EVALUATION OF PYRETHRIN AEROSOL INSECTICIDE AS AN ALTERNATIVE TO METHYL BROMIDE FOR PEST CONTROL IN FLOUR MILLS

by

KABITA KHAREL

M.S., Tribhuvan University, Nepal, 2009

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:

Co-Major Professor
Frank H. Arthur

Approved by:

Co-Major Professor
Kun Yan Zhu

Abstract

Experiments were conducted to assess the effects of direct and indirect exposure scenarios, different degrees of residual flour, open and obstructed positions, and seasonal temperature variations on the efficacy of synergized pyrethrin against the red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val. To evaluate effects of direct and indirect exposures of *T. castaneum* and *T. confusum* eggs, larvae, pupae, or eggs to the insecticide aerosol within a flour mill, the following treatments were made to each life stage: insects treated with aerosol and transferred to treated or untreated flour, untreated insects transferred to treated flour, and insects and flour combined and treated together. Different degrees of harborage or sanitation levels were created by exposing *T. confusum* larvae, pupae, and adults to pyrethrin aerosol in Petri dishes containing 0, 0.1, 1, 5, and 10 g of wheat flour. Effects of pyrethrin dispersal in open and obstructed positions and seasonal temperature variations were assessed by exposing *T. confusum* pupae and adults in open positions and inside wooden boxes (1 m long, 20 cm wide, and 5, 10, or 20 cm high) inside experimental sheds maintained at target temperatures of 22, 27, and 32 °C. Results showed that when *T. castaneum* and *T. confusum* were directly exposed to aerosol without the flour source, or with a low amount of flour at open exposed areas, the aerosol provided good control against all life stages of *T. castaneum* and *T. confusum*. However, when insects were indirectly exposed (treated together with flour or untreated insects were transferred to treated flour), or treated together with deeper flour amounts, and exposed inside the boxes, the efficacy was greatly reduced. Eggs and pupae of both the species were more susceptible compared to larvae and adults. Additionally, the moribund adults initially observed in indirect exposure treatments, or at the deeper flour depth

and exposure positions insides the boxes, were better able to recover. Generally, temperatures in the range of 22-32 °C had no significant effects on overall efficacy of pyrethrin aerosol.

Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Tables

Acknowledgements

I would like to express my sincere appreciation and gratitude to my major advisors Prof. Frank H. Arthur and Prof. Kun Yan Zhu for their continuous guidance and encouragement during my experiments and write-up. I am also very grateful to my committee members Prof. James F. Campbell and Prof. Bhadriraju Subramanyam for their support and insightful comments throughout the research. Apart from accomplishment of this thesis, my advisors also helped me in my personal development. The comments, suggestions and constructive guidance provided during my research presentations will provide me invaluable guidelines and confidence in the future too.

In addition, I owe my thanks to number of technicians, student workers and office staffs at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS. Especially, I would like to thank Brian Barnett, Rich Hammel, and Kris Hartzer for technical assistance with the research.

I would also like to thank Entech Corporation for providing the insecticide used in this study. This research was partially funded by the U. S. Department of Agriculture, National Institute of Food and Agriculture (NIFA) Methyl Bromide Transitions program (grant number 2010-51102-21660).

Lastly, I would like to thank my family for their never ending love and emotional support. A special thanks to Bikram and Bibiana.

Literature Review

Flour beetles: biology and behavior

The red flour beetle (RFB), *Tribolium castaneum* (Herbst) and the confused flour beetle (CFB), *Tribolium confusum* Jacquelin du Val, are members of the Order Coleoptera and Family Tenebrionidae. These two species are identical in many ways in their biology and behaviors. A female *Tribolium* lays on an average 2-3 eggs per day (Mukerji and Sinha 1953) and a maximum of 1000 eggs during her life time (Sallam 1999), nevertheless, fecundity may vary with temperature and other environmental conditions. Eggs are microscopic, white or colorless, and covered with sticky secretions which are deposited within flour or other food material. The sticky materials allow eggs to cling on surfaces and also allow flour particles to adhere to eggs. Under a favorable conditions, eggs hatch within a week into skinny, whitish to yellowish larvae which are about 0.25 inch in length. The number of larval instars varies from 5-11, but 7-8 instars are common (Hill 2002). Larvae are active, voracious feeders that can cause significant economic losses in flour mills. Larvae pupate into white to light brown, unschlerotized, and non-feeding pupae. Pupae then emerge as reddish brown flat bodied adults. Adults are approximately 0.18 inches in length and may live for several months and sometimes up to 3 years (Staunton et al. 2008). They are photo-negative and try to avoid direct light by hiding within food patches and inside shaded portions of facilities (Park 1934). Under optimal conditions (27±1 ºC, 60±5% RH, 12:12 h L: D), *T. castaneum* and *T. confusum* complete their life cycles in 5-6 and 6-7, weeks respectively (Bry and Davis 1985). However, the life cycle can be shorter with increased temperature (Table 1).

Table 1. Developmental times (days) for different stages of *T. castaneum*

Stages	Time (days)	
	30 °C	34 °C
Egg	3	2
Larva	20	15
Pupa	4	3
Reproductive maturation	5	4
Total time (egg to egg)	32	24

(Source: Beeman et al. 2012)

Despite many similarities in their biology and behavior, the two species can be readily differentiated in the adult stage. The antennae of *T. confusum* are four segmented and proportionate, while *T. castaneum* exhibit three segmented and peculiarly large antennae (Sallam 1999). Other morphologically differences are that the side of thorax of *T. castaneum* is curved while that of *T. confusum* is nearly straight (Mason 2003), and the distance between eyes, when viewed ventrally, are narrow in *T. castaneum* and wide in *T. confusum* (Rees 1995). The development of *T. confusum* has been found to be relatively slower than that of *T. castaneum* (Bry and Davis 1985). In the US, *T. castaneum* is more common in warmer southern states, while *T. confusum* is more common in the northern states (Park 1934). An adult of *T. castaneum* can fly short distances at higher temperature but adult *T. confusum* do not fly (Rees 2004). Therefore, *T. confusum* is more preferred species for higher temperature studies.

Occurrence of *T. castaneum* and *T. confusum* in flour mills

T. castaneum and *T. confusum* are well adapted to arid conditions of flour milling facilities. In an active flour mill, flour dust and spillages are continuously generated during various milling operations such as grinding, milling, sifting, and moving of processed food products. Also, frequent in-depth cleaning and removal of accumulated dusts from and underneath the heavy equipment and machinery may be impractical (Hawkin 2008). Those food resources can be successfully utilized by the *Tribolium* adults (Campbell and Hagstrum 2002; Fedina and Lewis 2008) and the immature stages (Toews et al. 2010). Practically, *T. castaneum* and *T. confusum* can be present throughout the mill flow (Good 1937) and throughout the facility, but they are abundantly present in unloading, milling, processing, pelleting, weighing, and storing sites (Trematerra and Sciarretta 2004).

Economic damage

T. castaneum and *T. confusum* can cause significant damage by direct consumption of flour and finished products. A larva and an adult of *T. castaneum* can consume approximately 13 and 315 mg of flour, respectively, during their lifetimes (Hagstrum and Subramanyam 2000). However, substantial economic losses can be caused through contamination of processed commodities with metabolic byproducts and body parts (Neethirajana et al. 2007). Adults of both the species secrete a compound called benzoquinones from specialized prothoracic and abdominal stink glands (Sonleitner and Gutherie 1991). The properties of benzoquinones are not fully understood, but it may play role in defense against microbes and predators (Pappas and Morrison 1995; Yezerski et al. 2004). This compound is heat stable and is of concern because it can taint food product, producing unappealing odors (Sallam 1999), and may have carcinogenic effects (Lis et al. 2011). The contaminated products may result in loss of consumers' goodwill and failure to meet regulatory requirements (Campbell and Arbogast 2004), thereby increasing the losses due to infestation.

Pest control in milling facilities

Methyl bromide (MB) was a primary fumigant for pest control in milling facilities until 2005, when it began to phase out within the US as part of the Montreal Protocol. This was an international agreement to reduce the use of this powerful ozone depleting agent (Fields and White 2002). MB had many desirable characteristics of a fumigant such as good penetration ability and effectiveness against a broad spectrum of storages insects and their life stages (eggs, larvae and adults) (CNMA 2007). With the phase-out of MB, milling facilities' pest control reliance has shifted to whole structure treatments with fumigants and heat, or on non structural treatments such as aerosols, residual contact insecticides as surface treatments, and increased sanitation.

Whole structure treatments

Facility fumigation: Fumigation is the process of applying insecticides in the form of toxic gases (White and Leesch 1995). Currently, the only fumigants registered for use inside flour mills in the US are sulfuryl fluoride (SF) and phosphine. SF is an inorganic, nonflammable, non corrosive gas which readily vaporizes under normal fumigation conditions. It penetrates into insects through spiracles in postembryonic life and through shells in eggs, where it breaks down into the fluoride anion (Prabhakaran 2006). The fluoride anion can disrupt the glycolysis and fatty acid cycles and deprive the affected insect of necessary cellular energy (Prabhakaran 2006).

In the US, the SF formulation Vikane®, was available for use in structures since late 1950s (Derrick 1990), before a newer formulation, Profume®, was registered for use in food and feed facilities in 2004. SF has been cited as a less effective fumigant against the egg stage of insects compared to MB (Emekci 2010). Additionally, there are concerns regarding fluoride residues in food due to SF treatment (USEPA 2012), and current atmospheric concentration of this potential greenhouse gas (GHG) (Andersen et al. 2009). In January, 2011, EPA proposed gradual phase out of all food fumigation with SF within three years (USEPA 2012).

