Session 10：Quarantine and Regulatory

Postharvest treatment research at USDA-ARS: stored product fumigation

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Abstract

The overall goal of this USDA-ARS research is to ensure the protection and quality of stored product foodstuffs. The results of this research directly enhance production, distribution, and safety of foodstuffs, promote and retain access of United States-grown crops to domestic and foreign markets, and protect the United States and trading partners from the agricultural, ecological and economic threat posed by quarantine and invasive pests. In general, USDA-ARS research related to the fumigation of stored products focuses on the development of techniques to rapidly disinfest raw products of field pests, control pests in processed products amenable to re-infestation and microbial infection, and reduce reliance on fumigation as a stand-alone measure for postharvest disinfestations and disinfections. Specific research objectives include: comparative evaluation of alternative fumigants to methyl bromide in postharvest applications, development of novel technologies to reduce and eliminate atmospheric emissions from chambers used in postharvest fumigation, and design production strategies that allow for a more strategic postharvest use of methyl bromide and alternative fumigants. Recent research findings will be presented and discussed, including: exposure requirements of phosphine on key stored product pests (as related to resistance management), the insecticidal efficacy of fumigant mixtures, and the juxtaposition of methyl bromide regulations and maximum residue level (MRL) regulations. Detailed below is a case study related to the fumigation of walnuts to control navel orangeworm, *Amyelois transitella*, in exports from the United States to control navel orangeworm, which will promote more strategic technical and economic Quarantine Pre-shipment (QPS) use of MB.

Keywords: food security, food safety, maximum residue levels (MRLs), postharvest methyl bromide

1. Introduction

Navel orangeworm (NOW), *Amyelois transitella*, is a pest of concern to walnut growers and shippers from California USA. Several key export markets, regulate NOW as a quarantine pest. Consequently, movement of walnuts from California typically requires a preshipment treatment to mitigate risk. Postharvest fumigation with methyl bromide (MB) is a treatment option to control NOW in walnuts and the use of MB in this capacity is regulated internationally via the Montreal Protocol on ozone-depleting substances under the Quarantine Pre-Shipment (QPS) Exemption. Sulfuryl fluoride (SF) is a promising alternative to MB for postharvest fumigation, particularly for the control of NOW. This study details experimental procedures, predictive models, and toxicological results to support the use of postharvest SF and MB chamber fumigation to control NOW in walnuts from California USA.

2. Materials and Methods

2.1. Insects and mortality

NOW was cultured as described in Tebbets et al. (1978) and USDA (2010); eggs were deposited on ~3x3 cm^2 filter paper sheets over a 48-h ovipositional period. Mortality of non-exposed (i.e., untreated control) and fumigant-exposed eggs was assessed following treatment after incubation for 7 d at 27.0 ± 1.0°C and 80 ± 2% r.h. ($\bar{x} \pm s$). Insects were more likely to survive and there was greater certainty in diagnosing survivorship after the treatment if incubated under conditions described above rather than if refrigerated post-fumigation at 5-10°C under simulated commercial transport and storage conditions, which confound the effect of a fumigation event on mortality.

Using a microscope, exposed-egg mortality was diagnosed by the development of white coloration and survivability by vacated egg cases. Control-egg mortality was diagnosed similarly and was assumed to be equal to that in fumigation trials and was treated numerically using Abbott's method (1925) as described by Finney (1944, 1971). Dose-mortality curves were generated using probit analyses (Polo Plus, LeOra Software, 2002-2007) with control mortality as a modeled response.

2.2. Exploratory fumigations

To determine the most MB- as well as SF-tolerant NOW life stage in prelude to confirmatory testing, dose-response data was generated in a series of exploratory fumigations performed in modified Labonco® 28.32-L vacuum chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2010). Discrete developmental stages of NOW (pupae, larvae, old eggs) were fumigated concomitantly within a chamber for a particular fumigation trial. Larvae and individuals that had recently pupated were extracted from colonies and transferred to 7-cm diameter Petri-dish cages modified with five 1-cm gas-portals in the lid each covered with 40-mesh stainless steel strainer cloth. Test specimens were acclimated, or tempered, to fumigation temperature of either 15.6 ± 0.5 or 21.0 ± 0.5°C ($\bar{x} \pm s$) for 12 h prior to treatment. Eggs sheets were collected and transferred to a 20-dram clear plastic vial with similar gas-portals on the bottom, snap cap, and side.

