application procedures for VAPORPH3OS® and ECOFUME® (commercial formulations) were consistent with guidelines in the USDA-APHIS treatment manual [13].

After the addition of phosphine, irrespective of formulation, normal atmospheric pressure (NAP) was reestablished in each chamber before the valve was closed; this marked the start of the fumigation and the beginning of the exposure period. A gas sample (40 mL) was taken from the chamber headspace through the sampling valve using a B-D® 100 mL gas-tight syringe (Hamilton, Reno, NV, USA) and the headspace concentration of phosphine, [PH_3], was quantified with GC (vide infra) at temporal intervals: 0.12 (initial), 2, 6, and finally 12 h. Carbon dioxide and oxygen concentration were measured at 15 min intervals with a gas-sampling pump connected in series with an atmospheric gas analyzer, which was looped into the chamber system. Mean carbon dioxide levels were calculated and reported as described below for air temperature. After a duration of 12 h, chamber valves were opened to atmosphere and vacuum was pulled for 1 h to aerate the chamber. Chamber lids were opened; the treated as well as non-treated infested oranges were collected, transferred, and evaluated for mortality, as described above and below.

2.4. Mortality Evaluation

Mortality was assessed 12 to 16 h after completion of a treatment. For the exploratory studies, specimens were removed from the incubation room and carefully transferred, whenever possible, from the vial to a single white sheet of paper that offered a color contrast relative to dark bodies of the adults. Using a stereoscope (Fisherbrand™ Basic Fixed Magnification Stereo Microscope) to aid observation, survivability was diagnosed by locomotion or prodding-induced motion. Mortality was diagnosed by lack of prodding-induced motion and was calculated by subtracting the number of survivors from the number of treated specimens. Control mortality was included as a response in duration–mortality, which was conducted with Polo Plus (LeOra Software, 2002–2007) (vide infra). With respect to the confirmatory fumigations, specimens were carefully transferred from the nylon mesh and the external surface of the orange, using a fine brush (Daler Rowney, Script/Liner, Lake Mary, FL, USA), only when necessary, to the paper sheet. Thereafter, 1- to 2-mm slices of peel were removed with a razor to allow for transactional observation (and collection) of specimens through the depth of the navel (Supplementary Material Figure S3). Survivability of fumigated and non-treated control specimens was diagnosed as described above, and mortality of non-treated control specimens was treated numerically using Abbott's method [14]. Mortality, calculated as a percentage of the response per treatment, was expressed as a function of the number of specimens treated via probit analysis of Finney [15,16] at the 95% confidence level (CL), as further derived in Couey and Chew [17].

2.5. Chemical Analysis and Calibration of Standards

Cytec Canada, Inc. (Niagara Falls, Ontario, Canada) provided the respective 300-lb cylinders of 1.6% phosphine by volume in nitrogen, >99.9% phosphine registered and commercialized as VAPORPH3OS® (note the HDS is required to safely dilute formulations > ca. 2% phosphine into air [18]), and 2.0% phosphine by volume in carbon dioxide, registered and commercialized as ECOFUME® (carbon dioxide can influence the marketability of fresh

fruit). Source cylinders (136.1 kg, 300 lb) of breathing air and carbon dioxide were obtained from Airgas (Fresno, CA, USA). The 1.6% phosphine by volume in nitrogen was sourced for gas chromatography calibrations, as well as for exploratory and confirmatory fumigations. VAPORPHOS® and ECOFUME® were only used in the confirmatory fumigations, as noted below. As is the case for any restricted use pesticide used commercially in California, VAPORPH3OS® and/or ECOFUME® labels approved by the California Department of Pesticide Regulation must be followed. Furthermore, $[PH_3]$ and steady-state concentrations thereof, $[PH_3]_{ss}$, were measured using gas chromatography (GC); retention time (PH3, t_r = 3.2 ± 0.2 min, $\overline{x} \pm s$, 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. Triplicate response curves were generated for each respective sampling interval and each sample of [PH3] referenced to the respective responses. Moreover, $[PH_3]$ levels were reported as the mean (±) a standard deviation ($\overline{x} \pm s$) across the respective responses. Phosphine analyses were performed with a Varian 3800 GC 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) (13 mL min^{-1} H_2, 20 mL min^{-1} air, and 10.0 mL min^{-1} N_2 make-up) at 250 °C that received only 10% of the 15 mL min^{-1} He column flow.