Similarly, phosphine is an unappealing fumigant to millers because it is corrosive to copper, silver, and gold (Fields and White 2002), and can cause potential damage to the electrical wiring system, computers and other instrumentation inside the food facilities. Also, phosphine resistance has been documented in major storage insects including *T. castaneum* (Subramanyam and Hagstrum 1995; Benhalima et al. 2004; Opit et al. 2012). Besides, SF and phosphine fumigants need tight sealing of the facilities prior to application to ensure that the gaseous insecticide does not contaminate outside environment. Therefore, the labor costs associated with sealing can increase the cost of fumigations (Watters 1968).

Heat treatment: Heat treatment can be defined as increasing the temperature of the facilities to levels that kill all life stages of flour beetles The current recommended level is raising air temperature up to 50–60 °C for at least 24 hours (Fields and White 2002; Dosland et al. 2006, Mahroof et al. 2003, Subramanyam et al. 2011). Different heating devices such as electrical, forced air gas, and steam heaters are being used for heat treatments (Hulasare et al. 2010). The high heat denatures proteins and disrupts the enzymatic functions in insects leading to their death (Fields and White 2002). Furthermore, it reduces oviposition and progeny production in survivors (Mahroof et al. 2005). Heat treatment has several other advantages. It is safe, residues free, requires no regulatory approval, and resistance development in insects is less likely (Fields and White 2002). However, there are practical considerations for using heat treatment. Achieving and holding the temperature as high as 50°C for at least 24 hours poses technical and economical challenges to a commercial facility (Brijwani et al. 2009). High temperature can cause adverse impacts on structural components and packaging materials in milling facilities (Dowdy and Fields 2002; Hulasare et al. 2010). In addition, the efficacy of heat can be greatly reduced if it is not dispersed uniformly among the crowded and complex setting within a milling facilities by the use of air movers or fans (Mahroof et al. 2003; Roesli et al 2003).

Non-structural treatments

Residual surface treatments: Certain residual insecticides can be applied as general surface treatments within a milling facility (Arthur 2012). These residual treatments must leave a residue on the surface that will be lethal enough to kill crawling insects (Toews et al. 2003). Some of the commonly used insecticides for surface treatments are diatomaceous earth (DE) (Arthur 1998; Dowdy and Fields 2002), spinosad (bacterial fermentation insecticide, registered in USEPA in 2005) (Bonjour et al. 2006), cyfluthrin (Arthur 1998), and insect growth regulators (IGR) such as hydroprene (Arthur 2001; Toews et al. 2005), methoprene (Jenson et al. 2009), and pyriproxfen (Arthur et al. 2009). The effectiveness of residual treatment may vary depending upon several conditions such as nature of treated surface, insecticidal formulation, target species, environmental conditions, etc. (Arthur 2012). White and Leesch (1995) stated that residual activity of many insecticides decreases rapidly due to hydrolysis on concrete surfaces which have pH 10.5 or greater. However, Toews et al. (2003) reported greater contact toxicity of spinosad on *Tribolium* adults exposed on concrete compared to those exposed on steel, unwaxed tile, or waxed tile. In another study, Arthur (2000) exposed adult *T. castaneum* and *T. confusum* to diatomaceous earth at 22, 27, and 32°C on filter paper inside plastic Petri dishes and held them for one week. Mortality was lowest at 22°C but increased with temperature. Therefore, the choice of insecticides for residual treatment should be based on biotic and abiotic factors within the facilities. Also, residual treatment can be challenging to control immature stages of insects located inside food patches or hidden areas that may not be treated directly.

Aerosol insecticides: Aerosol insecticides, also known as fogging, space sprays, or ultra-low volume spray (ULV), involve dispensing liquid insecticide formulation through a mechanical device in the form of fog or mist (Peckman and Arthur 2005). The applicator can be small handheld fog generators, or wall mounted permanent systems (Toews et al. 2010). The cost of applying aerosols is far less when compared to fumigation with MB, SF, or to heat treatment (Boina and Subramanyam 2012). Dichlorvos and pyrethrins are two of the common aerosol insecticides recently being used in flour mills. Dichlorvos is an acetylcholinesterase (AChE) inhibitor. Toxicity to exposed insects is through accumulation of acetylcholine at various sites as a result of AChE inhibition (Wang et al. 2004; Goel and Aggarwal 2007). However, many detoxification enzymes in insects can detoxify insecticides such as dichlorvos resulting in the reduced efficacy of insecticides to inhibit AChE (Zhu and Gao 1999). Dichlorvos resistance has been documented in many storage insects (Srivastava and Sinha 2000). Additionally, dichlorvos is cholinesterase inhibitor and an accidental exposure can be extremely hazardous to human and animals (Chattopadhyay et al. 1982). It has a positive correlation with temperature (Lellinger 1972) which means it can be less effective during cool season and in poorly heated facilities

(Wiliams and Walsh 1989). Also, it is highly volatile compound (White and Leesch 1995). Therefore, in the recent times, there is growing interest in the use of pyrethrin insecticides for aerosol spray as an alternative to dichlorvos (Boina and Subramanyam 2012).

Pyrethrin is a natural insecticide produced by grinding dried flowers of certain species of chrysanthemum plants. The extracted dusts usually have about 30% active ingredient (A.I) which comprises of pyrethrin 1, pyrethrin 2, cinerin 1, cinerin 2, jasmolin 1, and jasmolin 2 (Gunasekara 2004). These active insecticidal ingredients are collectively called as pyrethrins. Different commercial formulations of pyrethrins are available for the control of storage pests and may be referred both as pyrethrins or pyrethrin as different ingredients are formulated as a single insecticide.

Pyrethrin is popular for use in flour mills as it possesses low toxicity to mammals and at the same time is toxic to a broad range of stored product insects (Casida 1980). When pyrethrin penetrates into the insect's cuticle, it reaches the nervous system, and binds to sodium channels resulting in hyperexcitation of nerve cells (Goel and Aggarwal 2007). The sequential intoxication symptoms in insects include excitation, uncoordination in movement, and prostration (Blum and Kearns 1956). The principle drawbacks of pyrethrin usage are instability, rapid loss of insecticide activity and quick recovery from knockdown (Rathore and Ullah 2009). Therefore, pyrethrin insecticide is often used with the synergist piperonyl butoxide (PBO) (Gist and Pless 1985). It is reported that PBO can increase the potency of pyrethrin by more than fourfold when added at two to ten parts per part of pyrethrin (Casida 1980; Cox 2002).

Factors influencing efficacy of pyrethrin aerosols: Pyrethrin is well known for its negative temperature coefficient. Guthrie (1950) applied pyrethrin topically in German cockroaches and found that it was more toxic at 14 and 22°C than at 32°C. Similar results were reported by Hinks (1985) who emphasised on spraying pyrethrin and pyrethroid insecticides during cool weather and during later part of the day. But, when PBO is added to pyrethrin, its toxicity is increased at higher temperature (Blum and Kearns 1956). The possible reason for this might be; PBO, in synergized pyrethrin, blocks the detoxifying enzymes in insects and the inhibition of the detoxifying mechanism is more efficient at high temperature (Blum and Kearns 1956). Therefore, the seasonal temperature variations within the milling facilities can influence the effectiveness of synergized pyrethrin. However, there are few documented scientific reports regarding the interactions of temperature and synergized pyrethrin and their efficacy on stored product insects. In addition, the structural and machinery barriers in flour mills could affect the movement of aerosols. Watters (1968) conducted a study using the rusty grain beetles, *Cryptolestes ferrugineus*, to understand the dispersion of synergized pyrethrin aerosols. Watters (1968) reported mortality of the insects only on the floor and up to 1.5 m from application position. Similar results have been reported by Toews et al. (2010), who reported less than 75% mortality in all life stages of *T. castaneum* in Petri dishes exposed under wooden pallets as compared to at least 80% mortality in open positions. Furthermore, the effectiveness of synergized pyrethrin aerosols may vary with the insect species and life stages. Arthur (2008) reported greater susceptibility of *T. castaneum* adults compared to *T. confusum* for synergized pyrethrins.

Sanitation: Sanitation refers to cleaning and eliminating extraneous food materials within food facilities (Cramer 2006). Eliminating flour patches within the facilities also eliminates the hiding and breeding sites of many storage insects because they are the favored sites for oviposition and development (Tenhet et al. 1958). In an unclean facility, the accumulated food dusts and spillages can compromise the effectiveness of any kinds of pest control methods applied such as heat treatments (Brijwani et al. 2012), residual surface treatments (Arthur 1998), and aerosols (Arthur and Campbell 2008; Toews et al. 2010). Moreover, food patches may provide shelter (Barson 1991) and nutrition (Arthur 2000) to enable survivors of treatments to recover after exposure to control methods. Therefore, integrating sanitation with chemical control methods may help to increase their effectiveness.

Summary

Synergized pyrethrin aerosol can be an economically and technically feasible alternative to MB. It may reduce or replace the current heavy reliance on whole structure treatments. However, different biotic and abiotic factors can influence the efficacy of an aerosol treatment. The influence of seasonal temperature variations, barriers, and different degrees of harborage on the efficacy of synergized pyrethrins has not been well documented. *T. confusum* and *T. castaneum* are two economically important and abundant species in flour mills. Adult and larval stages are mobile and voracious feeder. Many previous studies with fumigants and laboratory studies have documented significant differences in stages susceptible to different insecticides in storage insects including *T. confusum* and *T. castaneum*. However, most of the studies on the efficacy of aerosols were done using adult beetles, and those results may not reflect efficacy against immature stages.

References

Andersen, M. P.S., D. R. Blake, F. S. Rowland, M. D. Hurley, and T. J. Wallington. 2009. Atmospheric chemistry of sulfuryl fluoride: reaction with OH radicals, Cl atoms and O_3, atmospheric lifetime, IR spectrum, and global warming potential. Environ. Sci. Technol. 43: 1067-1070.

Arthur, F.H. 1998. Residual toxicity of cyfluthrin wettable powder against *Tribolium confusum* (Coleoptera: Tenebrionidae) exposed for short time intervals on concrete. J. Stored Prod. Res. 34: 19-25.