After fumigation, lids and valves were opened to atmosphere and vacuum was pulled to aerate the chambers. Pupa-containing cages were inspected for adult emergence over the tempering and/or fumigation period; the adults were counted, physically removed with an aspirator, and placed in 20-dram clear plastic vials. All treated insects, as well as untreated controls, were then incubated at as described above.

2.3. Confirmatory export fumigations

Fumigations were conducted at treatment temperatures of 15.6 ± 0.5 or 21.0 ± 0.5°C ($\bar{x} \pm s$) using 241.9-L steel chambers (55.88l × 55.88w × 76.83h cm) housed in the facility described above. The chamber was first loaded with two 0.5 ft^3 sand bags each wrapped in plastic packaging that displaced ~28.3 L total of chamber volume. To simulate a commercial scenario, packaging materials were consistent with the export of California walnuts; each chamber contained four cardboard boxes, containing 25 lbs each of inshell Hartley variety walnuts (Diamond, Stockton USA) with plastic poly-liners (39.1l × 29.1w × 23.8h cm) and stacked on their sides in two columns. Boxes were opened prior to loading the chamber, poly-liners unfolded, and then cages containing 125-250 eggs each were buried amongst the uninfested walnuts. The liner was refolded, the boxes were then resealed, and subsequently transferred into the chamber. Chamber load, estimated as a percentage ($V_{commodity}/V_{chamber}$x100) (Monro, 1969), was 50.9 ± 0.7% ($\bar{x} \pm s$).

Chambers loaded with infested walnuts and test specimens, non-fumigated control specimens, source-gas cylinders, and gas-tight syringes were tempered at the respective treatment temperature for at least 12 h. Walnut meat temperature was confirmed prior to fumigation in three uninfested walnuts drilled to accommodate each of three probes (YSI scanning tele-thermometer) that recorded the respective temperature at different locations within boxes undergoing treatment. Temperature probes were then removed, circulation fans internal to the chamber were turned on, and chamber lids clamp-sealed in preparation for treatment. A vacuum of approximately 70 mmHg was established in each chamber. Gas-tight super-syringes (Hamilton® 500, 1,000, or 1,500 mL) were filled with a volume of either MB or SF to achieve the requisite dose as predetermined in preliminary calibration studies. A 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 either normal atmospheric pressure (NAP) or a pressure of 100 mmHg was established in each chamber before the valve was closed; this marked the beginning of the exposure period. Gas samples (40 mL) were taken temporally at standard intervals from the chamber headspace through a LuerLok® valve using a B-D® 100 mL gas-tight syringe and quantitatively analyzed for MB or SF with GC-FID or a GC-PFPD, respectively. For the vacuum fumigation trials, initial concentrations of fumigant in chamber headspace were based on the average headspace measurements recorded in five different fumigations at NAP having otherwise identical parameters and final 4-h concentrations were measured in samples withdrawn after the reestablishment of NAP in the chambers. Fumigant exposures were expressed as a concentration × time cross product, "CT", as calculated by the method of Monro (1969).

Following the final sampling for fumigant concentration, chamber valves were opened to atmosphere and a 1-h aeration period was initiated. Chamber lids were then opened and the treated and non-treated insect cages were collected, placed into respective pull-string cloth bags, and transferred to an incubator at 27.0 ± 1.0°C and 80 ± 2% r.h. ($\bar{x} \pm s$) for 7 d prior to mortality evaluation.