3. Results and Discussion

3.1. Exploratory Fumigations

The mean air temperature ($\overline{x}$), 4.9 °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 [19]. Respective duration–mortality regressions for (applied doses and) $[PH_3]_{ss}$ of 0.4 g m^{-3} (250 ppmv (µL L^{-1})), 0.8 gm^{-3} (500 ppmv (µL L^{-1})), and 1.5 g m^{-3} (1000 ppmv (µL L^{-1})) were modeled using Polo Plus (LeOra Software, 2002–2007). Polo Plus was selected for fitting the duration–mortality response; however, other probit (or logit) models [14–17] can be used to identify efficacious fumigation parameters that are subsequently corroborated in the confirmatory evaluations. The number of adult BT specimens treated (0.4 gm^{-3}: 1644 subjects and 1648 controls; 0.8 gm^{-3}: 1644 subjects and 1648 controls; 1.5 gm^{-3}: 1643 subjects and 1648 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 corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% CL are shown in Figure 1. Likelihood ratio-based hypothesis testing of equality was not rejected (P = 0.118, χ^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, χ^2 = 0.83, df = 2), indicating that the slopes of the regressions respective to $[PH_3]_{ss}$ were not significantly different.

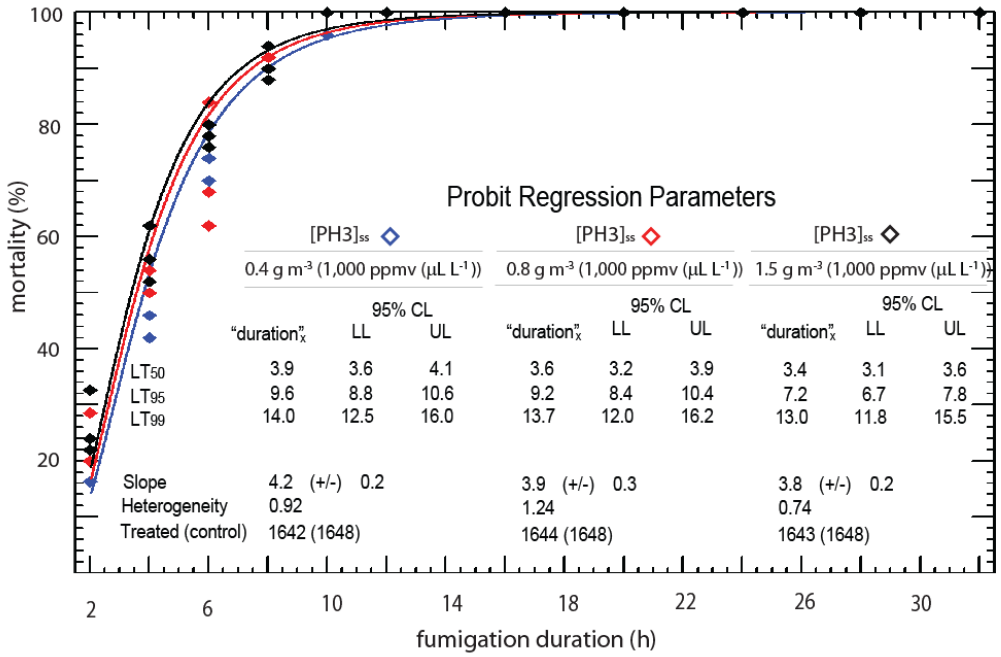

Figure 1. Mortality of adult bean thrips (BT), *Caliothrips fasciatus* (Pergande), adults following fumigation with 1.6% phosphine by volume in nitrogen at air temperature 4.9 ± 0.3 °C ($\overline{x} \pm 2s$) and probit regression analyses (Polo Plus, LeOra Software, 2002–2007) of the duration–mortality response respective to applied doses and steady-state headspace concentrations, $[PH_3]_{ss}$ of 0.4 (250), 0.8 (500), and 1.5 g m^{-3} (1000 ppmv (µL L^{-1})), 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 bounds (upper (UL) and lower (LL) limits) at the 95% confidence level (CL).