Arthur, F.H. 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. J. Econ. Entom. 93: 526-532.

Arthur, F.H. 2001. Susceptibility of last instar red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) to hydroprene. J. Econ. Entom. 94: 772-779.

Arthur, F.H. 2012. Aerosols and contact insecticides as alternatives to methyl bromide in flour mills, food production facilities, and food warehouses. J. Pest Sci. 85:323–329.

Arthur, F.H., 2008. Aerosol distribution and efficacy in a commercial food warehouse. Insect Sci. 15: 133-140.

Arthur, F.H., and J.F. Campbell. 2008. Distribution and efficacy of pyrethrin aerosol to control *Tribolium confusum* (Coleoptera: Tenebrionidae) in food storage facilities. J. Stored Prod. Res. 44: 58-64.

Arthur, F.H., J.F. Campbell, E.A. Fontenot, and M.D. Toews. 2013. Assessing effects of esfenvalerate aerosol applications on resident populations of *Tribolium castaneum* (Herbst), the red flour beetle, through direct and indirect sampling. J. Stored Prod. Res.53: 1-6.

Arthur, F.H., S. Liu, B. Zhao, and T.W, Phillips. 2009. Residual efficacy of pyriproxyfen and hydroprene applied to wood, metal and concrete for control of stored-product insects. Pest Manag. Sci. 65: 791-797.

Barson, G. 1991. Laboratory assessment of the residual toxicity of commercial formulations of insecticides to adult *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) exposed for short time intervals. J. Stored Prod. Res. 27: 205-211.

Beeman, R.W., and S. Haas, and K. Friesen. 2012. Beetle wrangling tips: an introduction to the care and handling of *Tribolium castaneum*. http://www.ars.usda.gov/Research/docs.htm?docid=12892 (Accessed, Feb, 19, 2013).

Benhalima, H., M.Q. Chaudhry, K.A. Mills, and N.R. Price. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stor. Prod. Res. 40: 241-249.

Blum, M.S., and C.W. Kearns. 1956. Temperature and the action of pyrethrum in the American cockroach. J. Econ. Entomol. 49:862-865.

Boina, D.R., and B. Subramanyam. 2012. Insect management with aerosols in food processing facilities, pp. 195-212. *In* F. Perveen (ed.), Insecticides: advances in integrated pest management. InTech. http://www.intechopen.com (Accessed, Jan, 2, 2013).

Bonjour E.L, T.W. Phillips, J.T. Pitts. 2006. Spinosad provides long-term protection for stored wheat, pp 1189–1193. *In* I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J.P. Santos, J.D. Biagi, J.C. Celaro, L.R.D.A Faroni, L.O.F. Bortolini, M.R. Sartori, M.C. Elias, R.N.C. Guedes, R.G. Fonseca, and V.M. Scussel (eds.), Proceedings of the 9th International Working Conference on Stored Product Protection. ABRAPOS, Campinas, Brazil.

Brijwani, M., B. Subramanyam, M. Hartzer, D. E. Maier, W. Chayaprasert, S. Savoldelli, P. Flinn, and J. Campbell. 2009. Efficacy of structural heat treatments against *Tribolium castaneum* life stages. Presentation at methyl bromide alternatives outlook conference, San Diego, CA., November 2009. Available at: http://mbao.org (Accessed, Nov, 15, 2012).

Brijwani, M., B. Subramanyam, P.W. Flinn. 2012. Impact of varying levels of sanitation on mortality of *Tribolium castaneum* eggs and adults during heat treatment of a pilot flour mill. J. Econ. Entomol. 105: 703-708.

Bry, R.E., and R. Davis. 1985. *Tribolium confusum* and *Tribolium castaneum*, 291-293. *In* P. Singh and P.W. Moore (eds.), Handbook of insect rearing, Vol 1. Elsevier, NY, USA.

Campbell, J.F., and D.W. Hagstrum D.W. 2002. Patch exploitation by *Tribolium castaneum*: movement patterns, distribution and oviposition. J. Stored Prod. Res. 38: 55-68.

Campbell, J.F., and R.T Arbogast. 2004. Stored product insects in a flour mill: population dynamics and response to fumigation treatments. Entomol. Exp. Appl. 112: 217-225.

Campbell, J.F., M.D. Toews, F.H. Arthur, and R.T. Arbogast. 2010a. Long term monitoring of *Tribolium castaneum* populations in two flour mills: rebound after fumigation. J. Econ. Entomol. 103: 1002-1011.

Campbell, J.F., M.D. Toews, F.H. Arthur, and R.T. Arbogast. 2010b. Long-term monitoring of *Tribolium castaneum* in two flour mills: seasonal patterns and impact of fumigation. J. Econ. Entomol. 103: 991-1001.

Casida, J. E. 1980. Pyrethrum flowers and pyrethroid insecticides. Environ. Health Perspect. 34:189.

Chattopadhyay, D.P., S.K. Dighe, D.K. Dube, and Purnanand, 1982. Changes in toxicity of DDVP, DFP, and parathion in rats under cold environment. Bull. Environ. Contam. Toxicol. 29: 605-610.

CNMA, 2007. Comparative evaluation of integrated pest management, heat treatments and fumigants as alternatives to methyl bromide for control of stored product pests in canadian flour mills. Canadian National Millers Association, Ottawa, Canada. http://www.canadianmillers.ca/english/pdf/issues/ComparativeEvaluation_March2007.pdf (Accessed, Aug, 5, 2012).

Cox, C. 2002. Insecticide synergist fact sheet: piperonyl butoxide. http://www.pesticide.org/get-the-facts/pesticide-factsheets/factsheets/piperonylbutoxide (Accessed, Jan, 23, 2013).

Cramer, M.M. 2006. Food plant sanitation: design, maintenance, and good manufacturing practices, pp 1-29. CRC Press, Boca Raton, FL

Derrick, M.R., H.D. Burgess, M.T. Baker, and N.E. Binnie. 1990. Sulfuryl fluoride (Vikane): a review of its use as a fumigant. J. Am. Inst. Conserv. 29: 77-90.

Dosland, O., B. Subramanyam, K. Sheppard, and R. Mahroof. 2006. Temperature modification for insect control, pp. 89-103. *In* J.W. Heaps (ed), Insect management for food storage and processing. AACC International, St. Paul, MN, USA.

Dowdy, A.K., and P.G. Fields. 2002. Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill. J. Stored Prod. Res. 38: 11-22.

Emekci, M. 2010. Fumigation, modified atmospheres and hermetic storage, pp. 303-313. *In* M.O. Carvalho, C.S. Fields Alder, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Nvarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, N.D.G. White (eds.), Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal. Julius-Kühn-Archiv, 425.

Fedina, T.Y., and S.M. Lewis. 2008. An integrative view of sexual selection in *Tribolium* flour beetles. Biol. Rev. 83: 151-171.

Fields, P.G., and N.D.G. White. 2002. Alternatives to methyl bromide treatments for stored product and quarantine insects. Annu. Rev. Entomol. 47:331-359.

Gist, G.L., and C.D. Pless 1985. Synergistic activity of piperonyl butoxide with nine synthetic pyrethroids against the fall armyworm, *Spodoptera frugiperda*. Fla. Entomol. 68: 316-320.

Goel A., and P. Aggarwal. 2007. Pesticide poisoning. Natl. Med. J. India. 20:182–191.

Good, N.E. 1937. Insects found in the milling streams of flour mills in the southwestern milling area. J. Kans. Entomol. Soc. 10: 135-148.

Gunasekara, A.S. 2004. Environmental Fate of Pyrethrins. California Environmental Protection Agency (Cal/EPA), Department of Pesticide Regulation. Sacramento, CA, USA.

Guthrie, F.E. 1950. Effect of temperature on toxicity of certain organic insecticides. J Econ Entomol. 43:559-560.

Hagstrum, D.W., and B. Subramanyam. 2000. Monitoring and decision tools, pp. 1-28. *In* B. Subramanyam and D.W. Hagstrum (eds.), Alternatives to pesticides in stored product IPM. Kluwer Academic Publishers, Norwell, MA, USA.

Hawkin, K. 2008. Monitoring populations of the flour beetles *Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst) in flour mills and in laboratory settings. M.S. thesis. University of Manitoba, Winnipeg, Manitoba, Canada.

Hill, D.S. 2002. Pests of stored foodstuffs and their control, pp 135-316. Kluwer Academic Publishers, Dordrecht, Netherlands.

Hinks, C. F. 1985. The influence of temperature on the efficacy of three pyrethroid insecticides against the grasshopper, Melanoplus sanguinipes (fab.)(Orthoptera: Acrididae), under laboratory conditions. Can. Entomol. 117:1007-1012.

Hulasare, R., B. Subramanyam, P.G. Fields, and A.Y. Abdelghany. 2010. Heat treatment: a viable methyl bromide alternative for managing stored-product insects in food-processing facilities, pp. 661-667. *In* M.O. Carvalho, C.S. Fields Alder, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Nvarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, N.D.G. White (eds.), Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal. Julius-Kühn-Archiv, 425.

Jenson, E.A., F.H. Arthur, and J.R. Nechols. 2009. Efficacy of methoprene applied at different temperatures and rates on surface substrates to control eggs and fifth instars of *Plodia interpunctella*. J. Econ. Entomol. 102: 1992-2002.

Lellinger, D.B. 1972. Dichlorovos and lindane as herbarium insecticides. Taxon 21:91-95.

Lis, L.B., T. Bakuła, M. Baranowski, and A. Czarnewicz. 2011. The carcinogenic effects of benzoquinones produced by the flour beetle. Pol. J. Vet. Sci. 14: 159-164.

Mahroof, R., B. Subramanyam, and D. Eustace. 2003. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. J. Stor. Prod. Res. 39: 555-569.

