View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by AIR Universita degli studi di Milano

Methyl bromide and sulfuryl fluoride effectiveness against red flour beetle life stages

Hartzer, M.*#[1], Subramanyam, B.[1], Chayaprasert, W.[2], Maier, D.E.[1], Savoldelli, S.[3], Campbell, J.F.[4], Flinn, P.W.[4]
[1] Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA
Email: mkaczmar@k-state.edu
[2] National Agricultural Machinery Center, Kasetsart University – Kamphaengsaen, Nakhon Pathom, Thailand
[3] Department of Food, Agricultural, and Urban Environmental Protection & Biodiversity Enhancement, Faculty of Agriculture, University of Milan, Via G. Celoria 2, I-20133, Milan, Italy
[4] USDA-ARS, Center for Grain and Animal Health Research, Manhattan, Kansas 66502, USA

* Corresponding author
# Presenting author

DOI: 10.5073/jka.2010.425.254

Abstract

The efficacy of methyl bromide (MB) and sulfuryl fluoride (SF) for managing all life stages of the red flour beetle, *Tribolium castaneum*, was investigated in the Hal Ross Flour Mill at Kansas State University. Eggs, young larvae, large larvae, pupae, and adults, confined in plastic compartments with dusting of flour and 2-cm deep flour, were exposed at 25 mill locations to two MB and two SF fumigations; in May and August 2009. MB and SF treatments were conducted by commercial fumigators, and each fumigation lasted 24 h. Gas monitoring lines were placed near the bioassay boxes to measure gas concentrations over time during fumigations. Both MB treatments killed 100% of all stages in the boxes except for large larvae in a few locations. In these locations, the mortality of large larvae ranged from 96-98%. SF treatments killed 100% of all stages except eggs. In the May treatment with SF, egg mortality ranged from 44-100% with only two boxes showing 100% mortality, because of under-dosing. Under-dosing occurred because the mill temperature was assumed to be greater than 27ºC when it was actually below 27ºC. In the second SF trial, only three boxes had egg mortalities that were less than 100%. However, data from the two replications showed that the mean mortalities of eggs and large larvae between MB and SF were not significantly different from each other.

Keywords**:** *Tribolium castaneum*, Methyl bromide, Sulfuryl fluoride, Ct product, Efficacy assessment.

1. Introduction

Methyl bromide (MB) is considered a Class I ozone depleting substance, and under the Montreal Protocol it was phased out in the United States in 2005 (UNEP 1998), except for certain critical uses under the critical use exemption category. Many MB alternatives for managing stored-product insects have been explored such as sulfuryl fluoride (SF), carbonyl sulphide, and heat treatment (Fields and White, 2002; Campbell and Arbogast, 2004; Boina et al., 2008). SF was registered by the United States Environmental Protection Agency under the trade name ProFume® by DowAgroSciences LLC, Indianapolis, Indiana, USA, in January 2004. SF was evaluated in flour mills and found to be effective, but the egg stage of the red flour beetle, *Tribolium castaneum* (Herbst), is reported to be relatively less susceptible when compared with postembryonic stages (Bell et al., 2003). Most comparisons of MB and SF treatments use different facilities for each of the different treatments or conduct treatments at different times of the year, and typically rely on pre- and post-trapping insect numbers to verify treatment effectiveness (Campbell and Arbogast, 2004; Small, 2007). The objective of the current study was to verify effectiveness of MB and SF fumigation in a pilot flour mill using insect bioassays under nearly identical environmental and sealing conditions.

2. Materials and methods

Bioassay boxes were constructed using large plastic craft boxes that contained twelve smaller compartments, each with individual lids (Fig. 1). Holes were cut in all four sides of the smaller boxes and the lids. These holes were covered with a metal mesh screen (90 µm opening) for ventilation and gas diffusion. Corresponding holes were also cut in the large outer box. In the top six small compartments, 2 cm of flour was added to simulate unsanitary mill conditions. In the remaining six compartments, a dusting of flour was added to simulate sanitary conditions. Eggs, small larvae (first instars), large larvae (late instars), pupae, and adults of *T. castaneum* were introduced into the separate compartments within each large box. Each compartment held 50 individuals of a life stage. Small data loggers to measure

temperatures (SmartButton, ACR Systems Inc., British Columbia, Canada) were added to the remaining two uninfested compartments. Data loggers were set to record temperature every two minutes. The boxes with insects were placed in 25 preselected locations across all five floors in the Hal Ross Flour Mill, a state-of-the-art pilot mill at Kansas State University. One box, placed in the laboratory growth chamber at 28ºC and 65% r.h., served as the control treatment.