Lethal time ratios (LTRs), where the response to $[PH_3]_{ss}$ of 0.4 g m^{-3} (250 ppmv (µL L^{-1})) was normalized to that of 0.8 g m^{-3} (500 ppmv (µ LL^{-1})) or 1.5 g m^{-3} (1000 ppmv (µL L^{-1})), were calculated with (±) 95% confidence intervals across the durations projected to cause 10 to 99% mortality in the treated population. The LTRs were used to identify that $[PH_3]_{ss}$ of 0.8 g m^{-3} (500 ppmv (µL L^{-1})), or 1.5 g m^{-3} (1000 ppmv (µL L^{-1})) were no more efficacious toward BT adults than a $[PH_3]_{ss}$ of 0.4 g m^{-3} (250 ppmv (µL L^{-1})), as LTRs respective to durations > LT_{85} all overlapped or superseded a value of 1 (unity) (Figure 2). Figure 3 shows the projected durations to cause 99% mortality in the treated population (LT_{99}) of adult BT did not vary as a function of $[PH_3]_{ss}$, indicating that a variability in $[PH_3]$ between 0.4 and 1.5 g m^{-3} (250 and 1000 ppmv (µL L^{-1})) did not change the efficacy.

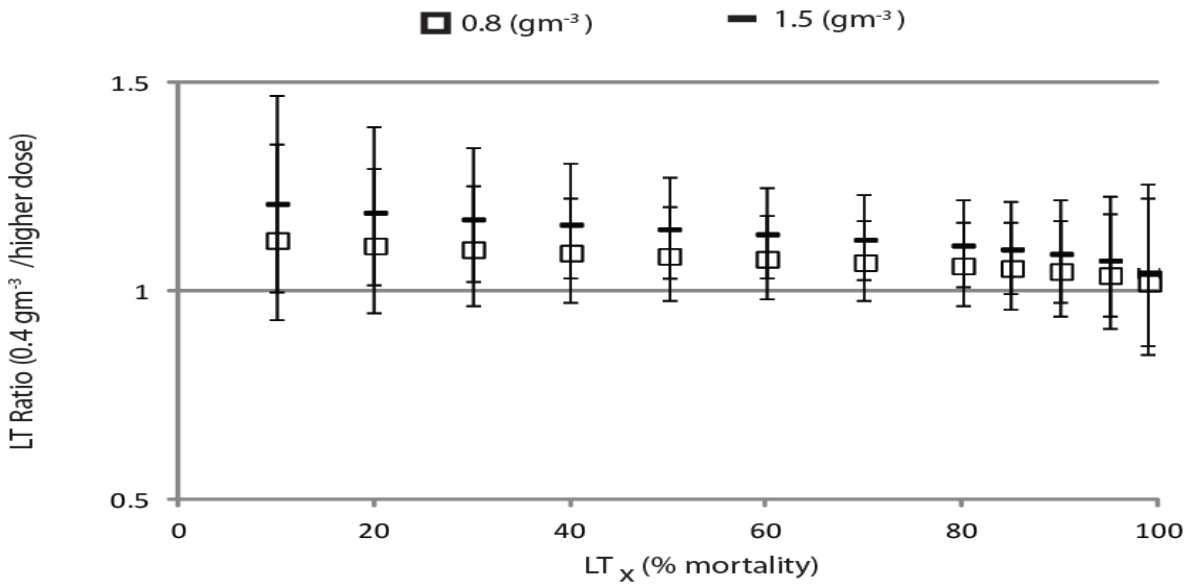

Figure 2. Lethal time ratios (LTRs) associated with steady-state headspace concentrations, $[PH_3]_{ss}$, of 0.4 (250), 0.8 (500), and 1.5 gm^{-3} (1000 ppmv (μL L^{-1})) were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of adult bean thrips at air temperature 4.9 ± 0.3 °C ($\bar{x} \pm 2s$). LTRs respective to durations predicted to yield >85% mortality all overlapped a value of 1 (unity), indicating that maintaining a $[PH_3]_{ss}$, of 0.4 g m^{-3} (250 ppmv (μL L^{-1})) was no more efficacious than maintaining [PH3]$_{ss}$ at 0.8 (500) or 1.5 g m^{-3} (1000 ppmv (μL L^{-1})).