Mahroof, R., B. Subramanyam, and P. Flinn. 2005. Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minimum heat treatment temperature as pupae and adults. J. Econ. Entom. 98: 626-633.

Mason, L. 2003. Insects and mites, pp. 293-315. *In* Y.H. Hui, B.L. Bruinsma, J.R.Gorham, W.K. Nip, P.S. Tong, and P. Ventresca (eds.), Food plant sanitation. Marcel Dekker, Inc., NY, USA.

Mukerji, D., and R.N. Sinha. 1953. Effect of food on the life history of the flour beetle, *Tribolium castaneum* Herbst. J. Kans. Entomol. Soc. 26: 118-124.

Neethirajan, S., C. Karunakaran, D. S. Jayas, and N. D. G. White. 2007. Detection techniques for stored-product insects in grain. Food Control 18:157-162.

Opit, G.P., T.W. Phillips, M.J. Aikins, M.M. Hasan. 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. J. Econ. Entomol. 105: 1107–1114.

Pappas, P.W., and S.E. Morrison. 1995. Benzoquinones of the beetles, *Tribolium castaneum* and *Tribolium confusum*. Prep Biochem. 3:155-68.

Park, T. 1934. Observations on the general biology of the flour beetle, *Tribolium confusum*. Q. Rev. Biol. 9: 36-54.

Peckman, P.S., and F.H. Arthur. 2005. Insecticide space treatments in food plants, pp. 175-182. *In* J.W. Heaps (ed), Insect management for food storage and processing. AACC International, St. Paul, MN. USA.

Prabhakaran, S. 2006. Commercial performance and global development status of ProFume gas fumigant, pp. 635-641 *In* I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J.P. Santos, J.D. Biagi, J.C. Celaro, L.R.D.A Faroni, L.O.F. Bortolini, M.R. Sartori, M.C. Elias, R.N.C. Guedes, R.G. Fonseca, and V.M. Scussel (eds.), Proceedings of the 9th International Working Conference on Stored Product Protection. ABRAPOS, Campinas, Brazil.

Rathore, H.S., and S. Ullah. 2009. Simple and affordable methods: spectrophotometric, thin-layer chromatographic and volumetric determination of pesticide residues, pp. 343-380. In L.M.L. Nollet and H.S. Rathore (eds.), Handbook of Pesticides: Methods of Pesticide Residues Analysis, CRC Press, Boca Raton, FL, USA.

Rees, D. 2004. Insects of stored products, p 192. CSIRO Publishing, Collingwood, Victoria, Australia.

Roesli, R., B. Subramanyam, F.J. Fairchild, K.C. Behnke. 2003. Trap catches of stored-product insects before and after heat treatment in a pilot feed mill. J. Stor. Prod. Res. 39: 521–540.

Sallam, M.N. 1999. Insect damage: damage on post-harvest. http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_Pests-Insects.pdf (Accessed, Aug, 17, 2013).

Sonleitner, F.J., and J. Gutherie. 1991. Factors affecting oviposition rate in the flour beetle *Tribolium castaneum* and the origin of the population regulation mechanism. Res.Popul.Ecol. 33: 1-11.

Srivastava, C., S.R. Sinha, and S.N. Sinha. 2000. Occurrence of insecticide resistance in lesser grain borer, *Rhyzopertha dominica* (Fab.). Ann. Agr. Res. 21: 93-95.

Staunton, I., J. Gerozisis, and P. Hadlington. 2008. Insect pests of commerce, industry, accommodation and recreation, pp. 148-207. *In* Urban pest management in Australia. The University of New South Gales Press Ltd., Sydney, Australia.

Subramanyam, B., and D.W. Hagstrum. 1995. Resistance measurement and management, pp 331-397. *In* B. Subramanyam and D. Hagstrum (eds.), Integrated management of insects in stored products. Marcel Dekker Inc, NY, USA.

Subramanyam, B., R. Mahroof, and M. Brijwani. 2011. Heat treatment of grain-processing facilities for insect management: a historical overview and recent advances Stewart Postharvest Review. 7:1-11.

Tenhet, J.N., C.O. Bare, and D.P. Childs. 1958. Further studies on the control of the cigarette beetle with DDVP. Tob Sci. 147: 106-10.

Toews M.D., J.F. Campbell, and F.H. Arthur. 2010. The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle, *Tribolium castaneum.* J. Insect Sci. 10: 1-14.

Toews, M. D., B. Subramanyam, and J. M. Rowan. 2003. Knockdown and mortality of adults of eight species of stored-product beetles exposed to four surfaces treated with spinosad. J. Econ. Entomol. 96: 1967-1973.

Toews, M.D., J.F. Campbell, F.H. Arthur, and M. West. 2005. Monitoring *Tribolium castaneum* (Coleoptera: Tenebrionidae) in pilot-scale warehouses treated with residual applications of (S)-hydroprene and cyfluthrin. J. Econ. Entomol. 98: 1391-1398.

Trematerra, P., and A. Sciarretta. 2004. Spatial distribution of some beetles infesting a feed mill with spatio-temporal dynamics of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum*. J. Stor. Prod. Res. 40: 363-377.

USEPA, 2012. EPA Proposes to withdraw sulfuryl fluoride tolerances. http://www.epa.gov/pesticides/sulfuryl-fluoride/evaluations.html (Accessed, March, 5, 2013).

Wang, J.J., W.X. Cheng, W. Ding, and Z.M. Zhao. 2004. The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, *Liposcelis bostrychophila* and *L. entomophila*. J. Insect Sci. 4: 1-5.

Watters, F. L. 1968. Pyrethrins-piperonyl butoxide applied as a fog in an empty grain bin. J. Econ. Entomol. 61:1313- 1316.

White, N.D.G., and J.G. Leesch. 1995. Chemical control, pp 287-330. *In* B. Subramanyam and D. Hagstrum (eds.), Integrated management of insects in stored products. Marcel Dekker Inc, NY, USA.

Williams, S.L., Walsh, E.A., 1989. Behavior of DDVP in storage cases. Curator: The Museum Journal. 32: 41–49.

Yezerski, A., T.P Gilmor, and L. Stevens. 2004. Genetic analysis of benzoquinone production in *Tribolium confusum*. J. Chem. Ecol. 30: 1035-1044.

Zhu, K.Y., and J.R. Gao. 1999. Increased activity associated with reduced sensitivity of acetylcholinesterase in organophosphate-resistant greenbug, *schizaphis graminum* (homoptera: aphididae). Pestic. Sci. 55: 11-17.

Objectives

1) To evaluate the efficacy of synergized pyrethrin aerosol at different direct and indirect exposure scenarios that could be encountered in a flour mill.
2) To compare the susceptibility between *T. castaneum* and *T. confusum*.
3) To determine the susceptibility of immature stages (eggs, larvae, and pupae) of *T. castaneum* and *T. confusum.*
4) To evaluate the effects of different degree of flour accumulation on the efficacy of synergized pyrethrin aerosol.
5) To determine the toxicity and dispersion of aerosol at 3 different temperature levels: 22, 27, and 32 °C.
6) To determine dispersion of aerosol insecticide at different open and obstructed positions.

Hypothesis

Ha_1: Synergized pyrethrin aerosol will be less effective if insects are not directly exposed

Ha_2: *T. castaneum* and *T. confusum* eggs will be less susceptible than immature stages

Ha_3: *T. confusum* will be less susceptible to synergized pyrethrins than *T. castaneum*

Ha_4: Efficacy of the pyrethrin aerosol will decrease with increased flour depth

Ha_5: Temperature variations will influence the toxicity of pyrethrin aerosol

Ha_6: Dispersion of aerosols will be low under barriers

Chapter 1 - Evaluation of the efficacy of pyrethrins used as aerosols for pest control in milling facilities

Abstract

Aerosol insecticides are being used in flour mill pest management programs, but there is limited information on their efficacy on different insect life stages. In this study, we evaluated the efficacy of synergized pyrethrin applied as an aerosol against eggs, larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val. Effects of direct and indirect exposure were evaluated by exposing each life stage to the aerosol and then transferring to untreated flour, transferring untreated insects to treated flour, or exposing both the insects and the flour to the aerosol. The synergized pyrethrin aerosol was effective against both species and all life stages when insects were directly treated with aerosol irrespective of transferring them to treated or untreated flour, with mortality generally >88%. However, the mortality was significantly reduced when insects were either treated together with flour or untreated insects were transferred to treated flour (indirect exposure to aerosol spray). Adults and larvae of both species were more tolerant compared to eggs and pupae. Additionally, more moribund adults in indirect exposure treatments were able to recover compared to moribund insects in the direct exposure treatments. Results show the importance of delivering the aerosol to the insects instead of treating flour or surfaces. Good sanitation prior to aerosol application could facilitate direct exposure of insects and thus increase effectiveness of aerosols applied inside flour mills.

Keywords: Aerosols, synergized pyrethrin, flour mill, *Tribolium castaneum, Tribolium confusum*

Introduction

Flour milling facilities typically consist of different units such as grain receiving and storing unit, cleaning towers, milling and processing arenas, and packaging and shipping warehouses (Williams and Rosentrater 2007). The constant availability of whole grains and milled products provides excellent feeding and oviposition sites for many stored product insects. The red flour beetle (RFB), *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val, are two of the most important pests in the United States (US) (Campbell and Runnion 2003; Arthur and Campbell 2008; Toews et al. 2009). These species can successfully exploit food patches of different size and quality (Campbell and Hagstrum 2002; Campbell et al. 2010; Ming and Cheng 2012) that are generated during various milling operations such as grinding, milling and moving of processed food products. In a flour mill, *T. castaneum* and *T. confusum* can cause economic impact by feeding and also through contamination and damage of finished products, which may result in return and rejection of goods, loss of customer good will, and failure to meet regulatory requirements (Campbell et al. 2002).