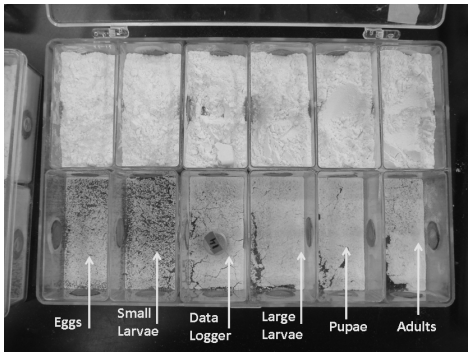

Figure 1 Rectangular plastic box with 12 compartments used in insect bioassays in the Hal Ross Flour Mill.

Some boxes were positioned directly on the floor, while others were placed within pieces of equipment. Gas monitoring lines were attached to the center of each box and during treatments, gas concentrations were continuously monitored. Treatments typically started in the late afternoon and each treatment lasted 24 h. Once the treatment was completed, the mill was opened for the gas to dissipate overnight and the boxes were retrieved the next morning when it was safe to reenter the mill. In the laboratory, boxes were placed in incubators set at 28ºC, and after 24 h adult mortality was determined by counting live and dead insects. For immature stages, insects and flour were removed from compartments and placed in 150 mL round plastic containers and reared to adulthood. Pupae were counted after 11 d, large larvae after 18 d, and small larvae and egg stages were counted after 45 d.

The first set of MB and SF treatments were performed during May 6-7 and May 27-28, 2009, respectively. The second set of treatments with MB and SF was conducted during August 11-12 and August 19-20, 2009, respectively. The final set of treatments will be conducted in May 2010. Data from four control treatments were averaged to obtain mean and associated standard errors for mortality of each of the five stages. Mortality data for each stage from the two MB and two SF replications were averaged to show the mean mortality and associated standard errors (SE). The effectiveness of MB and SF against each stage was compared by subjecting mortality data to two-way analysis of variance (ANOVA) using the GLM procedure (SAS Institute, 2002). Differences in mortality were considered to be significant at the $\alpha = 0.05$ level. Egg mortality was less than 100% in treatments with SF. Therefore, variation in egg mortality observed during May and August treatments were separately plotted against SF concentration x time (Ct) product at each of the 25 box locations.

3. Results and discussion

The fumigation starting time, mill temperature, amount of gas used, and Ct products for MB and SF are shown in Table 1. By using the same facility during the same month, the environmental conditions and experimental protocols followed were essentially similar, allowing us to do a true side-to-side comparison of MB and SF effectiveness against *T. castaneum* life stages.

Table 1 Starting time, amount of fumigant used, and range of mill temperatures and Ct products for May and August fumigations.

	May treatments		August treatments	
Variable	**MB**	**SF**	**MB**	**SF**
Starting time	6:40 p.m.	6:00 p.m.	2:50 p.m.	2:45 p.m.
kg of gas used	181.4	567.0	159	511
Mill temp. (°C)	22 - 23	23 - 26	27 - 31	28 - 32
Ct product (g-h/m^3)	283 - 327	923 - 1191	268 - 318	663 - 1003

The average control mortality across all life stages is shown in Figure 2. Mortality for both small larvae and eggs in compartments with flour dust was higher than expected (>40%). The higher mortality with these stages could be attributed to lack of sufficient food and possibly cannibalism. The achieved Ct product for MB in May and August resulted in 100% kill of all life stages, except for large larvae in a few locations (Table 2). However, the mean mortality for large larvae in compartments with flour dusting and in 2 cm deep flour was still close to 99.7%.

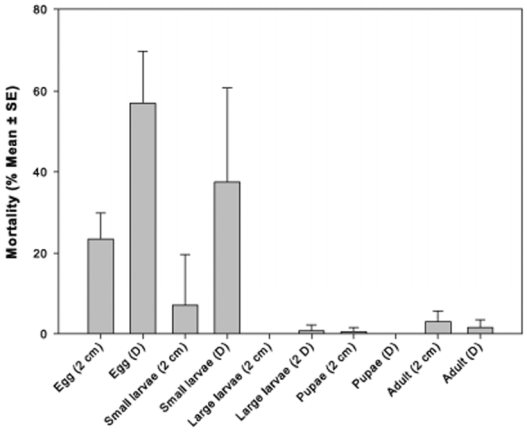

Figure 2 Mortality of life stages of *T. castaneum* in the control treatment (Each mean is based on four 4 replications).

Table 2 Average mortality of life stages of *T. castaneum* in bioassay boxes during the two MB and SF fumigations.