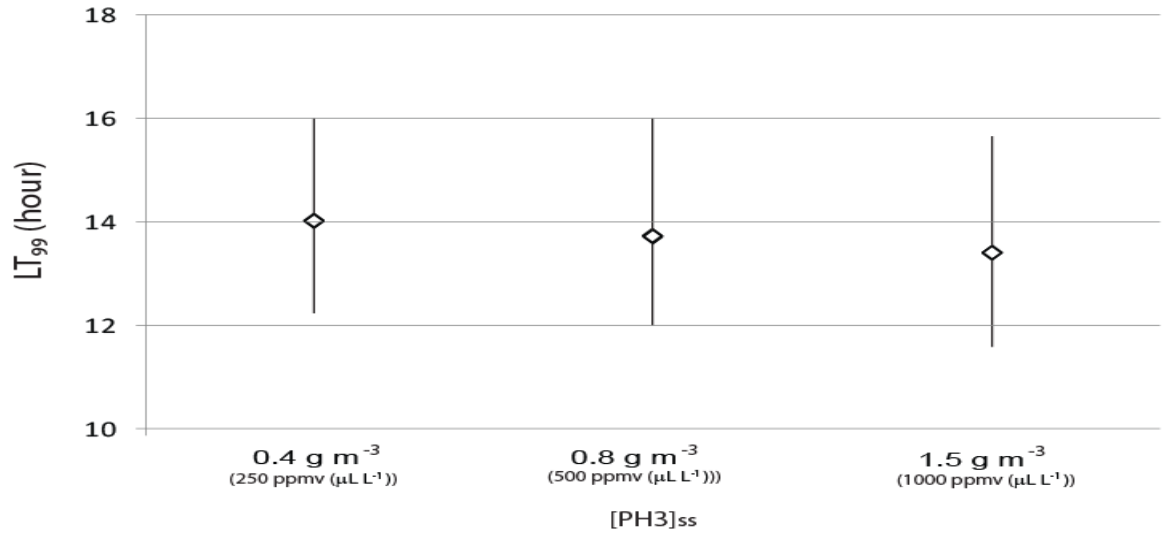

Figure 3. The projected durations to cause 99% mortality in the treated population (LT_{99}) of bean thrips (BT) adults did not vary as a function of steady-state headspace concentrations, $[PH_3]_{ss}$, over the range 0.4 to 1.5 g m^{-3} (250 to 1000 ppmv (μL L^{-1})), indicating that variability in phosphine levels within that range will not change the efficacy of fumigation. Error bars are the estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% confidence level (CL) (see Figure 1). These results indicate that the narcosis threshold for adult BT at air temperature 4.9 ± 0.3 °C ($\bar{x} \pm 2s$) spans $[PH_3] \geq 0.4$ and ≤1.5 g m^{-3} (250≥ and ≤1000 ppmv (μL L^{-1})). If headspace concentrations of phosphine, $[PH_3]$, are recorded outside this "optimal" range, a longer treatment duration could potentially be required to achieve 99% mortality.

To rationalize this result, note the seminal phosphine research of Winks [20–23] in the context of Haber's Rule in its form, $C^z t = \omega$, most usually used in fumigation science [24,25]. Haber's Rule relates concentration (C) and duration time (t), to a level of lethality (ω); in this case, a proxy for the mortality specific to an exposure (Ct). The relative effect of C

versus t toward the response evoked by a specific toxicant to subjects, z (unitless), is the negative slope ($-\Delta\ m/x = z_p^a$) from a least-squares analysis of "log C_0" plotted versus "log t". For phosphine, z changes as a function of C, $[PH_3]_{ss}$ [23]. Winks operationally defined the "narcosis threshold" as the region where $z \cong 0$, whereby increases or decreases in $[PH_3]_{ss}$ did not change the duration required for the particular level of control—99% mortality, in this case. Moreover, $[PH_3]_{ss}$ levels below the "narcosis threshold" required a longer duration to cause 99% mortality, while the $[PH3]_{ss}$ levels above resulted in the narcotic effect and a longer duration required to cause 99% mortality. In this case of treating fresh fruit with phosphine, or any other product in which minimizing the duration required for efficacy is desired, an "optimal" treatment maintains the $[PH_3]$ level within the upper and lower limits of the narcosis threshold. If the $[PH_3]$ level surpasses the limits, a longer, "sub-optimal", treatment duration would be required to achieve an equivalent level of efficacy.

