CAF2020 Paper No. P-6-7-54

Walse S (2021) Recent advances in methyl bromide alternatives for fresh fruit at USDA-ARS. Pp. 201-207. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Recent advances in methyl bromide alternatives for fresh fruit at USDA-ARS

Spencer S Walse

USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center,
9611 S. Riverbend Avenue, Parlier, CA 93648-9757, USA.
Corresponding author's email: spencer.walse@ars.usda.gov

Abstract

This research generally serves technical interaction between industry, ARS Office of Pest Management Policy (OPMP), the US State Department, USEPA, and the UN Environmental Program, Methyl Bromide Technical Options Committee to support postharvest compliance with the Montreal Protocol. Research on off-gassing potential and labeling of postharvest fumigants: methyl bromide (MB), phosphine, propylene oxide, and sulfur dioxide, which critically supported mandatory reviews by California and USEPA, was detailed specific to fresh fruit. Moreover, recent research findings for fresh fruit treatments were presented and discussed, including: the application of ethyl formate to fruit in field bins, the use of a sulfuryl fluoride-propylene oxide blend to disinfect and disinfest tree nuts and dried fruit, and as detailed in this article for drosophila control in fresh citrus, a Quarantine Pre-Shipment (QPS) application of phosphine.

Keywords: Postharvest, Methyl bromide alternatives, Phosphine, Ethyl formate, Sulfuryl fluoride

Introduction

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 (Horn and Horn, 2004) 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 maximum residue limit (MRL) of 10 µg/kg (ppb) is established in key markets, including those relevant to citrus exports from California, USA. A 12 h postharvest fumigation with cylinderized phosphine is now used in a quarantine and pre-shipment (QPS) capacity to control bean thrips *Caliothrips fasciatus* (Pergande) in "Navel" orange, *Citrus sinensis* (L.), exports from California, USA to key international markets (Walse and Jimenez, 2021). Spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is now a pest of concern to certain countries and below results are detailed to support an efficacious phosphine schedule with, and without, considering the additionally toxicity of *in transit* cold storage.

Materials and methods

Experimental details were as reported in Walse and Corbett (2019). Briefly, the methodologies used to develop phytosanitary treatments for control SWD, including general rearing and fruit infestation methods, are as originally described in Walse et al. (2012), and more recently outlined in Walse et al. (2021). Fruit was first transferred into a cage with ~2,000 adult SWD for a predetermined amount of time. Infestations were synchronized so that a treatment could be conducted on fruit infested with distinct age groupings, spanning from the egg through pupal life stages. Fruit respective to the age groupings were treated simultaneously, and the relative survivorship was used to identify the most treatment-tolerant age grouping. The variance associated with SWD development on fruit hosts (Bellamy et al. 2013) resulted in age groupings that are comprised of different proportions of life stages, whereby the most treatment-tolerant life stage was most prevalent tin the most treatment-tolerant age group. The most treatment tolerant life stage was used in all exploratory laboratory-scale fumigations to identify efficacious treatment parameters, facilitated by mortality-response models (e.g., probit). The evaluation of SWD mortality following a treatment, which required counting the numbers of adults that emerged from treated fruit, was as detailed in Walse et al. (2016). Results of exploratory treatments were verified in confirmatory treatments at the pilot- or commercial-scale. The overall number of SWD treated, or alternatively the number of SWD survivors, across a series of trials was calculated by summing the results and propagating the error (Walse et al. 2016). The evaluation of overall treatment efficacy following fumigation, cold, or sequential combinations thereof, were as described in Walse and Bellamy (2012). Procedural aspects of phosphine fumigation, as well as the sourcing of citrus fruit, were as described in Walse and Jimenez (2021) and were consistent with previous work to control stored product insects.