Treatment	Insect Stage	Flour Depth	Mean±SE Mortality (%)[a]
MB	Eggs	2 cm	100.00
	Eggs	Dusting	100.00
	Small larvae	2 cm	100.00
	Small larvae	Dusting	100.00
	Large larvae	2 cm	99.70 ± 0.16
	Large larvae	Dusting	99.96 ± 0.08
	Pupae	2 cm	100.00
	Pupae	Dusting	100.00
	Adults	2 cm	100.00
	Adults	Dusting	100.00
SF	Eggs	2 cm	91.00 ± 9.00
	Eggs	Dusting	92.55 ± 7.05
	Small larvae	2 cm	100.00
	Small larvae	Dusting	100.00
	Large larvae	2 cm	99.96 ± 0.04
	Large larvae	Dusting	100.00
	Pupae	2 cm	100.00
	Pupae	Dusting	100.00
	Adults	2 cm	100.00
	Adults	Dusting	100.00

[a]Each mean is based on *n*=2 replications.

The SF treatments were effective against all postembryonic stages. Interestingly, the effectiveness on eggs during the May SF treatment ranged from a low of 44 to a high of 100% (Fig. 3 and 4). During the August treatment egg mortality with SF only in three locations ranged from 94-98% (Fig. 5**).** The poor efficacy against eggs in May is due to using 14% less SF than that required to kill all of the eggs.

This occurred because a temperature of 26.7°C was planned and used for dosage calculation, but the actual mill temperatures ranged from 22.2-25.0°C.

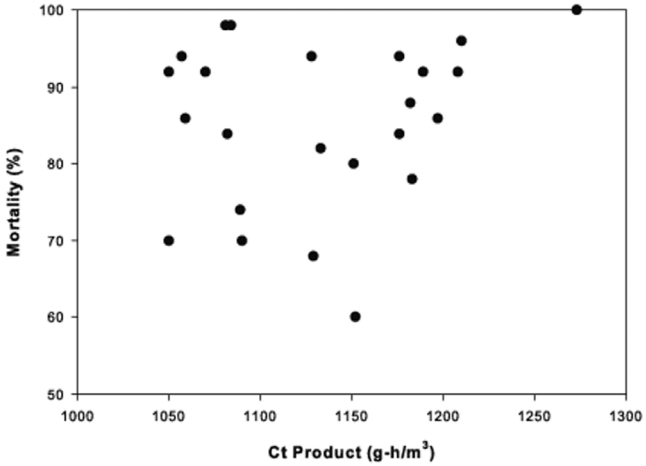

Figure 3 Scatter plot showing variation in egg mortality in compartments with flour dusting as a function of the concentration x time (Ct) product during May 6-7, 2009 fumigation with sulfuryl fluoride.

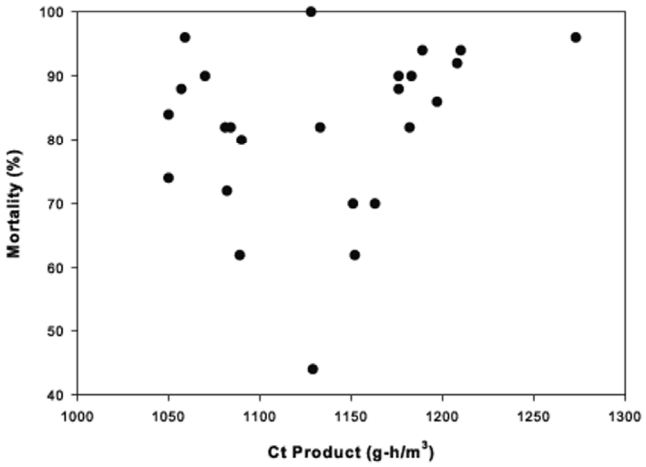

Figure 4 Scatter plot showing variation in egg mortality in compartments with 2 cm deep flour as a function of the concentration x time (Ct) product during May 6-7, 2009 fumigation with sulfuryl fluoride.

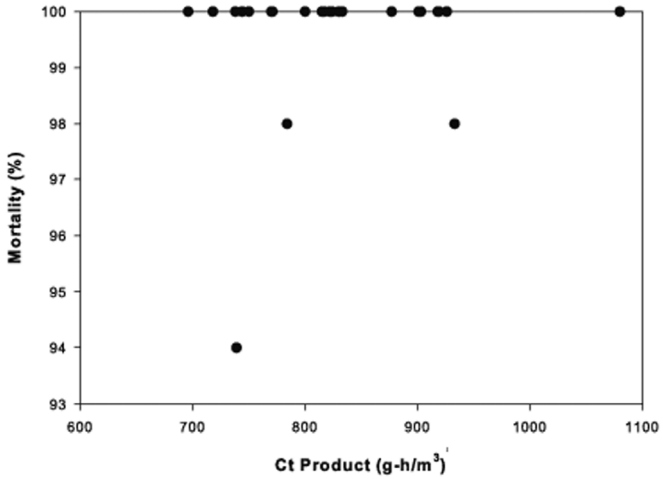

Figure 5 Scatter plot showing variation in egg mortality in compartments with flour dusting as a function of the concentration x time (Ct) product during August 19-20, 2009 fumigation with sulfuryl fluoride.