In the US, flour milling facilities utilize whole structure treatments such as heat or cold and fumigants, or more targeted treatments such as aerosols, sanitation, surface treatments. to control insect pests. Heat and cold treatments may not be suitable for older facilities and can be cost-intensive, while fumigants such as sulfuryl fluoride, and cylinderized phosphine require several of facility shutdown, and may cause potential negative impacts on equipments and structures. Hence, there is increasing interest in targeted treatments in general and aerosols in particular (Campbell et al 2010).

Aerosol application is a method of applying a liquid insecticide in the form of fog or mist consisting of particle sizes ranging from 5 to 50 microns (Peckman and Arthur 2005), which are deposited on the surface as liquid droplets. The disadvantage associated with the aerosols is that they may not distribute into areas that are obstructed by equipment or into sites that harbor hidden infestations in spillage and accumulated food dust. Nonetheless, the exposed food materials may have some residual effects and insects could be indirectly exposed through treated foods (Arthur 2010), but there are few published data regarding activity of aerosols through indirect exposure.

Currently, insecticides such as dichlorvos, synergized pyrethrin, pyrethroids (Arthur and Campbell 2008), and insect growth regulators (IGR) are being used as aerosol insecticides. Pyrethrins are of special interest due to their low mammialin toxicity and efficacy against a broad range of storage insects. Entech Fog 10 (EPA Reg. No. 40391-10) which is comprised of 1% pyrethrin and 2% piperonyl butoxide (PBO) is a newer formulation of pyrethrins that is commercially available in the US. The synergist PBO prolongs the biological activity of pyrethrins (Brooke 1958) and it has been labeled safe for use in food premises (Arthur 2010; Sutton et al. 2011). However, most of the data available on the efficacy of aerosol insecticides are from dichlorvos (Harein et al. 1970; Bond et al. 1972; Kane et al. 1977), which belongs to a different class of insecticide than pyrethrins.

Evaluation of the efficacy of pyrethrin aerosol against *T. castaneum* and *T. confusum* may be crucial to determine the potentials of pyrethrin aerosol in milling facilities. Most published studies regarding efficacy of pyrethrin have been conducted against adults, but in field conditions, adults may comprise 5 to 10% of the total population (Toews et al. 2005; Campbell et al. 2010ab; Arthur et al. 2013). There are no recent studies evaluating different life stages of *T. castaneum* and *T. confusum* for susceptibility to pyrethrin. Therefore, the objectives of this study were to determine: 1) efficacy of synergized pyrethrin aerosol at different direct and indirect exposure scenarios that could be encountered in flour mills; 2) compare the susceptibility between *T. castaneum* and *T. confusum*; and 3) determine susceptibility of immature stages.

Materials and Methods

This study was conducted during October and November 2011 in two experimental sheds at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS. The dimensions of the sheds were 6 x 2.9 x 2.6 m^3, and the floor, walls, and roof of each shed were lined with 0.6 mm polyethylene sheeting. Environmental conditions inside the shed were maintained at 27°C and 70% relative humidity (RH), and monitored with HOBO data loggers (Onset Computer Corporation, Bourne, MA).

Colonies of *T. castaneum* and *T. confusum* were established using existing laboratory strains that had been in culture at the CGHAR for more than 20 years. Colonies of each species were on a diet of wheat flour supplemented with 5% brewer’s yeast, and reared inside a laboratory incubator set 27 °C, 70% RH. The life cycle at these conditions takes approximately 6 weeks to complete. The life stages for each of the two beetle species used for the study were 2-d-old eggs, late-stage (4-week old) larvae, 5-week old pupae, and 1-week-old adults.

The insecticide used in the test, Entech Fog 10 (EPA Reg. No. 40391-10) is produced by Entech Systems Corporation, Kenner, LA, and comprised of 1.0% pyrethrin, 2.0% piperonyl butoxide (PBO) (technical), 3.33% N-octyl bicycloheptane dicarboximide, and 93.67% other ingredients. One of the sheds was used as the untreated control and other for exposure to the insecticide. Inside each shed, a 1.4 m x 0.70 m area was marked with a measuring tape. This grid was about 1 m from the end wall and 0.75 m from the side walls, and was divided into 10 equal columns and 4 rows.

An exposure arena consisted of glass Petri dishes with an approximate surface area of 63 cm^2. Twenty individuals of a particular life stage for each species were prepared in a Petri dish with or without flour. Also, Petri dishes containing only flour but no insects were prepared. Five grams of wheat flour was used per dish in all flour dishes. Altogether, there were 40 dishes exposed per shed: 16 dishes containing only a life stage for *T. castaneum* or *T. confusum*, 16 dishes containing only flour, and 8 dishes with a life stage of an insect and flour together. All the 40 dishes were placed randomly in a 10 x 4 layout. At 1.5 m from the door, and 2.8 m from the grid, a line was marked for the aerosol spray position.

The required volume of insecticide per shed (47 mL) was calculated on the basis of labeled rate of Entech Fog 10 (1.04 mL/m^3). The insecticide was delivered using a hand-held aerosol applicator, Fogmaster jr 5330 (The Fogmaster Corporation, Deerfield Beach, FL) with delivery capacity of 27 g/min. Since 1 mL of Entech Fog 10 weighs 0.76 g, a total of 20.8 mL Entech Fog 10 could be delivered per min. Hence, total time required to deliver 47 mL of insecticide was 2 min and 16 sec. The aerosol was applied by standing at the marked spray position and holding the Fogmaster at about 3 m above the floor and pivoting the unit slowly from side to side. The insects and flour dishes were held in both treated and control sheds for 2 h post aerosol application.

After 2 h of exposure, the dishes were removed from the sheds. To mimic different direct and indirect ways that insects could be exposed to aerosol insecticides within a flour mill the following treatments were created for each life stage: insects treated with aerosol and transferred to treated flour (TI + TF), treated insects transferred to untreated flour (TI + UF), insects untreated and transferred to aerosol treated flour (UI +TF), untreated insects transferred to untreated flour (UI +UF) (control treatment), insects and flour combined and both treated together (TIF), and insects and flour combined and both untreated (UIF) (control treatment). After these transfers were completed, all treatment arenas were placed in an incubator set at 27°C and 70% RH. This entire process was repeated six times at weekly intervals.

For each replicate, adults of each species were assessed at 2, 5, 8, and 15-d after exposure to the aerosols, and classified as follows: live (morphologically normal adults), moribund adults (which were on their backs and capable of reflex movement), and dead (unable to move when prodded with a probe). Untreated controls were classified in the same manner. Exposed pupae, larvae and eggs were held in the incubator for 21, 28, and 40 d respectively, to assess the number of dead individuals of the exposed immatures. To observe the effects of different treatments and different between two *Tribolium* species, total affected individuals were calculated for all developmental stages in each treatment. For adults, affected individuals comprised the total dead and moribund individuals, while for immature stages total affected comprised of all the dead, moribund, and arrested individuals which could not complete development to the normal adult stage. Control mortality was calculated for each stage to compare susceptibility of different developmental stages to the aerosol.

Statistical analyses were conducted with the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS 9.2 software, SAS Institute, Cary, NC). The number of responses, affected, or mortality in each treatment were converted to percentage values were transformed by arcsine square root to perform statistical analyses. However, the data presented in figures and text are the untransformed values. The total affected data by species and exposure methods were subjected to two-way analysis of variance (ANOVA) to determine difference in affected individuals produced for each life stage. After two-way ANOVA, one-way ANOVAs were performed to determine differences among exposure method and species. Means were separated using the Waller-Duncan *k*-ratio *t*-test in SAS ($P < 0.05$). For immature stages, the mortality data were analyzed by two-way analysis of variance (ANOVA). For each exposure method, mortality data were then analyzed by one-way ANOVA to determine the susceptibility among the stages. Differences among means were considered significant at $P < 0.05$ level.

Results

Effects of direct and indirect exposure methods

Total affected individuals. For adult exposure, the two-way ANOVA by exposure methods and species showed no interaction effect for the total affected adults produced at 15-d post exposure to aerosol ($F = 1.04$, df = 5, 60, $P = 0.40$). The affected adults were significantly different for exposure methods ($F = 179.44$, df = 5, 60, $P < 0.01$) but not for species ($F = 0.77$, df = 1, 60, $P = 0.38$). The one-way ANOVA showed significant differences among exposure methods for both *T. castaneum* and *T. confusum* ($F = 61.67$, df = 5, 30, $P < 0.01$; $F = 101.08$, df = 5, 30, $P < 0.01$, respectively). Exposing adults directly to aerosol affected virtually all of the exposed individuals, whether or not they were transferred to treated or untreated flour. However, exposing beetles with flour, or transferring untreated beetles to Petri dishes containing treated flour, reduced the total affected individuals in both *T. castaneum* (85% and 56% respectively) and *T. confusum* (91% and 75% respectively) (Table 1).

For the pupal stage exposure, the two-way ANOVA showed no interaction effect between exposure methods and species at 21-d post aerosol application (F = 0.35, df = 5, 60, P = 0.87). The total affected pupae were significantly different for exposure methods (F = 137.53, df = 5, 60, P < 0.01) but not for species (F = 0, df = 1, 60, P = 0.95). The one-way ANOVA showed significant differences among exposure methods for both *T. castaneum* and *T. confusum* (F = 87.21, df = 5, 30, P < 0.01; F = 103, df = 5, 30, P < 0.01, respectively). Exposing pupae directly to aerosol produced significantly higher number of affected individuals, generally greater than 98%, irrespective of transferring them to treated or untreated flour, compared to exposing pupae to aerosol with flour (80% *T. castaneum*; 88% *T. confusum*), or transferring untreated pupae to treated flour (80% *T. castaneum*; 78% *T. confusum*) (Table 1).