Results and discussion

Exploratory fumigations

The average air temperature, 4.8ºC, 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*, 0.2ºC, the 95% confidence interval (Quinn, 1983). For each of the four isolated age groupings, duration-mortality regressions for (applied doses) PH_3 steady-state concentration ($[PH_3]_{ss}$) of 0.4 (250), 0.8 (500), and 1.5 g/m^3 (1000 ppmv(μL/L^1)) were modeled using Polo Plus (LeOra Software, 2002-2007). 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}, LT_{99}, LT_{P9}), and the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% confidence level (CL) are shown in Table 1. Across the age groupings, for each of the three $[PH_3]_{ss}$, likelihood ratio-based hypothesis testing of equality was rejected (0.4 g/m^3: $P = 0.000$, $\chi^2 = 122$, $df = 6$; 0.8 g/m^3: $P = 0.000$, $\chi^2 = 103$, $df = 6$; 1.5 g/m^3: $P = 0.000$, $\chi^2 = 77$, $df = 6$), indicating that the slopes as well as the intercepts of the regressions respective to $[PH_3]_{ss}$ were significantly different. Likelihood ratio-based hypothesis testing of parallelism was also rejected (0.4 g/m^3: $P = 0.000$, $\chi^2 = 23$, $df = 3$; 0.8 g/m^3: $P = 0.000$, $\chi^2 = 19.2$, $df = 3$) indicating that the slopes of the regressions respective to the age grouping were significantly different for the two lowest levels of $[PH_3]_{ss}$. However, at 1.5 g/m^3 parallelism was not rejected ($P = 0.5$, $\chi^2 = 2.2$, $df = 3$), indicating that the slopes of the regressions respective to the age groupings were not significantly different.

Table 1 shows the probit regression parameters (^) for mortality of spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), following fumigation with 1.6% (v/v) phosphine balanced with nitrogen at air temperature 4.8 ± 0.2 ºC ($\overline{x} \pm 2s$) and probit regression analyses 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)).

Table 1. Probit regression parameters for mortality of spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura)

Probit Regression Parameters

		12- to 60-h old (eggs)			36- to 84-h old (small larvae)			108- to 156-h old (large larvae)			300- to 348-h old (pupae)		
			95% CL			95% CL			95% CL			95% CL	
		“duration”$_x$	LL	UL	“duration”$_x$	LL	UL	“duration”$_x$	LL	UL	“duration”$_x$	LL	UL
250 ppmv $[PH3]_{ss}$	LT_{50}	4.9	4.2	5.6	2.7	1.8	3.5	3.9	3.0	4.7	4.3	3.4	5.2
	LT_{95}	13.3	11.9	15.3	10.7	9.5	12.4	10.6	9.4	12.5	12.9	11.3	15.4
	LT_{99}	19.9	17.1	24.0	19.0	15.8	25.0	15.9	13.2	22.2	20.2	16.6	27.2
	Slope	3.9 (+/-) 0.2			2.8 (+/-) 0.2			3.9 (+/-) 0.2			3.5 (+/-) 0.2		
	Heterogeneity	4.35			3.4			4.9			5.6		
	Treated	6420			6616			6708			6312		
500 ppmv $[PH3]_{ss}$			95% CL			95% CL			95% CL			95% CL	
		“duration”$_x$	LL	UL	“duration”$_x$	LL	UL	“duration”$_x$	LL	UL	“duration”$_x$	LL	UL
	LT_{50}	5.7	5.3	6.1	4.6	4.0	5.0	5.5	4.9	5.5	4.6	4.3	4.9
	LT_{95}	13.1	12.2	14.4	10.4	9.6	11.4	10.2	9.6	11.0	11.7	11.0	12.4
	LT_{99}	18.6	16.7	21.2	14.5	12.9	17.0	13.5	12.3	15.1	17.1	15.8	18.9
	Slope	4.5 (+/-) 0.2			4.6 (+/-) 0.3			5.7 (+/-) 0.3			4.1 (+/-) 0.2		
	Heterogeneity	2.28			2.0			1.5			0.7		
	Treated	6420			6616			6708			6312		
1000 ppmv $[PH3]_{ss}$			95% CL			95% CL			95% CL			95% CL	
		“duration”$_x$	LL	UL	“duration”$_x$	LL	UL	“duration”$_x$	LL	UL	“duration”$_x$	LL	UL
	LT_{50}	5.7	5.2	6.3	4.8	4.0	5.4	4.8	3.8	5.5	5.1	4.8	5.5
	LT_{95}	14.1	12.9	15.9	11.8	10.6	13.8	11.1	9.7	13.7	13.2	12.4	14.2
	LT_{99}	20.5	17.9	24.6	17.2	14.6	22.0	15.8	13.0	21.9	19.5	17.7	21.9
	Slope	4.2 (+/-) 0.6			4.2 (+/-) 0.2			4.5 (+/-) 0.3			3.5 (+/-) 0.2		
	Heterogeneity	3.78			4.9			1.4			5.6		
	Treated	6420			6616			6708			6312		

