# A COMPARISON OF METHYL BROMIDE AND PROFUME® EFFICACY AGAINST THE EGGS OF NAVEL ORANGEWORM AND THE DIAPAUSING LARVAE OF CODLING MOTH IN WALNUT FUMIGATIONS CONDUCTED AT NAP AND UNDER VACUUM: MULTIFACTOR EXPLORATION OF THE INSECTICIDAL EFFICACY AND DEGRADATION OF SULFURYL FLUORIDE IN STORED WALNUTS

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## ABSTRACT

Multivariable experimental designs, which facilitate the analyses and interpretation of data, can be used to simultaneously delineate the contribution of various factors that influence the overall effectiveness of a fumigant. Using this statistics-based approach, existing or novel fumigants can be rapidly and thoroughly screened for optimal dose-duration responses, applicability toward a particular commodity, and physicochemical behavior within a commodity, the target organism(s), and the environment. Sulfuryl fluoride, or ProFume®, a fumigant that been used to target other postharvest insect pests,[1-3] has been proposed as an alternative to methyl bromide for treating dried walnut commodity infested with navel orangeworm (*Amyelois transitella*) eggs and diapausing coddling moth (*Cydia pomonella*) larvae. Here we detail ProFume® treatment schedules for these species under both atmospheric pressure (NAP) and reduced pressure (-100 mmHg) environments. In addition, we report the relative influence of dose, pressure, temperature, and exposure duration on both insect mortality, as well as, levels of $SF_2O_2$, $FSO_3^{1-}$, and $F^{1-}$ residues.[4,5]

## OBJECTIVES

1) Determine the efficacy (LD50 and LD99.99) of methyl bromide and ProFume® against eggs of *Amyelois transitella* on in-shell walnut at 15.6 ºC using NAP for 24 h and reduced pressure (660 mmHg) for 4 h.
2) Determine the efficacy (LD50 and LD99.99) of methyl bromide and ProFume® against diapausing larvae of *Cydia pomonella* in in-shell walnuts at 15.6 ºC using NAP for 24 h and reduced pressure (660 mmHg) for 4 h.
3) Determine an empirical relationship between ProFume® efficacy on these "tolerant" life stages and dose, pressure, temperature, and exposure duration.

## PROCEDURES

Labonco® 1-cu. ft. chambers (27.93 L) were used for fumigations and were loaded with in-shell Hartley walnuts (Diamond, Stockton USA) at ~33% by volume (13 ± 0.1 lbs.). For the determination of treatment schedules/protocols, chambers contained either 125-250 1-2 day-old eggs that had been deposited on ~3x3 $cm^2$ paper sheets, or 50 walnuts that were drilled (1/4" bit) ~1 cm to accommodate a single larvae prior to being resealed with sticky-tac. The seeded larvae

had been reared for 6 weeks on a bran and honey diet and then held in diapause (8 h photophase, 18ºC) for ~4 weeks. For the multifactorial experimental runs, the eggs and the larva were loaded together into the chambers.

Mortality of the exposed and non-exposed (i.e., control) egg and larvae stages was assessed following treatment after a weeklong incubation at 28ºC and 18ºC, respectively. Using a microscope, exposed-egg mortality was diagnosed by the development of white coloration and survivability by vacated egg cases. Control-egg mortality, which typically is ~40%, was diagnosed similarly and was treated numerically using Abbott's method. Larval mortality was also diagnosed visually (white color) and survivability by locomotion or by prodding-induced motion. Moribund larvae, which were categorized via inconclusive diagnostics, were incubated for an additional two weeks at 28ºC with a bran food source. Following this period, survivability was assessed by cocoon formation and/or adult emergence. Dose-mortality curves were generated using Probit 2007 software.