In the two-way ANOVA test, the percent affected larvae at 28-d were not significant for interaction between exposure methods and species (F = 0.25, df = 5, 60, P = 0.93). The total affected larvae were significantly different for exposure methods (F = 112.93, df = 5, 60, P < 0.01) but not for species (F = 0.41, df = 1, 60, P = 0.52). The one-way ANOVA showed significant differences among exposure methods for both *T. castaneum* and *T. confusum* (F = 59.32, df = 5, 30, P < 0.01; F = 73.07, df = 5, 30, P < 0.01, respectively). Direct exposure of larvae to aerosol produced significantly higher number of affected individuals (at least 96% affected) compared to exposing them with flour (75% for *T. castaneum*; 83% for *T. confusum*), or transferring untreated larvae to treated flour (75% for *T. castaneum*; 69% for *T. confusum*) (Table 1).

Similarly, for the egg stage exposure, the two-way ANOVA showed no interaction effect between exposure methods and species at 35-d post aerosol application ($F = 0.57$, df = 5, 60, $P = 0.72$). The total affected eggs were significantly different for exposure methods ($F = 149.09$, df = 5, 60, $P < 0.01$) but not for species ($F = 1.09$, df = 1, 60, $P = 0.29$). The one-way ANOVA showed significant differences among exposure methods for both *T. castaneum* and *T. confusum* ($F = 126.98$, df = 5, 30, $P < 0.01$; $F = 169.33$, df = 5, 30, $P < 0.01$, respectively). Exposing eggs directly caused significantly high effects (at least 99% affected), while the percent affected were significantly reduced when eggs were either exposed together with flour (85% for *T. castaneum*; 86% for *T. confusum*), or when untreated eggs were transferred to treated flour (94% for *T. castaneum*; 91% for *T. confusum*). However, for eggs, high mortality was observed in both controls (untreated eggs transferred to untreated flour or eggs and flour kept together in Petri dishes and unexposed to the aerosol) (Table 1).

Recovery in Adults. When beetles were exposed without food and transferred to treated or untreated flour post exposure, no normal live beetles were found at 2, 5, 8, and 15-d. However, when exposed to aerosol with flour, or when untreated beetles were transferred to treated flour (indirect exposure), many adults were in the moribund state. Those moribund beetles were able to recover with time (Fig. 1A, 1B: *T. castaneum*; Fig. 1C, 1D: *T. confusum*). Recovery was about 15% in *T. castaneum* and 8% in *T. confusum* with food, and about 43% in *T. castaneum* and 25% in *T. confusum* when untreated beetles were transferred to treated flour (Fig. 2A: *T. castaneum*; Fig. 2B: *T. confusum*).

Developmental stage susceptibility

The susceptibility of the immature stages was compared across the exposure methods. For *T.* castaneum, the mean ± SE mortality for controls UI+UF and UFI were 5.00 ± 1.82 and 2.50 ± 1.18%, respectively, for larvae, 4.16 ± 2.38 and 5.83 ± 2.38%, respectively, for pupae, but 28.33 ± 2.47 and 16.66 ± 1.05%, respectively, for eggs. Similarly, for *T. confusum*, the mean ± SE mortality for controls UI+UF and UFI were 1.66 ± 1.05% for both controls in larvae, 2.50 ± 1.70 and 4.16 ± 0.83% for pupae, but 20.83 ± 4.16 and 19.2 ± 3.51%, respectively, for eggs. Therefore, the mortality data were corrected for control mortality (Abbott 1925) for the further analysis.

For *T. castaneum*, the two-way ANOVA by stages and exposure methods showed no interaction effect for the mortality produced among the immature stages (F = 1.24, df = 6, 60, P = 0.29), while the stages and exposure methods both were significant (F = 6.34, df = 2, 60, P < 0.01; F = 16.63, df = 3, 60, P < 0.01, respectively). The one-way ANOVA for stages showed no differences among the stages when treated insects were transferred to treated flour, treated insects were transferred to untreated flour, insects were treated together with flour, or insects were transferred to treated flour (F = 1, df = 2, 15, P = 0.39; F = 2.90, df = 2, 15, P = 0.08; F = 0.18, df = 2, 15, P = 0.83; F = 2.88, df = 2, 15, P = 0.08, respectively) (Table 2).

For *T. confusum*, the two-way ANOVA by stages and exposure methods showed significant interaction between the stages and exposure methods in terms of mortality produced among the immature stages (F = 2.76, df = 6, 60, P = 0.02). Also, the stages and exposure methods were significant (F = 21.16, df = 2, 60, P < 0.01; F = 38.86, df = 3, 60, P < 0.01, respectively). The one-way ANOVA for stages showed no differences among the stages when treated insects were transferred to treated flour and treated insects were transferred to untreated flour (F = 2.14, df = 2, 15, P = 0.15; F = 1.68, df = 2, 15, P = 0.22, respectively). However, when insects and flour were exposed together and when untreated insects were transferred to treated flour, larvae were the least susceptible stage followed by pupae (F = 6.72, df = 2, 15, P < 0.01; F = 10 df = 2, 15, P < 0.01, respectively). The eggs were the most susceptible of all the developmental stages (Table 2).

Discussion

Our results indicated that direct exposure to pyrethrin aerosol was effective on all developmental stages of *T. castaneum* and *T. confusum*. Similar results have been reported by Toews et al. (2010), who showed mortality generally greater than 80% on all developmental stages of *T. castaneum* exposed to pyrethrin aerosol. Also, our study shows reduced efficacy when various developmental stages were either treated together with flour or untreated insects was transferred to treated flour (indirect exposure to aerosol). Additionally, the moribund adults initially observed in indirect exposure treatments were able to recover as healthy adults over time, which is consistent with other reports showing greatly reduced efficacy of pyrethrin when adult flour beetles were provided with a food source after they were exposed (Arthur and Campbell 2008).

These results emphasize on the importance of hygienic and sanitary measures in milling facilities in conjunction with insecticide applications, as the presence of food materials limits the exposure to insecticides, enables insects to remove insecticide particles from body surfaces, and provides nutritional support for recovery (Arthur 2000). In addition, cleaning procedures may influence insect movement through increased searching for food patches, which potentially increases their chances of contacting the applied aerosols (Roesli et al. 2003). However, milling facilities have considerable structural complexity because of the different types of processing equipment, thereby generating continual spillage and food residues. Hence, populations could develop in hidden areas and there would be reduced exposure to the aerosol. Our study shows a sharp increase in recovery of moribund adults after 5-d when these adults were indirectly exposed to pyrethrin, which may be due to little residual activity of synergized pyrethrin. This indicates that the moribund insects should be removed from the facilities shortly after aerosol treatment.

Furthermore, we observed no differences between the two exposed *Tribolium* species to pyrethrin aerosol, which is differs from previous published studies using the same laboratory strains and exposure method. Previous results reported greater susceptibility of *T. castaneum* compared to *T. confusum* for synergized pyrethrin (Arthur 2008) as well as the combination of pyrethrin and methoprene (Sutton et al. 2011). The discrepancy in the species susceptible observed in those studies may be due to several reasons. Arthur (2008) held the insects overnight in the facility after they were exposed to pyrethrin, and removed them the following day. In the current test, we removed the insects after 2 h. The longer holding time in the treated facility may have contributed for the increased susceptibility of *T. castaneum*. Similarly, Sutton et al. (2011) conducted tests by exposing larvae of both species on concrete dishes containing wheat flour that had been previously exposed to a combination of either 1% pyrethrin plus methoprene or 3% pyrethrin plus methoprene. Larvae were added to the treated flour, for 0-16 weeks post exposure, using different dishes each time. Less than 1.5 % of exposed larvae of *T. castaneum* developed to the adult stage at either pyrethrin formulations for all the residual exposures. However, adult emergence of *T. confusum* larvae generally increased as the weeks progressed, and was reduced at the 3% pyrethrin rate compared to the 1% rate. Residual control is generally assumed to be through the IGR component when added to pyrethrin, but results of that study indicated some residual effect of pyrethrin or an additive effect between the two components that may have affected the relative susceptibility of the two species. Our study was conducted with the only 1% pyrethrin formulation.

Additionally, our study showed larvae were more tolerant than the immobile stages pupae and eggs in *T. confusum*. Even though, the susceptibility of the stages was not significantly different in *T. castaneum*, there was a clear trend that the eggs were the most susceptible stage followed by the pupae. Furthermore, less number of affected individuals produced in adults compared to eggs and pupae and the recovery observed in adults also suggested that adults were less susceptible to some extent. Inconsistent to our finding, many previous studies have cited eggs and pupae as the most tolerant stages of stored product insects exposed to the fumigants phosphine (Rajendran 1992; Pike 1994; Bell 2000), carbonyl sulfide (Zettler et al. 1997; Zettler and Arthur 2000), sulfuryl fluoride (Bell and Savvidou 1999; Tsai et al. 2011), and propylene oxide (Isikber et al 2004). Greater tolerance of eggs and pupae to insecticides has often been attributed to less metabolic activities in those stages. In our study adults and larvae were more tolerant life stages, especially when they were indirectly exposed to aerosol (when treated with flour and when untreated insects were transferred to treated flour). It is plausible that during aerosol treatment, the adults and larvae burrowed into the flour (5 g flour available per dish), and reduced their exposure to the aerosol. We assumed the pyrethrin would have limited penetration into the flour.

In conclusion, results of this study show that synergized pyrethrin aerosol was only effective when all life stages of *T. castaneum* and *T. confusum* were directly exposed. The indirect exposure was much less effective, indicating that the presence of food material may have compromised aerosol efficacy. Integrating sanitation with aerosols could facilitate more direct exposure to aerosols by reducing available food material. This will increase the effectiveness of the aerosol treatment in the flour mills. In addition, good sanitation can reduce infestation by physically removing insects from the facilities as adults, and basically the immature stages of storage insects are commonly located inside the food patches (Campbell and Hagstrum 2002; Toews et al. 2010).

References

Arthur, F.H., 2000. Impact of high temperatures on efficacy of cyfluthrin and hydroprene applied to concrete to control *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 39: 193–204.