^Number of specimens treated, the regression heterogeneity (H), the projected durations to cause 50, 95, and 99% mortality in the age groupings (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).

To identify the most phosphine-tolerant age grouping at each of the three $[PH_3]_{ss}$, lethal time ratios (LTRs), where the response to 12 to 60 h old specimens was normalized to that of older groupings, 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, irrespective of $[PH_3]_{ss}$, that the older age groupings were less phosphine-tolerant than 12 to 60 h old specimens, as LTRs respective to durations > LT_{85} all overlapped or superseded a value of 1 (unity).

Lethal time ratios (LTRs), where the response to $[PH_3]_{ss}$ of 0.4 g/m^3 (250 ppmv) was normalized to that of 0.8 (500) or 1.5 g/m^3 (1000 ppmv), were calculated with (±) 95% confidence intervals across the durations projected to cause 10 to 99% mortality in the treated population of 12 to 60 h old specimens and provide potential insight into the "narcosis threshold", or "sweet spot", associated with this pest (Winks and Waterford, 1986). The tabulated regression data as well as Fig. 1 shows the projected durations to cause 99% mortality in the treated population (LT_{99}) did not vary as a function of $[PH_3]_{ss}$, indicating that variability in $[PH_3]$ between 0.4 and 1.5 g/m^3 (250 and 1000 ppmv) did not change the efficacy. The LTRs are consistent with the above finding, and identify that $[PH_3]_{ss}$ of 0.8 (500), or 1.5 g/m^3 (1000 ppmv) were no more efficacious toward the most phosphine-tolerant age of SWD, 12 to 60 h old specimens, than a $[PH_3]_{ss}$ of 0.4 g/m^3 (250 ppmv), as LTRs respective to durations > LT_{30} all overlapped or superseded a value of 1 (unity). However, both LT ratios decrease as mortality in the treated population increases, suggesting that if $[PH_3] < 0.4$ g/m^3 (250 ppmv and > 1.5 g/m^3 (1000 ppmv), longer treatment times may be required to achieve the same level of control as observed when $[PH_3] \geq 0.4$ g/m^3 (250 ppmv) and ≤ 1.5 g/m^3 (1000 ppmv), or at least ≅ 0.8 g/m^3 (500 ppmv (μL/L)). 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.