Across the three tested $[PH_3]_{ss}$ levels, 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 3134 specimens survived fumigation with $[PH_3]$ of $\geq$ 0.4 g m^{-3} (250 ppmv (μL L^{-1})) for a duration of $\geq$10 h. These results indicate that the narcosis threshold for adult BT spans $[PH_3] \geq$ 0.4 g m^{-3} (250 ppmv (μL L^{-1})) and $\leq$1.5 g m^{-3} (1000 ppmv (μL L^{-1})). Future work will more clearly outline the upper and lower limits of $[PH_3]$ for an "optimal" treatment, as well as define how much longer of a treatment (than 12 h) is required when $[PH_3]$ is "sub-optimal", or at least $\leq$0.4 g m^{-3} (250 ppmv (μL L^{-1})) and $\geq$1.5 g m^{-3} (1000 ppmv (μL L^{-1})).

3.2. Confirmatory Fumigations

Exploratory results provided evidence that the phosphine fumigation of fresh citrus at $T \geq$ 5.0 °C will control adult BT infestations if $[PH_3]$ is maintained at $\geq$0.4 g m^{-3} (250 ppmv (μL L^{-1})) and $\leq$1.5 g m^{-3} (1000 ppmv (μL L^{-1})) for a duration of $\geq$12 h. To test this, a series of confirmatory trials were conducted to verify efficacy toward adult BT infesting the navel of sweet oranges following the application of ca. 1.5 g m^{-3} (1000 ppmv (μL L^{-1})) or 0.5 g m^{-3} (300 ppmv (μL L^{-1})) phosphine for 12 h at $T \leq$ 5.0 °C (Tables 1 and 2). The mean T was calculated over the course of each trial, as described above (for air temperature). Demonstrating the mortality of quarantine insect pests as a function of probit analyses and associated confidence levels is often requested to qualify phytosanitary treatment efficacy, particularly when a commodity is moved internationally [17,26]. To this end, confirmatory testing resulted in 0 survivors from 38,993 (probit 8.60, 95% CL; probit 9, 72%CL) and 31,204 (probit 8.56, 95% CL; probit 9, 70% CL) BT treated with an applied dose of ca. 1.5 g m^{-3} (1000 ppmv (μL L^{-1})) and 0.5 g m^{-3} (300 ppmv (μL L^{-1})), respectively.

Table 1. Efficacy analysis and parametrics associated with confirmatory fumigations of adult bean thrips (BT), *Caliothrips fasciatus,* (Pergande) infesting navels of sweet oranges, *Citrus sinensis* (L.) having pulp temperature ($\bar{x} \pm 2s$), *T*, and applied doses of ca. 1.5 g m^{-3} (1000 ppmv (μL L^{-1})) delivered by three formulations of cylinderized phosphine −1.6% phosphine by volume in nitrogen—Scheme 1, VAPORPH$_3$OS®—Scheme 2, and ECOFUME®—Scheme 3—with respective carbon dioxide levels, [CO_2] (ppmv = μL L^{-1}), as well as headspace concentrations of phosphine, [PH_3] (ppmv = μL L^{-1})($\bar{x} \pm s$).

					Applied	2 h	6 h	12 h	Adult BT		Adult BT			
	PH_3	[CO_2]	T	Load	[PH_3]$_0$	[PH_3]$_t$	[PH_3]$_t$	[PH_3]$_t$	Control		Treated			
Trial #	Scheme #	(μL L^{-1})	(°C)	(%)	(μL L^{-1}) ($\bar{x} \pm s$)				# obs. : mort.	(% surv.)	# obs. : surv.	$P_{(surv)}$	% mort. $_{corr}$	Probit
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	99.755	7.60
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	99.749	7.59
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	99.758	7.60
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	99.747	7.59
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	99.753	7.60
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	99.749	7.59
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	99.756	7.60
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	99.745	7.59
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	99.741	7.58
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	99.751	7.59
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	99.745	7.59
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	99.751	7.59
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	99.760	7.60
14	2	396 ± 2	4.8 ± 0.3	48.1	986 ± 6	971 ± 10	963 ± 16	928 ± 11	200 : 7	96.50	1296 : 0	0.002309	99.761	7.60
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	99.749	7.59
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	99.753	7.59
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	99.763	7.61
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	99.752	7.59
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	99.744	7.59
20	2	397 ± 2	4.9 ± 0.2	48.1	983 ± 14	972 ± 8	956 ± 17	924 ± 18	198 : 5	97.47	1303 : 0	0.002296	99.764	7.61
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	99.730	7.57
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	99.748	7.59
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	99.761	7.60
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	99.746	7.59
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	99.755	7.60
26	3	48,651 ± 65	4.9 ± 0.2	48.1	995 ± 7	967 ± 10	921 ± 11	845 ± 15	205 : 14	93.17	1302 : 0	0.002298	99.753	7.60
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	99.755	7.60
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	99.742	7.61
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	99.748	7.60
30	3	48,905 ± 82	4.8 ± 0.2	48.1	968 ± 11	954 ± 11	931 ± 11	905 ± 10	199 : 6	96.98	1300 : 0	0.002302	99.763	7.58
								Σ	6000 : 434	92.77	38,993 : 0	0.000077	99.992	8.60