2.4. Chemical analysis

Fumigant levels in headspace of fumigation chambers were measured using gas chromatography; retention time was used for chemical verification and the integral of peak area, referenced relative to liner least-squares analysis of a concentration – detector response curve, will be used to determine concentration (Walse et al., 2012). Detector response and retention indices were determined each day in calibration studies by diluting known volumes of fumigants into volumetric gas vessels. MB analyses was with a Varian 3800 and splitless injection (150°C) using a gas sampling port (110°C) with a 0.1 mL-sample loop, a 2 mm id x 2 m Teflon® column packed with 10% OV-101 on Gas-Chrom Q® (100/120 mesh) held at 100°C for 10 min. The FID detector, which is plumbed to receive only 10% of the 15 $mlmin^{-1}$ He carrier flow, was at 275°C with respective flows of 30 $mLmin^{-1}$ H_2, 250 $mLmin^{-1}$ air, and 5.0 $mLmin^{-1}$ N_2 make-up. SF analyses will be with a Varian 3800 and splitless injection (150°C) using a gas sampling port (125°C) with a 250 μL-sample loop, a packed GSQ analytical column (L = 30 m, ID = 4.5 mm) held at 100°C for 10 min, and a PFPD detector (30 mL/min H_2, 250 mL/min air, and 5.0 mL/min N_2 make-up) at 250°C that receives only 10% of the 1.2 ml He/min column flow.

3. Results and Discussion

3.1. Most SF-and MB-tolerant NOW life stage

Direct methods of analysis were used to identify eggs (60 to 108 h-old at fumigation start) as the age of NOW most tolerant toward MB; discrete developmental stages and ages were

concomitantly fumigated over a range of applied doses as described above. Dose-mortality analyses demonstrated that pupae and larvae of NOW were generally less tolerant than eggs toward MB under reduced pressure of ~100mmHg at 15.6±0.5°C ($\bar{x} \pm s$) (Table 1). A similar effect was observed under atmospheric pressure at the same temperature.

Probit regressions of the dose-mortality response were used to quantify the relative tolerance of NOW eggs and CM eggs to MB fumigation for 24 h under normal atmospheric pressure at 15.6 ± 0.5 as well as 21.0 ± 0.5°C ($\bar{x} \pm s$). Figure 1 shows the number of specimens treated, the regression heterogeneity (H), the projected CT exposures to cause 50, 95, 99, and 99.9968% mortality in the treated population (respectively LE_{50}, LE_{90}, $LE_{99,}$ and LE_{P9}), and the corresponding estimates of the upper (UL) and lower limits (LL) at the 95% level of confidence (LOC). Likelihood ratio-based hypothesis testing of equality and parallelism was rejected ($P < 0.5$), indicating that the slopes and the intercepts are not the same. Lethal exposure ratios (LERs) were calculated with 95% LOC intervals and used to identify statistically significant decrease in the exposure projected to cause > 50% mortality in the treated population of NOW eggs at 21.0 ± 0.5°C relative to 15.6 ± 0.5°C.

Table 1. Lethality of MB to NOW life stages under 100 mmHg vacuum at 15.6°C

stage	exposure (mg L^{-1}h)	# treated	% surviving	% mortality (corr.)
larvae	64	50	0	100
	84	50	0	100
	0 (contr.)	25	88	-
pupae	64	50	0	100
	84	50	0	100
	0 (contr.)	25	85	-
egg	64	100	43.4	28.8
	84	100	31.4	48.5
	0 (contr.)	50	61.0	-

It is generally accepted that insect eggs are relatively more tolerant to SF than other life stages of the same species (UNEP 2011; Walse et al., 2009). Probit regressions of the dose-mortality response were used to quantify the tolerance of NOW eggs to SF fumigation at 15.6 ± 0.5°C ($\bar{x} \pm s$) for 24 h under normal atmospheric pressure as well as for 4 h under 100 mmHg. Figure 2 shows the number of specimens treated, the regression heterogeneity (H), the projected CT exposures to cause 50, 95, 99, and 99.9968% mortality in the treated population (respectively $LE_{50,}$ LE_{90},$LE_{99,}$ and LE_{P9}), and the corresponding estimates of the upper (UL) and lower limits (LL) at the 95% LOC.