Arthur, F.H., 2008. Aerosol distribution and efficacy in a commercial food warehouse. Insect Sci. 15: 133-140.

Arthur, F.H., 2010. Residual efficacy of aerosols to control *Tribolium castaneum* and *Tribolium confusum*, pp. 789-792. *In* M.O. Carvalho, C.S. Fields Alder, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Nvarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, N.D.G. White (eds.), Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal. Julius-Kühn-Archiv, 425.

Arthur, F.H., and J.F. Campbell. 2008. Distribution and efficacy of pyrethrin aerosol to control *Tribolium confusum* (Coleoptera: Tenebrionidae) in food storage facilities. J. Stored Prod. Res. 44: 58-64.

Bell, C.H. 2000. Fumigation in the 21st century. Crop Prot. 19: 563-569.

Bell, C.H., and N. Savvidou. 1999. The toxicity of Vikane (sulfuryl fluoride) to age groups of eggs of the Mediterranean flour moth (*Ephestia kuehniella*). J. Stored Prod. Res. 35: 233-247.

Bond, E.J., H.A.U. Monro, T. Dumas, J. Benazet, and E.E. Turtle. 1972. Control of insects in empty cargo ships with dichlorvos. J. Stored Prod. Res. 8: 11-18.

Brooke, J.P. 1958. The stabilization of pyrethrins by piperonyl butoxide in water, with special reference to their use as mosquito larvicides. Ann. Appl. Biol. 46:254.

Campbell, J.F., and C. Runnion. 2003. Patch exploitation by female red flour beetles, *Tribolium castaneum*. J. Insect Sci., Available online: insectscience.org/3.20

Campbell, J.F., and D.W. Hagstrum D.W. 2002. Patch exploitation by *Tribolium castaneum*: movement patterns, distribution and oviposition. J. Stored Prod. Res. 38: 55-68.

Campbell, J.F., M.A. Mullen, and A.K. Dowdy. 2002. Monitoring stored product pests in food processing plants with pheromone trapping, contour mapping, and mark recapture. J. Econ. Entomol. 95: 1089- 1101.

Campbell, J.F., M.D. Toews, F.H. Arthur, and R.T. Arbogast. 2010. Long-term monitoring of *Tribolium castaneum* in two flour mills: seasonal patterns and impact of fumigation. J. Econ. Entomol. 103: 991-1001.

Harein, P.K., H.B. Gillenwater, and E.G. Jay. 1970. Dichlorvos: methods of dispensing, estimates of concentration in air, and toxicity to stored product insects. J. Econ. Entomol. 63:1263-1268.

Isikber, A.A., S. Navarro, S. Finkelman, M. Rindner, A. Azrieli, and R. Dias. 2004. Toxicity of propylene oxide at low pressure against life stages of four species of stored product insects. J. Econ. Entomol. 97:281-285.

Kane, J., F.B. Fishwick, A.A. Green, and P.R. Warre. 1977. Automatic dispensing of dichlorvos to control *Ephestia kuehniella*Zell. (Lepidoptera: Phycitidae) in flour mills. J. Stored Prod. Res. 13:59-64.

Ming Q.L., and C. Cheng. 2012. Influence of nutrition on male development and reproduction in *Tribolium castaneum.* J. Econ. Entomol. 105: 1471-1476.

Peckman, P.S., and F.H. Arthur. 2005. Insecticide space treatments in food plants, pp. 175-182. *In* J.W. Heaps (ed), Insect management for food storage and processing. AACC International, St. Paul, MN, USA.

Pike, V. 1994. Laboratory assessment of the efficacy of phosphine and methyl bromide fumigation against all life stages of *Liposcelis entomophilus* (Enderlein). Crop Prot. 13: 141–145.

Rajendran, S. 1992. Selection for resistance to phosphine or methyl bromide in *Tribolium castaneum* (Coleoptera: Tenebrionidae). Bull. Entomol. Res. 82: 119-124.

Roesli, R., B. Subramanyam, J.F. Campbell, and K. Kemp. 2003. Impact of selected IPM practices on insect populations in retail pet stores, pp. 410-419. *In* P.F. Credland, D.M. Armitage, C.H. Bell, P.M. Cogan, and E. Highley E(eds.), Proceedings, 8th International Working Conference on Stored Product Protection, 22-26 July 2002. CAB International, York, UK.

SAS Institute, 2002-2008. SAS 9.2 for Windows. SAS Institute, Cary, NC, USA.

Sutton, A.E., F.H. Arthur, K.Y. Zhu, J.F. Campbell, and L.W. Murray. 2011. Residual efficacy of synergized pyrethrin and methoprene aerosol against larvae of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 47: 399-406.

Toews, M.D., F.H. Arthur and J.F. Campbell. 2009. Monitoring *Tribolium castaneum* (Herbst) in pilot-scale warehouses treated with *b*-cyfluthrin: are residual insecticides and trapping compatible? Bull. Entomol. Res. 99: 121–129.

Toews, M.D., J.F. Campbell, and F.H. Arthur. 2010. The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle, *Tribolium castaneum.* J. Insect Sci. 10: 1-14.

Toews, M.D., J.F. Campbell, F.H. Arthur, and M. West. 2005. Monitoring *Tribolium castaneum* (Coleoptera: Tenebrionidae) in pilot-scale warehouses treated with residual applications of (S)-hydroprene and cyfluthrin. J. Econ. Entomol. 98: 1391-1398.

Tsai, W.T., L.J. Mason, W. Chayaprasert, D.E. Maier, and K.E. Ileleji. 2011. Investigation of fumigant efficacy in flour mills under real-world fumigation conditions. J. Stored Prod. Res. 179-184.

Williams, G.D., and K.A. Rosentrater. 2007. Design considerations for the construction and operation of flour milling facilities. part i: planning, structural, and life safety considerations. Paper presented at the Annual International Meeting of American Society of Agricultural and Biological Engineers. ASABE Paper No. 074116, 17 - 20 June 2007, Minneapolis Convention Center, Minneapolis, MN, USA.

Zettler, J.L., and F.H. Arthur. 2000. Chemical control of stored product insects with fumigants and residual treatments. Crop Prot. 19: 577-582.

Zettler, J.L., J. Larry, J.G. Leesch, R.F. Gill, and B.E. Mackey. 1997. Toxicity of carbonyl sulfide to stored product insects. J. Econ. Entomol. 90: 832-836.

Table 1. Percent affected individuals (mean ± SE) produced after exposure of different life stages of *T. castaneum* and *T. confusum* to each exposure methods

Exposure methods	Adults		Pupae		Larvae		Eggs	
	T. castaneum	*T. confusum*	*T. castaneum*	*T. confusum*	*T. castaneum*	*T. confusum*	*T. castaneum*	*T. confusum*
Direct exposure								
TI+TF	100 a	100 a	100 a	100 a	100 a	99.2 ± 0.8 a	100 a	100 a
TI+UF	100 a	100 a	100 a	98.3 ± 1.0 ab	97.4 ± 1.7 a	96.6 ± 2.4 ab	100 a	99.2 ± 0.8 ab
Indirect exposure								
TFI	85.6 ± 7.3 a	91.9 ± 4.3 a	80.0 ± 8.2 b	88.3 ± 4.4 bc	75.8 ± 8.7 b	83.3 ± 4.9 b	85.0 ± 5.2 b	86.7 ± 4.0 c
UI+TF	56.9 ± 12 b	75.0 ± 10.8 b	80.0 ± 7.5 b	78.3 ± 9.8 c	75.0 ± 10 b	69.2 ± 11 c	94.2 ± 5.8 a	91.7 ± 3.1bc
Controls								
UI+UF	2.5 ± 1.7 c	0.8 ± 0.8 c	6.6 ± 2.8 c	4.1 ± 1.5 d	5.0 ± 1.8 c	1.7 ± 1.0 d	28.3 ± 2.5 c	21.7 ± 3.6 d
UFI	0.8 ± 0.8 c	0.0 c	4.2 ± 2.4 c	4.1 ± 0.8 d	2.5 ± 1.1 c	1.7 ± 1.0 d	16.7 ± 1.0 d	20.8 ± 3.5 d

For a given life stage, means among exposure methods within species with same letter are not significantly different ($P \geq 0.05$, $n = 6$, Waller-Duncan k-ratio t-test)

Treatments: TI+TF = insects treated with aerosol and added to treated flour; TI+UF = insects treated and added to untreated flour; TFI = insects and flour treated together; UI+TF = untreated insects added to treated flour; UI+UF = untreated insects added to untreated flour; UFI = insects and flour combined and untreated

Figure caption

Fig. 1. Response of adult beetles exposed to different exposure method at different time intervals. A: *T. castaneum* at 5-d; B: *T. castaneum* at 15-d; C: *T. confusum* at 5-d; D: *T. confusum* at 15-d

Exposure methods: TA+TF = adults treated with aerosol and added to treated flour; TA+UF = adults treated and added to untreated flour; TFA = adults and flour combined and both treated together; UA+TF = untreated adults added to treated flour