Table 2. Efficacy analysis and parametrics associated with confirmatory fumigations of adult bean thrips (BT), *Caliothrips fasciatus,* (Pergande) infesting navels of sweet oranges, *Citrus sinensis* (L.) having pulp temperature ($\bar{x} \pm 2s$), *T*, and applied doses of ca. 0.5 gm^{-3} (300 ppmv ($\mu L\ L^{-1}$)) delivered by three formulations of cylinderized phosphine—1.6% phosphine by volume in nitrogen—Scheme 1, VAPORPH_3OS®—Scheme 2, and ECOFUME®—Scheme 3—with respective carbon dioxide levels, [CO_2] (ppmv = $\mu L\ L^{-1}$), as well as headspace concentrations of phosphine, [PH_3] (ppmv = $\mu L\ L^{-1}$)($\bar{x} \pm s$).

					Applied	2 h	6 h	12 h	Adult BT		Adult BT			
	PH_3	[CO_2]	T	Load	[PH_3]$_0$	[PH_3]$_t$	[PH_3]$_t$	[PH_3]$_t$	Control		Treated			
Trial #	Scheme #	($\mu L\ L^{-1}$)	(°C)	(%)	($\mu L\ L^{-1}$)($\bar{x} \pm s$)				# obs. : mort.	(% surv.)	# obs. : surv.	$P_{(surv)}$	% mort. corr	Probit
1	1	348 ± 5	4.8 ± 0.2	48.1	301 ± 8	289 ± 5	276 ± 7	260 ± 6	202 : 10	95.05	1300 : 0	0.002302	99.758	7.60
2	1	347 ± 6	4.9 ± 0.3	48.1	305 ± 11	291 ± 6	284 ± 7	265 ± 6	200 : 12	94.00	1299 : 0	0.002304	99.755	7.60
3	1	353 ± 9	4.9 ± 0.2	48.1	300 ± 9	290 ± 6	274 ± 5	258 ± 6	199 : 8	95.98	1302 : 0	0.002298	99.761	7.60
4	1	357 ± 7	5.0 ± 0.3	48.1	305 ± 11	285 ± 5	270 ± 5	251 ± 5	201 : 5	97.51	1301 : 0	0.002300	99.764	7.61
5	1	360 ± 5	5.0 ± 0.2	48.1	295 ± 7	290 ± 11	281 ± 8	261 ± 7	203 : 10	95.07	1299 : 0	0.002304	99.758	7.60
6	1	349 ± 4	4.7 ± 0.3	48.1	310 ± 10	302 ± 8	288 ± 9	276 ± 7	198 : 5	97.47	1298 : 0	0.002305	99.763	7.61
7	1	348 ± 6	4.9 ± 0.3	48.1	292 ± 11	280 ± 10	271 ± 8	254 ± 5	200 : 17	91.50	1297 : 0	0.002307	99.748	7.59
8	1	351 ± 8	4.7 ± 0.3	48.1	289 ± 10	278 ± 8	264 ± 8	252 ± 7	200 : 5	97.50	1302 : 0	0.002298	99.764	7.61
9	1	367 ± 9	5.0 ± 0.2	48.1	302 ± 5	291 ± 7	274 ± 8	259 ± 7	199 : 8	95.98	1303 : 0	0.002296	99.761	7.60
10	1	358 ± 4	5.0 ± 0.2	48.1	293 ± 8	282 ± 10	271 ± 9	260 ± 5	203 : 10	95.07	1305 : 0	0.002293	99.759	7.60
11	2	392 ± 3	5.0 ± 0.3	48.1	305 ± 10	295 ± 7	281 ± 8	262 ± 5	201 : 9	95.52	1302 : 0	0.002298	99.759	7.60
12	2	399 ± 1	4.7 ± 0.2	48.1	310 ± 9	302 ± 10	296 ± 7	277 ± 5	200 : 9	95.50	1301 : 0	0.002300	99.759	7.60
13	2	405 ± 2	4.7 ± 0.2	48.1	299 ± 8	289 ± 9	271 ± 7	263 ± 6	203 : 5	97.54	1295 : 0	0.002311	99.763	7.60