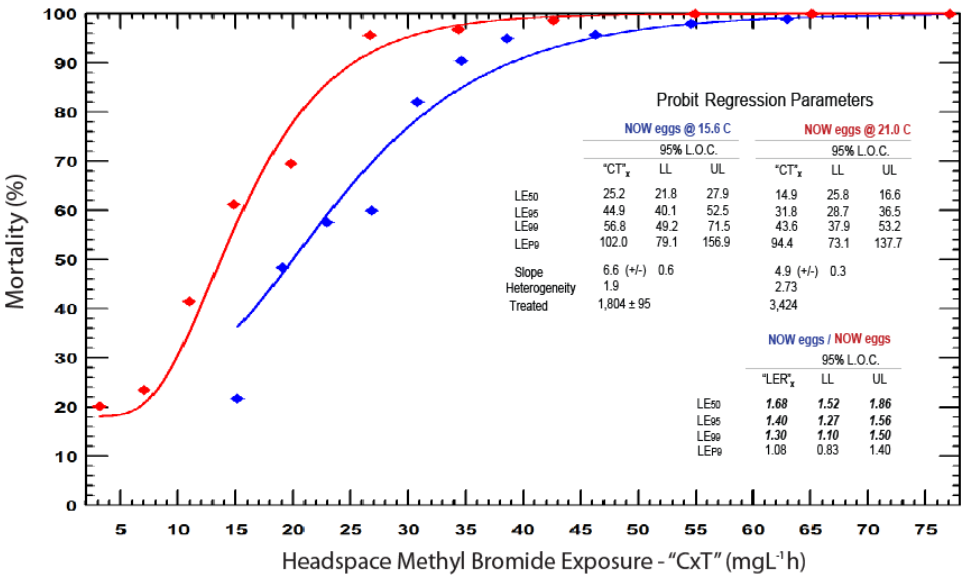

Figure 1 Mortality of NOW eggs (60 to 108 h-old at start of fumigation) following methyl bromide (MB) fumigation and corresponding probit regression analyses; NOW eggs were statistically more tolerant toward MB at 15.6 ± 0.5°C relative to 21.0 ± 0.5°C as indicated by 95% confidence intervals of lethal exposure ratios that do not bracket unity (***bold italics***).

Demonstrating 99.9968% (i.e., Probit 9) mortality of insect pests is often required to qualify phytosanitary treatment efficacy, particularly when commodity is moved internationally (Couey and Chew, 1986; Follet and Nevin, 2006). Projected LE_{P9} values required for efficacy against the most MB- and SF-tolerant life stage (12-60 h old eggs at fumigation start) were superseded, or at least bracketed at the at the 95% LOC, by the exposures observed in the confirmatory fumigations (*vide infra*) and support the conclusion that the proposed confirmatory fumigations will result in Probit 9-level mortality (95% LOC) of NOW infested walnuts at temperature ≥ 15.6 ± 0.5°C.

3.2. Confirmatory fumigations

Chamber fumigations were conducted in the context of confirming the efficacy of MB as well as SF toward NOW eggs at temperature > 15.6°C. Differential sorption by walnut loads resulted in a range of CTs across replicate trials for each fumigant (Table 2, 3). Under 100 ± 5 mmHg vacuum at treatment temperature of 15.6 ± 0.5°C with 4-h treatment durations, SF exposures ≥ 405 $mgL^{-1}h$ (433 $mgL^{-1}h$ max.) and MB exposures ≥ 140 $mgL^{-1}h$ (151 $mgL^{-1}h$ max.) resulted in complete mortality of 35,775 (Probit 9 at the 68% LOC or Probit 8.7 at the 95% LOC) and 41,242 (Probit 9 at 73% or Probit 8.8 at the 95% LOC) NOW eggs, respectively. Under normal atmospheric pressure at treatment temperature of 15.6 ± 0.5°C with 24-h treatment durations, SF exposures ≥ 670 $mgL^{-1}h$ (716 $mgL^{-1}h$ max.) and MB exposures ≥ 104 $mgL^{-1}h$ (126 $mgL^{-1}h$ max.) resulted in complete mortality of 38,942 (Probit 9 at the 71% LOC or Probit 8.8 at the 95% LOC) and 35,050 (Probit 9 at the 68% LOC or Probit 8.7 at the 95% LOC) NOW eggs, respectively. In addition, duplicate fumigation trials with an applied dose of 16 mgL^{-1} MB for 24 h under normal atmospheric pressure at treatment temperature of 21.0 ± 0.5°C resulted in complete control of 6,956 total NOW eggs and an average exposure of 88.2 $mgL^{-1}h$, which is consistent with the regression-projected 95 % confidence interval of Probit 9 control *(vide supra).*