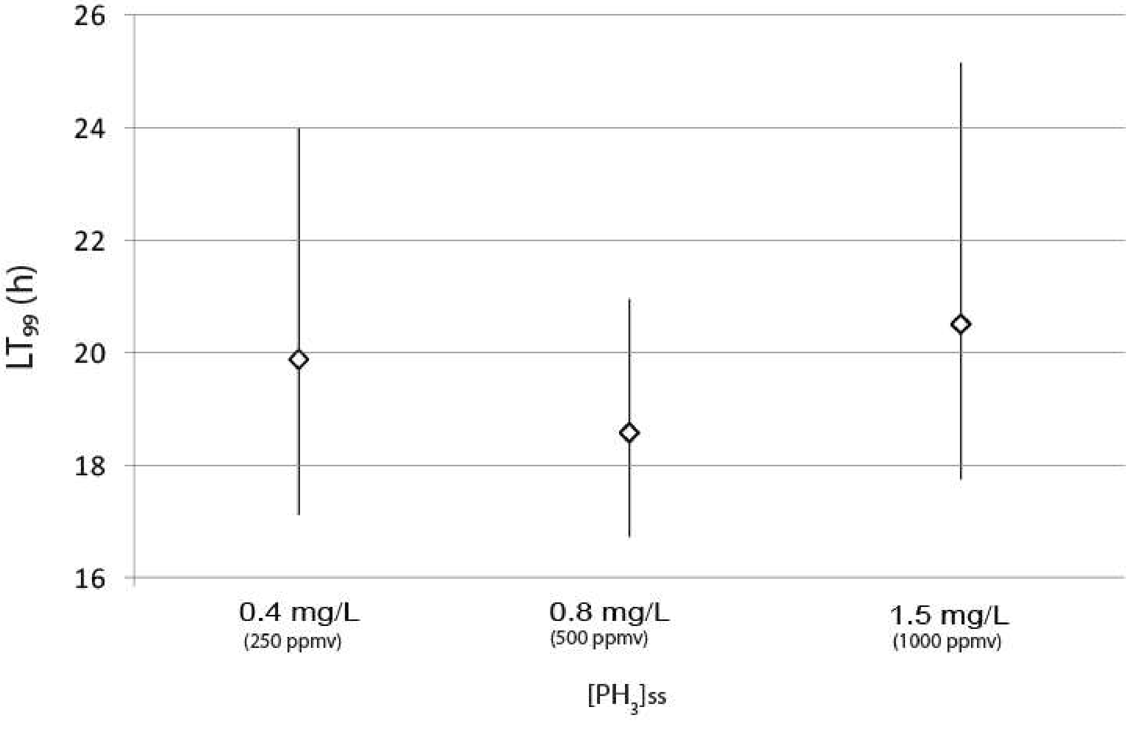

Fig. 1. Projected durations to cause 99% mortality in the treated population (LT_{99}) of population of 12 to 60 h old SWD (the most phosphine-tolerant age).

The projected durations did not significantly 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)), suggesting 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). These results indicate that the narcosis threshold for adult BT spans [PH_3] ≥ 0.4 and ≤ 1.5 g/m^3 (250 ≥ and ≤ 1000 ppmv (µL/L)). 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.

Collectively, these results suggest that the narcosis threshold for 12 to 60 h old specimens, the most phosphine-tolerant SWD age, was bounded by [PH_3] ≥ 0.4 and ≤ 1.5 g/m^3 (250 ≥ and ≤ 1000 ppmv). Future work will more clearly outline the upper and lower limits of [PH_3] for an "optimal" treatment, as well as define the how much longer (than 36 h) is required when [PH_3] is "sub-optimal", or at least ≤ 0.4 (250 ppmv) and ≥ 1.5 g/m^3 (1000 ppmv). 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. Accordingly, the efficacy of phosphine toward SWD at T of ca. 5°C is nearly equivalent across all fruit types, let alone citrus types.

Confirmatory fumigations: 36 h Phosphine fumigation

Exploratory results provided evidence that phosphine fumigation of fresh citrus at $T \geq 5.0$ºC will control SWD infestations if [PH_3] is maintained ≥ 0.4 g/m^3 (250 ppmv) and ≤ 1.5 g/ m^3 (1000 ppmv (µL/L)) for a duration ≥ 36 h. To test this prediction, a series of confirmatory trials were conducted to verify efficacy toward 12- to 60-h old SWD, the most phosphine-tolerant age, infesting the navel of sweet oranges following application of ca. 1.5 (1000 ppmv) or 0.8 g/m^3 (500 ppmv (µL/L)) phosphine for 36 h at $T \leq 5.0$ºC. The average temperature was calculated over the course of each trial as described above. Demonstrating 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 commodity is moved internationally (Couey and Chew, 1986; Follet and Nevin, 2006). Toward this end, confirmatory testing resulted in 0 survivors from 103910 ± 2627 ($n \pm s$) (probit 9, 96.4% CL) and 103850 ± 2271 ($n \pm s$) (probit 9, 96.4% CL) SWD treated, respectively, with an applied dose of ca. 1.5 (1000 ppmv) and 0.8 g/m^3 (500 ppmv).