14	2	398 ± 2	4.7 ± 0.3	48.1	287 ± 10	275 ± 8	265 ± 8	253 ± 6	197 : 11	94.42	1298 : 0	0.002305	99.756	7.60
15	2	396 ± 3	4.8 ± 0.3	48.1	295 ± 9	280 ± 8	269 ± 7	251 ± 6	198 : 13	93.43	1301 : 0	0.002300	99.754	7.60
16	2	389 ± 3	4.9 ± 0.2	48.1	301 ± 6	288 ± 9	271 ± 6	252 ± 4	200 : 9	95.50	1300 : 0	0.002302	99.759	7.60
17	2	405 ± 3	4.9 ± 0.2	48.1	305 ± 7	299 ± 11	291 ± 7	281 ± 6	202 : 8	96.04	1300 : 0	0.002302	99.760	7.60
18	3	49,857 ± 102	4.9 ± 0.3	48.1	297 ± 8	284 ± 7	278 ± 6	272 ± 5	203 : 8	96.06	1300 : 0	0.002302	99.760	7.60
19	3	49,002 ± 98	5.0 ± 0.2	48.1	290 ± 11	275 ± 5	264 ± 9	255 ± 7	200 : 12	94.00	1302 : 0	0.002298	99.756	7.60
20	3	48,985 ± 76	4.8 ± 0.3	48.1	285 ± 7	271 ± 8	263 ± 6	250 ± 4	199 : 15	92.46	1297 : 0	0.002307	99.750	7.60
21	3	48,236 ± 97	4.8 ± 0.2	48.1	301 ± 6	291 ± 11	282 ± 9	261 ± 7	201 : 9	95.52	1298 : 0	0.002305	99.759	7.60
22	3	49,206 ± 84	4.9 ± 0.3	48.1	289 ± 9	271 ± 9	261 ± 7	252 ± 5	201 : 5	97.51	1302 : 0	0.002298	99.764	7.61
23	3	49,047 ± 75	4.9 ± 0.3	48.1	295 ± 10	287 ± 9	281 ± 7	275 ± 5	200 : 19	90.50	1304 : 0	0.002295	99.746	7.59
24	3	49,308 ± 79	4.7 ± 0.3	48.1	279 ± 10	271 ± 8	260 ± 7	251 ± 6	198 : 10	94.95	1298 : 0	0.002305	99.757	7.60
								Σ	4808 : 232	95.17	31,204 : 0	0.000096	99.990	8.56

It is critical to note efficacy was consistent across the three different phosphine formulations (1.6% phosphine by volume in nitrogen, VAPORPH3OS®, and ECOFUME®), with each formulation resulting in 0 survivors from ca. 10,000 total treated specimens. From a technical perspective, this result supports the decision to use 1.6% phosphine by volume in nitrogen during exploratory fumigations to outline efficacious fumigation parameters, at least with respect to this pest. Moreover, it provides evidence that shows increasing carbon dioxide levels in chamber headspace, over the range of ca. 0.4 to ca. 63 g m^{-3} (365 to 49,000 ppmv (μL L^{-1}); 0.036 to 5% carbon dioxide by volume in air), did not impact treatment efficacy. Important from an operational perspective, this later result indicates that both commercially available formulations of cylinderized phosphine, VAPORPH3OS® or ECOFUME®, are equivalently efficacious toward adult BT, and either could be used for the proposed fumigation schedule.