Gas chromatography was used to quantify ProFume®; during the fumigation trials, doses were confirmed and monitored using a gas sampling valve injector with a 10 μL sample loop, a packed GSQ analytical column held at 100°C (L = 15 m, ID = 4.5 mm), and a PFPD detector receiving only 10% of the column flow. For residue determinations, walnuts (25 g) were homogenized in 500-mL glass vessels with 200 mL of 0.01M NaHCO3 buffer at pH 7 and 0.1μ (NaCl-adjusted). Vessels were stored at 19°C for 24 h and then, for the analysis of ProFume®, duplicate 500 μL aliquots of headspace were withdrawn and injected (splitless) onto a megabore GSQ analytical column (L = 30 m, ID = 0.53 mm, df = 0.25 μm) held at 100°C with μECD detection.

For the determination of $FSO_3^{1-}$ and $F^{1-}$ residues, EPA method 300.1 "anions in drinking water" was used with 250 μL full-loop injections after purification of aqueous homogenate. A Buchner funnel with a #1 filter was used to remove solids, which were rinsed with an additional 200 mL of DI water (18 mΩ). After dilution of the solution to 500 mL, triplicate aliquots (100 mL) were then each passed at a ~0.5 mL/min flow rate through a DSC-18 12-mL solid-phase extraction cartridge (Supelco®) containing 2 g of packing material that had been preconditioned with sequential methanol (2 x 10 mL), 50%:50% acetonitrile:water (2 x 10 mL), and water (4 x 10 mL) rinses. The cartridge was eluted with an additional 5 mL of water. Each sample was then passed at a ~0.5 mL/min flow rate through a series of 1-mL solid-phase extraction cartridges (Dionex®), a cation-exchange followed by a fruit juice (i.e., C 40-50), which were preconditioned as described above. After an additional 5 mL of water was used to rinse the cartridges, the sample was concentrated to dryness via vacuum evaporation, and reconstituted in 500 μL of DI water prior to analysis using ion chromatography. $FSO_3^{1-}$ ($R_t$ 13.1 min) was quantified routinely via this approach; however, $F^{1-}$ ($R_t$ 3.69 min) was prone to poor chromatographic resolution. Thus, it was also analyzed indirectly as $AlF^{2+}$ by ICP-MS. Sample preparation involved introducing $Al(CHO_2)_3$ at 500ppm into the reconstituted 500 μL sample. The complexed $AlF^{2+}$ was then resolved from excess $Al^{3+}$ by ion chromatography with 250 μL full-loop injections and a cation-exchange column as described in the EPA D6919-03. Eluant fractions containing $AlF^{2+}$ were collected, concentrated, and analyzed for mass spectrometric confirmation of Al.

The multifactorial experimental design was generated and the results were analyzed using Design Expert 7.0 (Stat-Ease, Inc.). A four-factor central composite design was employed,[6,7] which contained five levels ($-\alpha$, -1, 0, 1, $\alpha$) of the four factors, $x_1$–$x_4$, and six replicates of the center-point. Conditions of temperature, duration, and pressure were chosen to accommodate, or span, those applicable to standard industrial practice, at least with respect toward analogous methyl bromide protocols. The maximum dose value of 96 mg/L (i.e., g/m$^3$, oz./1000-cu. ft.) was selected because it supersedes the highest dose value for LC99.99 obtained during schedule development.

| *Factor (original units)* | *Factor levels* | | | | |
|---|---|---|---|---|---|
| | $-\alpha$ | -1 | 0[a] | 1 | $\alpha$ |
| $x_1$: dose (mg/L) | 0 | 24 | 48 | 72 | 96 |
| $x_2$: temp (°C) | 5 | 10 | 15 | 20 | 25 |
| $x_3$: duration (h) | 1 | 12 | 24 | 36 | 48 |
| $X_4$: pressure (- inch. Hg) | 0 | 7 | 14 | 21 | 28 |

[a] 0 = center point

The design involved a total of 30 experiments, which were run in a randomized order in three different time blocks. The modeled response(s) ($y$) was insect mortality or residue levels. The full second-order model with all possible two-factor interactions contained 15 parameters:

$$y = \beta_0 + \beta_1 x_1 + \beta_{11} x_1^2 + \beta_2 x_2 + \beta_{22} x_2^2 + \beta_3 x_3 + \beta_{33} x_3^2 + \beta_4 x_4 + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4$$