It is critical to note efficacy was consistent across the three different phosphine formulations (1.6% (v/v) balanced in nitrogen, VAPORPH3OS®, and ECOFUME®), with each formulation resulting in 0 survivors from ca. 30,000 total treated specimens. From a technical perspective, this result supports the decision to use 1.6% (v/v) phosphine balanced with nitrogen in 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 ca. 0.4 to ca. 63 g/m^3 (365 to 49,000 ppmv; 0.036 to 5%) 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 SWD and either could be used for the proposed schedule.

Confirmatory fumigations: 12 h PH_3 fumigation followed by 10 d refrigeration at 5°C

Exploratory results provided evidence that phosphine fumigation of fresh citrus at $T \geq 5.0$ºC will control ca. 95% of 12- to 60-h old SWD, the most phosphine-tolerant age, if [PH_3] is maintained ≥ 0.4 g/m^3 (250 ppmv) and ≤ 1.5 g/m^3 (1000 ppmv) for a duration of 12 h. To test this prediction, a series of confirmatory trials were conducted to verify efficacy following application of ca. 1.5

(1000 ppmv) or 0.5 g/m^3 (300 ppmv) phosphine for 12 h at $T \leq 5.0$ ºC. Confirmatory testing resulted in 18 survivors from 50560 ± 1313 ($n \pm s$) (probit 8.20, 95% confidence level) and 15 survivors from 51210 ± 1167 ($n \pm s$) (probit 8.24, 95% CL) treated SWD, respectively, with an applied dose of ca. 1.5 (1000 ppmv) and 0.5 g/m^3 (300 ppmv).

Previous work on SWD suggested that (the few) survivors of a 12 h fumigation were not likely to also survive a subsequent refrigeration. To test this hypothesis, half of the infested fruit was transferred to refrigeration at ~5°C for 10-d following the 12-h fumigation. Testing resulted in 0 survivors from 50560 ± 1313 ($n \pm s$) treated (probit 8.68, 95% CL; probit 9, 80.2% CL) when refrigeration at $T = (4.9 \pm 1.2)$ ($\overline{x}_{GM} \pm 2\, s_{pooled}$) followed an applied dose of ca. 1.5 (1000 ppmv). An applied dose of 0.5 g/m^3 (300 ppmv) followed by $T = 4.9 \pm 1.2$ ($\overline{x}_{GM} \pm 2\, s_{pooled}$), resulted in 0 survivors from 51210 ± 1167 ($n \pm s$) (probit 8.69, 95% CL; probit 9, 80.5% CL) treated SWD. It is critical to note that citrus exported to key export markets is refrigerated during the 14 to 21 d required to reach port, with pulp temperature (T) rarely outside the range of 0.5 to 5°C.

Discussion

Results indicate that SWD can be controlled in citrus. For the 36 h fumigations, across all formulations, an applied dose of ca. 1.5 (1000 ppmv) resulted in 0 survivors from 103,910 ± 2,627 ($n \pm s$) (probit 9, 96.4% confidence level (CL)) treated SWD, while an applied dose of 0.8 g/m^3 (500 ppmv) resulted in 0 survivors from 103,850 ± 2,271 ($n \pm s$) (probit 9, 96.4% CL) treated. The 12 h fumigations at $T \leq 5$ °C, across all formulations, resulted in ca. 95% control as expected. Importantly, however, when the 12 h fumigation was followed by 10 d of refrigeration at $T \cong 5$°C, an applied dose of ca. 1.5 (1000 ppmv) resulted in 0 survivors from 50,560 ± 1,313 ($n \pm s$) treated (probit 8.68, 95% CL; probit 9, 80.2% CL), while an applied dose of 0.5 g/m^3 (300 ppmv) resulted in 0 survivors from 51,210 ± 1,167 ($n \pm s$) treated (probit 8.69, 95% CL; probit 9, 80.5% CL).