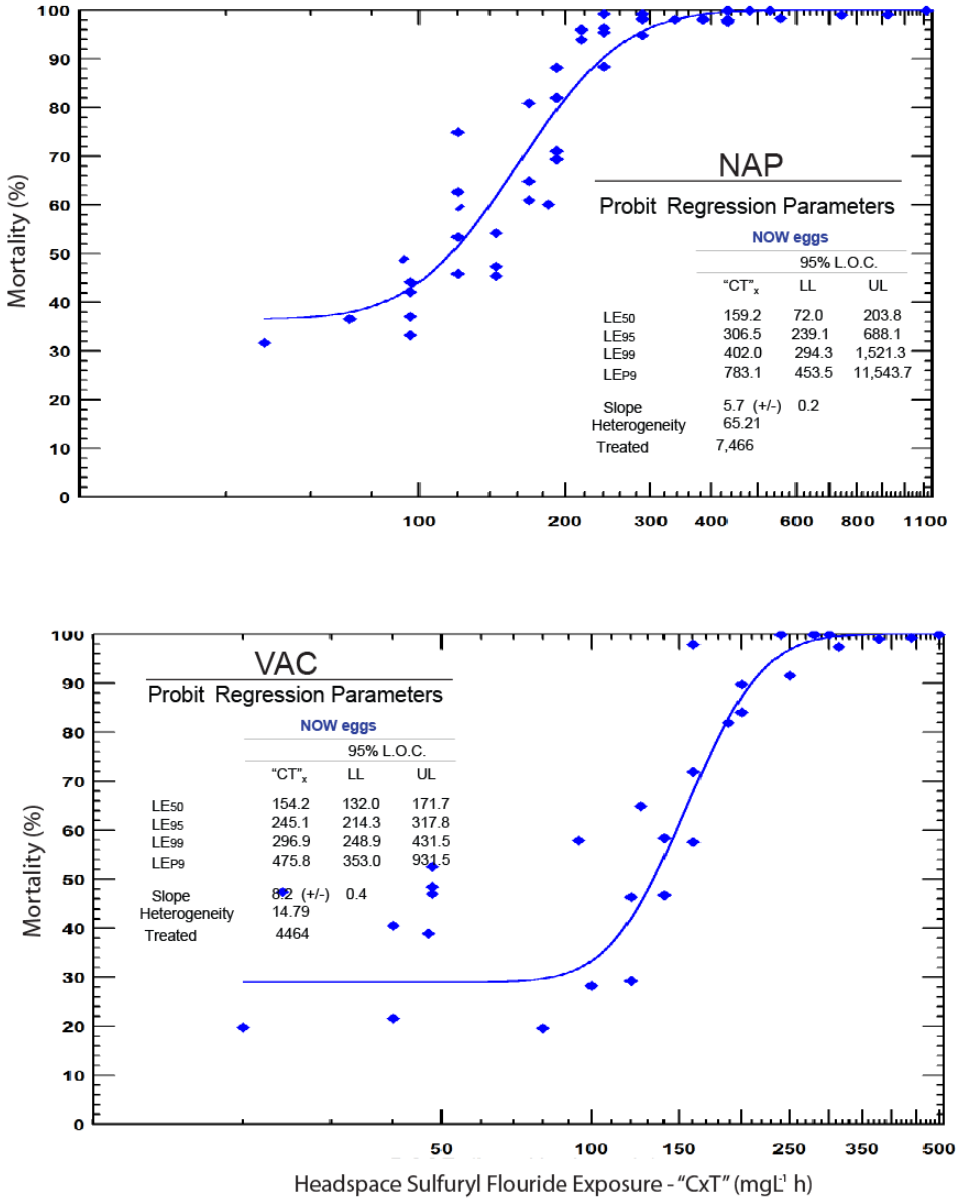

Figure 2 Dose-mortality response of NOW eggs (60 to 108 h-old at start of fumigation) following sulfuryl fluoride (SF) fumigation at 15.6 ± 0.5°C under normal atmospheric pressure (*top panel*) as well as at pressure of 100mmHg (*bottom panel*).

Table 2 Listing of methyl bromide (MB) fumigation trials with corresponding exposure and efficacy data for NOW eggs.