Commercial use of VAPORPH3OS® versus ECOFUME® is a function of many factors, including economics, the type of structure/enclosure, the type of commodity being fumigated, and the duration of fumigation as related to the range of [PH_3] required for efficacy. With respect to the fumigation of adult BT infesting the navel of sweet oranges at $T \leq 5.0$ °C, the exploratory data indicated that nearly equivalent efficacy is expected when [PH_3] was maintained ≥0.4 and ≤1.5 g m^{-3} (250≥ and ≤1000 ppmv (μL L^{-1})), conditions that were achieved during the confirmatory trials where, regardless of applied phosphine formulation, [PH_3] levels dropped only ca. 50 to 160 ppmv (μL L^{-1}) over the 12 h treatment. Such minimal variation in [PH_3] loss was expected as leakage of fumigant from the chamber, and sorption by (and residue formation within) nearly equivalent loads was consistent. Moreover, it should be noted that loads of fresh fruits that vary by amount (load factor) and type (variety, size, etc.) are known to only minimally influence [PH_3] levels, as equilibrium between headspace in the enclosure and the load is typically reached within 30 min of application. The 12 h duration required for the stated control of adult BT infesting sweet oranges at $T \leq 5.0$ °C, which is relatively short compared to requirements for other horticultural pests at cold-storage temperature [27,28], indicating that an initial phosphine application of ≤1.5 g m^{-3} (1000 ppmv (μL L^{-1})) and ≥0.5 g m^{-3} (300 ppmv (μL L^{-1})) will not likely require additional applications or "top-ups", as they are known, to fumigation service providers in order to maintain the [PH_3] level ≥0.4 g m^{-3} (250 ppmv (μL L^{-1})) over the course of treatment.

Phosphine can be applied in several types of fumigation enclosure and, in all cases, preparations should be made to minimize the potential for phosphine-catalyzed corrosion of metals within the structure [29–31]. With respect to existing infrastructure geared to conduct QPS methyl bromide chamber fumigations, either VAPORPH3OS® or ECOFUME® can be used. If only a few fumigations are required, or as chamber size decreases from a geometry of ca. 280 m^{-3} (280,000 L; 10,000 ft^{-3}) typical to the California industry, it becomes increasingly difficult to justify the use of VAPORPH3OS®, which requires the HDS air dilution system for application, in favor of ECOFUME®, which is distributed as a carbon dioxide dilution. On the other hand, as chamber size increases, or the number of fumigations increase, it becomes increasingly difficult to justify the use of ECOFUME® in favor of VAPORPH3OS®, as the mass of phosphine in ~40 cylinders of ECOFUME® equals that in a single VAPORPH3OS® cylinder.

This rationale extends to controlled-atmosphere rooms or any other gas-tight enclosure where the load is typically < ca. 50% and the fumigator introduces "plugs" of the phosphine formulation at the maximum level that can be safely delivered into an atmosphere with ≥ambient levels of oxygen (ca. 25 g m^{-3}, 2.0% phosphine by volume in air, ca. 20,000 ppmv ((μL L^{-1})), regardless of whether the phosphine dilution is with air, via HDS and VAPORPH3OS®, or by carbon dioxide in the case of ECOFUME®. Considerations may change with respect to tarpaulins, shipping containers, and reefer containers, as well as gas-tight enclosures, where the load is typically > ca. 50%. In such instances, where "free" headspace is relatively limited, the structures are more often "purged" with the phosphine formulation. The use of VAPORPH3OS® via HDS uniquely allows for phosphine to be

introduced at levels of <25 g m^{-3}, which serves to decrease the amount of phosphine exhausted during the application process.

4. Conclusions

The results provide evidence to support the control of adult BT infesting California sweet oranges at a pulp temperature (T) of $\geq$ 5.0 °C following fumigation with an applied dose of $\leq$1.5 g m^{-3} (1000 ppmv (μL L^{-1})) phosphine, at least when headspace levels are maintained at $\geq$0.4 g m^{-3} (250 ppmv (μL L^{-1})) for $\geq$12 h. Operationally, these fumigations can be conducted at ambient temperature throughout most of the export season, in a variety of enclosures and with a choice of commercially available phosphine formulations. Requisite food tolerances and MRLs are established for citrus and, although additional phytosanitary treatment demonstrations may be requested by Australia per guidelines of International Standards for Phytosanitary Measures No. 28 [3], interest from California packers, shippers, importers, and consumers will ultimately drive the commercial adoption of phosphine as a QPS treatment, both for the control of adult BT in citrus and beyond.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7060134/s1, Figure S1: Method of collecting adult BT, Figure S2: Containing the BT in the navel, Figure S3; Evaluating BT in the navel.

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Notes: Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

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