References

Bliss CI (1940) The relation between exposure time, concentration, and toxicity in experiments in insecticides. Ann Entomol Soc Am **33**: 721-766.

Bellamy DE, Sisterson MS, Walse SS (2013) Quantifying Host Potentials: Indexing postharvest fresh fruits for spotted wing drosophila, *Drosophila suzukii*. PLoS ONE. **8** (4): e61227 doi:10.1371/journal.pone.0061227. 2013.

Couey MC, Chew V (1986) Confidence limits and sample size in quarantine research. J Econ Entomol **79**: 887-890.

Follett PA, Neven LG (2006) Current trends in quarantine entomology. Annu Rev Entomol **51**: 359-385.

Miller FJ, Schlosser PM, Janszen DB (2000) Haber's rule: a special case in a family of curves relating concentration and duration of exposure to a fixed level of response for a given endpoint. Toxicology **149**: 21-34.

Quinn TJ (1983) Temperature (monographs in physical measurement) Academic Press London LTD. Pp. 241-280.

Horn F, Horn P (2004) Fresh fruit fumigation with phosphine as alternative for methyl bromide. Proc Methyl Bromide Alternatives and Emissions Res Conf, Orlando, Florida, USA.

Walse SS, Bellamy DE (2012) Combining fumigation and cold-treatment for postharvest control of spotted wing drosophila, Drosophila suzukii, in California-grown table grapes. USDA-APHIS report for Biosecurity Australia and Department of Agriculture, Forestry, and Fisheries, April 13, 2012.

Walse SS, Krugner R., Tebbets JS (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 SS, Jimenez LR, Hall WA, Tebbets JS, Obenland DM (2016) Optimizing postharvest methyl bromide treatments to control spotted wing drosophila, *Drosophila suzukii*, in sweet cherries from Western USA. J. Asian-Pac. Entomol. **19**: 223-232.

Walse SS (2018) QPS use of phosphine for T. Variable. Pp. 569-576. In: Adler CS, Opit G, Fürstenau B, Müller-Blenkle C, Kern P, Arthur FH, Athanassiou CG, Bartosik R, Campbell J, Carvalho MO, Chayaprasert W, Fields P, Li Z, Maier D, Nayak M, Nukenine E, Obeng-Ofori O, Phillips T, Riudavets J, Throne J, Schöller M, Stejskal V, Talwana H, Timlick B, Trematerra P (eds) Proc 12th Intern Working Conf Stored-Prod Prot, Berlin, Germany.

Walse SS, Cha DH, Lee, BH, Follett PA (2021) Postharvest quarantine treatments for Drosophlia suzukii in fresh fruit. Pp. 265-267. In: Garcia M, Roberto F (eds) Drosophila suzukii Managment, Springer Nature, Switzerland.

Walse SS, Jimenez LR (2021) Postharvest fumigation of fresh citrus with cylinderized phosphine to control bean thrips (Thysanoptera: Thripidae) Horticulturae **7**(134) https://doi.org/10.3390/horticulturae7060134

Walse SS, Jimenez LR (2021) Postharvest fumigation of fresh citrus with cylinderized phosphine to control bean thrips (Thysanoptera: Thripidae) Horticulturae **7**(134) *20220*

Walse S, Corbett S (2019) Postharvest fumigation of fresh citrus with cylinderized phosphine to control spotted wing drosophila, *Drosophila suzukii.* Report for USDA-APHIS and Plant and Food New Zealand, October 1.

Winks RG, Waterford CJ (1986) The relationship between concentration and time in the toxicity of phosphine to adults of a resistant strain of *Tribolium castaneum* (Herbst). J Stored Prod Res **22**: 85-92.