Trial	Gas	Temp.	P_0	Dose	$[X]_{t<5min}$	$[X]_{t\ 0.5h}$	$[X]_{t\ 1h}$	$[X]_{t\ 2h}$	$[X]_{t\ 4h}$	$[X]_{t\ 24h}$	CxT	sorption	P_{final}	# treated	survivors
		^0C	mmHg				mgL^{-1}				$mgL^{-1}h$	%	mmHg		
1	MB	21.0	NAP	16.0	19.3	10.1	7.2	5.2	3.9	2.2	88.0	88.6	-	4587	
2	MB	21.0	NAP	16.0	19.3	9.6	7	5.1	3.9	2.3	88.4	87.9	-	2369	
3	MB	15.6	NAP	24.0	26.2	16.3	13.2	9.8	5.3	2.8	125.6	89.3	-	4341	
4	MB	15.6	NAP	24.0	26.7	16.5	14.0	10	5.0	2.5	120.4	90.6	-	5437	
5	MB	15.6	NAP	24.0	25.4	15.7	13.1	9.4	4.7	2.0	109.8	92.1	-	2324	
6	MB	15.6	NAP	24.0	25.3	15.5	12.8	9.1	4.4	1.9	104.7	92.5	-	4752	
7	MB	15.6	NAP	24.0	26.9	16.8	14.4	10.4	4.9	2.6	121.4	90.3	-	3584	
8	MB	15.6	NAP	24.0	27.1	17.0	14.5	10.2	4.7	2.3	116.2	91.5	-	5465	
9	MB	15.6	NAP	24.0	27.0	16.9	14.3	9.9	4.8	2.5	118.6	90.7	-	4775	
10	MB	15.6	NAP	24.0	26.0	15.9	13.6	9.6	4.9	2.8	121.0	89.2	-	4372	
											117.2 ±6.8 ($\bar{x}\pm s$)	90.8 ±1.2	-	Σ 35050	Σ 0
11	MB	15.6	100	56.0	59.8 ŧ	-	-	-	12.6		144.8	78.9	104	2638	
12	MB	15.6	99	56.0	59.8	-	-	-	9.9		139.4	83.4	110	3455	
13	MB	15.6	100	56.0	59.8	-	-	-	10.5		140.6	82.4	103	2056	
14	MB	15.6	100	56.0	59.8	-	-	-	15.7		151.0	73.7	101	3295	
15	MB	15.6	99	56.0	59.8	-	-	-	10.6		140.8	82.3	108	4581	
16	MB	15.6	99	56.0	59.8	-	-	-	11.5		142.6	80.8	102	2654	
17	MB	15.6	100	56.0	59.8	-	-	-	13.8		147.2	76.9	103	3626	
18	MB	15.6	99	56.0	59.8	-	-	-	10.7		141.0	82.1	101	3159	
19	MB	15.6	100	56.0	59.8	-	-	-	9.9		139.4	83.4	105	2573	
20	MB	15.6	99	56.0	59.8	-	-	-	13.4		146.4	77.6	102	3762	
21	MB	15.6	100	56.0	59.8	-	-	-	12.5		144.6	79.1	102	2459	
22	MB	15.6	100	56.0	59.8	-	-	-	12.9		145.4	78.4	104	3649	
23	MB	15.6	100	56.0	59.8	-	-	-	13.7		147.0	77.1	101	3335	
											143.9 ±3.6	79.7±3.0		Σ41242	Σ 0

ŧ initial concentrations of fumigant in chamber headspace were based on the average headspace measurements recorded in five different fumigations at NAP

Table 3 Listing of sulfuryl fluoride (SF) fumigation trials with corresponding exposure and efficacy data for NOW eggs.