The only two stages that survived included the egg stage (with SF) and large larvae (with MB and SF). All other stages were completely controlled by MB and SF. Therefore, differences in the effectiveness of MB and SF on eggs or large larvae were compared. The comparisons for each stage show that the mortality of eggs or large larvae was not significant ($P > 0.05$) between MB and SF treatments (Table 3). Similarly, flour depth did not influence mortality of eggs or large larvae. The interaction term was also not significant, indicating that the mortality of eggs and large larvae were consistent between the two treatments (MB and SF) and at the two flour depths. The results from the two replications showed that under nearly identical environmental and sealing conditions, the effectiveness of MB and SF on the mortality of five life stages of *T. castaneum* was similar, despite differences in the survival of eggs with SF and large larvae with MB and SF.

Table 3 Two-way analysis of variance statistics comparing mortality of eggs and large larvae between MB and SF treatments in compartments with flour dusting and 2 cm deep flour.

Stage	Source	df	Mean square	*F*-value	*P*-value*
Egg	Treatment[a]	1	135.30	2.07	0.22
	Depth[b]	1	1.20	0.02	0.90
	Treatment x Depth	1	1.20	0.02	0.90
	Error	4	65.35	-	-
Large larvae	Treatment[a]	1	0.09	5.14	0.09
	Depth[b]	1	0.02	1.08	0.36
	Treatment x Depth	1	0.01	0.40	0.56
	Error	4	0.02	-	-

[a]Treatments are MB and SF. [b]Depths represent flour dusting and 2 cm deep flour in bioassay compartments.
*None of the *P*-values is significant at $P = 0.05$.

Acknowledgments

This study was funded by a 2008 USDA-CSREES Methyl Bromide Transitions Program under agreement number 2008-51102-04583 for a project titled, "Critical Evaluation of Methyl Bromide, Sulfuryl Fluoride, and Heat Treatment for Disinfesting Food-Processing Facilities." The authors thank The Industrial Fumigant Company, Olathe, Kansas, USA, and Presto-X, Omaha, Nebraska, USA, for providing fumigation services. The cooperation of Dow AgroSciences, Indianapolis, Indiana, USA, and Chemtura, Middlebury, Connecticut, USA, in providing the fumigants for use in this project. We thank Xue Meng and Monika Brijwani, Moses Khamis, and Eldon James Weaver for excellent laboratory assistance.

❖ Mention of a proprietary product name does not constitute an endorsement by Kansas State University, Kasetsart University, University of Milan, or USDA.

References

Bell, C.H., Wonter-Smith, T.J., Savvidou, N., 2003. Some properties of sulfuryl fluoride in relation to its use as a fumigant in the cereals industry. In: Credland, P.F.A., Armitage, D.M., Bell, C.H., Cogan, P.M., Highley, E. (Eds), Proceedings of the Eighth, International Working Conference on Stored-product Protection, 22-26 July 2002, York, UK, CAB International, Wallingford, UK, pp. 910-915.

Boina, D., Subramanyam, Bh., Alavi, S., 2008. Dynamic model for predicting survival of mature larvae of *Tribolium confusum* during facility heat treatments. Journal of Economic Entomology 101, 989-997.

Campbell, J.F., Arbogast, R.T. 2004. Stored-product insects in flour mill: population dynamics and response to fumigation treatment. Entomologia Experimentalis et Applicata 112, 217-225.

Fields, P.G., White, N.D.G., 2002. Alternatives to methyl bromide for stored-product and quarantine insects. Annual Review of Entomology 47, 331–359.

Small, G. J. 2007. Evaluation of the impact of sulfuryl fluoride fumigation and heat treatment on stored-product insect populations in UK flour mills. Journal of Stored Products Research 43, 410-416.

SAS Institute, 2002. SAS/STAT user's guide, version 9.1. SAS Institute, Cary, North Carolina.

UNEP, 1998. Report of the Methyl Bromide Technical Options Committee, 1998 Assessment of Alternatives to Methyl Bromide, United Nations Environment Programme, Nairobi.