The parameters of this full second-order model include: $\beta_0$, a constant or offset term; $\beta_1$, $\beta_2$, $\beta_3$, $\beta_4$ estimate the linear effects of the factors; $\beta_{11}$, $\beta_{22}$, $\beta_{33}$, $\beta_{44}$ estimate the quadratic (curvature) effects of the factors; and $\beta_{12}$, $\beta_{13}$, $\beta_{23}$, $\beta_{24}$, $\beta_{34}$ estimate the interaction effects between every pair of two factors.

## RESULTS

Briefly, the LD99.99 for ProFume® against larvae of *Cydia pomonella* in in-shell walnuts at 15.6 ºC was 8 mg/L at NAP for 24 h and 24 mg/L at –100 mmHg reduced pressure (660 mmHg) for 4 h. Previous work on equivalent methyl bromide schedules established 56 mg/L treatments for both scenarios; we will confirm these schedules in the near future. The LD99.99 for ProFume® against eggs of *Amyelois transitella* on in-shell walnuts at 15.6 ºC was 32 mg/L at NAP for 24 h and 80 mg/L at –100 mmHg reduced pressure (660 mmHg) for 4h. Corresponding methyl bromide schedules were respectively 16 mg/L and 32 mg/L; however, more repetitions are needed at NAP for 24 h.

Following the egg (80 mg/L) and larvae (32 mg/L) LD99.99 treatments for 4 h at reduced pressure, ProFume® residues decreased below the EPA prescribed maximum threshold level (3 ppm) in 168 h and 24 h, respectively. $F^{1-}$ residue levels were typically between 10-70 ppb, below the EPA prescribed maximum threshold level of 10 ppm for walnuts. $FSO_3^{1-}$ residue

levels, a valuable molecular diagnostic of ProFume®-derived hydrolytic contribution to the $F^{1-}$ residues, were below 10 ppb in every case.

## DISCUSSION

The results of this analysis are presented from several perspectives, including environmental health concerns surrounding sub-ppm chronic exposures to $F^{1-}$ in diet and the practicality of ProFume® as a methyl bromide replacement for treating stored walnuts infested with these pests. Physicochemical data on ProFume®, such as the homogeneously- and heterogeneously-catalyzed hydrolysis rate constants, water solubility, and Henry's Law constants, are presented in the context of their compulsory significance to each perspective. Furthermore, the marked potential for multivariate experimental techniques in streamlining the development of biocidal treatments for perishable and durable commodities is discussed, particularly their predictive and confirmatory power.

## REFERENCES


1) Scheffrahn, R.H.; Hsu, R.C.; Osbrink, W.L.A.; Su, N. *J Agric. Food Chem.*, 37, 203-206, 1989.
2) Bell, C.H.; Savvidou, N. *J. Stored Prod. Res.*, 35, 233-247, 1999.
3) Barak, A.V.; Wang, Y.; Zhan, G.; Wu, Y.; Xu, L.; Huang, Q. *J. Econ. Entomol.* 99(5), 1628-1635, 2006.
4) Heuser, S. G. *Anal. Chem.* 35(10), 1477-1480, 1963.
5) Cady, G.H.; Misra, S. *Inorg. Chem.* 13(4), 837-841, 1974.
6) Deming, S. N.; Morgan, S. L. *Experimental Design: A Chemometric Approach*; 2nd ed.; Elseiver Science Publishers B.V.: Amsterdam, 1993.
7) Montgomery, D. C. *Design and Analysis of Experiments*; 5th ed.; John Wiley & Sons, Inc.: New York, 2001.
8) *Fluoride Risks Are Still a Challenge*; Chemical and Engineering News Editorial, 84 (36), 34-37 2006. http://pubs.acs.org/cen/government/84/8436gov1.html