Trial	Gas	Temp.	P_0	Dose	$[X]_{t<5min}$	$[X]_{t\,0.5h}$	$[X]_{t\,1h}$	$[X]_{t\,2h}$	$[X]_{t\,4h}$	$[X]_{t\,24h}$	CxT	sorption	P_{final}	# treated	survivors
		°C	mmHg				mgL^{-1}				$mgL^{-1}h$	%	mmHg		
1	SF	15.6	NAP	32.0	39.2	33.4	32.5	31.9	30.8	26.4	701.5	32.7	-	3215	
2	SF	15.6	NAP	32.0	38.8	35.6	33.5	31.5	29.2	26.5	686.1	31.7	-	3691	
3	SF	15.6	NAP	32.0	39.6	31.9	30.5	29.6	29.1	28.0	693.2	29.3	-	2572	
4	SF	15.6	NAP	32.0	39.5	34.5	33.6	32.4	30.4	25.7	692.3	34.9	-	3312	
5	SF	15.6	NAP	32.0	40.4	33.9	33.1	32.3	29.6	24.5	670.9	39.4	-	3564	
6	SF	15.6	NAP	32.0	41.5	33.9	33.0	32.5	29.2	25.7	679.0	38.1	-	3599	
7	SF	15.6	NAP	32.0	40.6	34.2	32.9	31.9	30.0	27.3	702.8	32.8	-	3614	
8	SF	15.6	NAP	32.0	41.6	34.6	33.5	32.7	29.9	25.2	682.8	39.4	-	3839	
9	SF	15.6	NAP	32.0	41.4	35.1	34.3	33.3	31.5	26.6	716.1	35.7	-	3961	
10	SF	15.6	NAP	32.0	41.9	33.8	32.5	31.8	29.5	25.1	675.0	40.1	-	4154	
11	SF	15.6	NAP	32.0	42.9	32.9	32.5	31.9	30.2	28.0	711.6	34.7	-	3421	
											691.9 ± 14.8 ($\bar{x} \pm s$)	35.3 ±3.6		Σ 38942	Σ 0
12	SF	15.6	99	104.0	123.3 [t]	-	-	-	91.6		429.8	25.7	102	3183	
13	SF	15.6	100	104.0	123.3	-	-	-	81.3		409.2	34.1	101	3682	
14	SF	15.6	100	104.0	123.3	-	-	-	85.5		417.6	30.7	105	3527	
15	SF	15.6	100	104.0	123.3	-	-	-	79.4		405.4	35.6	110	3714	
16	SF	15.6	99	104.0	123.3	-	-	-	80.2		407.0	35.0	105	2106	
17	SF	15.6	100	104.0	123.3	-	-	-	82.9		412.4	32.8	103	3652	
18	SF	15.6	100	104.0	123.3	-	-	-	80.2		407.0	35.0	102	1689	
19	SF	15.6	100	104.0	123.3	-	-	-	81.0		408.6	34.3	101	2965	
20	SF	15.6	99	104.0	123.3	-	-	-	86.8		420.2	29.6	103	3471	
21	SF	15.6	100	104.0	123.3	-	-	-	86.3		419.2	30.0	102	2561	
22	SF	15.6	100	104.0	123.3	-	-	-	92.3		431.2	25.1	106	2642	
23	SF	15.6	100	104.0	123.3	-	-	-	93.4		433.4	24.2	107	2583	
											416.8 ± 10.2	31.0 ±4.1		Σ 35775	Σ 0

t initial concentrations of fumigant in chamber headspace were based on the average headspace measurements recorded in five different fumigations at NAP

4. Conclusions

Methyl bromide (MB) and sulfuryl fluoride (SF) chamber fumigations were evaluated for postharvest control of navel orangeworm (NOW), *Amyelois transitella*, in inshell walnut exports from California USA. The dose-mortality response of NOW eggs, the most MB- and SF-tolerant life stage of NOW (12 to 60-h old at fumigation), was established using probit regression analyses in the absence of walnut load and were used to guide confirmatory testing. To simulate infestation of inshell walnuts, eggs were contained in gas-permeable cages and buried amongst uninfested walnuts at a 50% chamber load. Fumigations were conducted using MB or SF at 15.6 ± 0.5 °C ($\bar{x} \pm s$). Doses of 24 mgL^{-1} (1.5 lbs/1,000 ft^3) MB or 32 mgL^{-1} (2 lbs/1,000 ft^3) SF applied for 24 hr under normal atmospheric pressure corresponded to respective exposures, expressed as a concentration x time products (CTs), ≥ 105 $mgL^{-1}h$ or ≥ 670 $mgL^{-1}h$ and resulted in complete mortality of 35,050 and 38,942 NOW specimens, respectively. Doses of 56 mgL^{-1} (3.5 lbs/1,000 ft^3) MB or 104 mgL^{-1} (6.5 lbs/1,000 ft^3) SF applied for 4 hr under vacuum of 100 ± 5 mmHg resulted in respective exposures ≥ 140 $mgL^{-1}h$ or ≥ 405 $mgL^{-1}h$ and complete mortality of 41,242 and 35,775 NOW specimens, respectively.

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