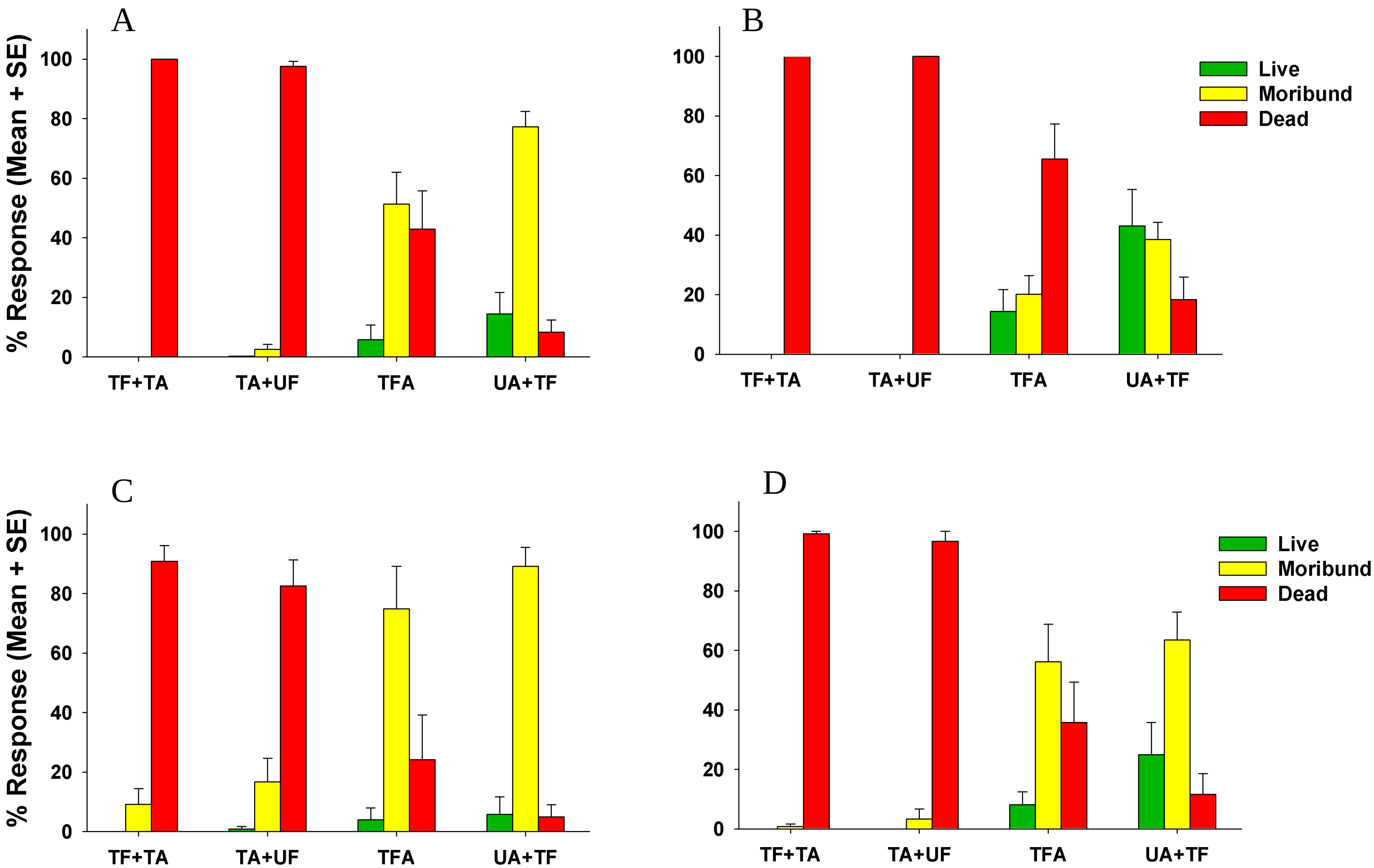

A
B
C
D
% Response (Mean + SE)
0
20
40
60
80
100
TF+TA
TA+UF
TFA
UA+TF
Live
Moribund
Dead

Figure caption

Fig.2. Recovery of treated adults over time. A: *T. castaneum*; B. *T. confusum*

Treatments: TI+TF = insects treated with aerosol and added to treated flour; TI+UF = insects treated and added to untreated flour; TFI = insects and flour treated together; UI+TF = untreated insects added to treated flour

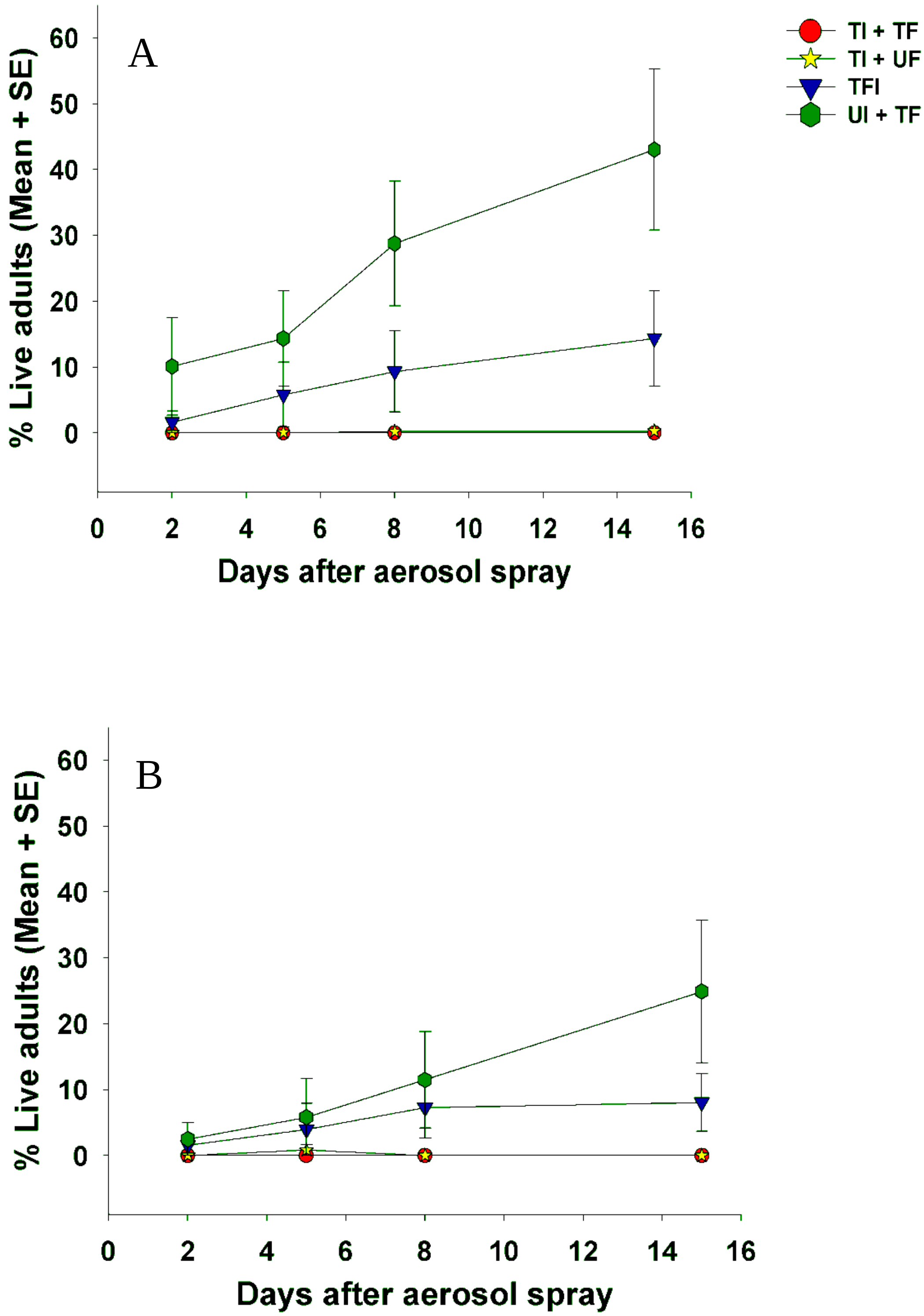

A
B
TI + TF
TI + UF
TFI
UI + TF
% Live adults (Mean + SE)
Days after aerosol spray

Table 2. Percent mortality (mean ± SE) observed after exposure of immature stages of *T. castaneum* and *T. confusum* to synergized pyrethrin aerosol

Exposure methods	Eggs	Pupae	Larvae
		Mortality[a] ± SE	
		T. castaneum	
TF + TI	100 a	100 a	95.4 ± 4.6 a
TI + UF	100 a	100 a	90.3 ± 5.7 a
TFI	73.1 ± 10.9 a	71.8 ± 8.9 a	65.2 ± 10.33 a
UI + TF	89.9 ± 8.8 a	70.1 ± 9.2 a	48.2 ± 17.1 ab
		T. confusum	
TF + TI	100 a	100 a	93.9 ± 4.2 a
TI + UF	98.9 ± 1.0 a	93.0 ± 3.7 a	88.7 ± 5.7 a
TFI	78.6 ± 4.3 a	66.8 ± 12.2 a	29.2 ± 11.4 b
UI + TF	86.5 ± 6.3 a	54.2 ± 12.3 b	22.6 ± 10.6 c

[a] Corrected mortality mean (Abbott 1925)

Means among stages within treatment with same letter are not significantly different ($P \geq 0.05$, $n = 6$, Waller-Duncan *k*-ratio *t*-test

Exposure methods: TI+TF = insects treated with aerosol and added to treated flour; TI+UF = insects treated and added to untreated flour; TFI = insects and flour combined and both treated together; UI+TF = untreated insect added to treated flour

Chapter 2 - Susceptibility of different life stages of Tribolium confusum to pyrethrin aerosol: effects of flour source on insecticidal efficacy

Abstract

Many previous studies have shown that accumulated dust and food residues in flour mills can potentially decrease the efficacy of contact insecticides used for control of adult and immature stages of stored product insects. A study was conducted to evaluate the effects of flour residues on the efficacy of synergized pyrethrin aerosol against different life stages of the confused flour beetle, *Tribolium confusum* Jacquelin du Val. Twenty individual adults, pupae, or larvae of *T. confusum* were exposed separately inside an empty shed to an aerosol spray in Petri dishes containing 0, 0.1, 1, 5 or 10 g of wheat flour. After 2 h of exposure, the dishes were removed from the shed and placed in an incubator set at 27 °C. Mortality of *T. confusum* adults decreased with increasing depth of flour. Also, recovery of moribund adults at 15-d exposure was greater in the 5 and 10 g flour dishes (15 and 46% respectively) compared to the 0, 0.1, or 1 g flour dishes (0, 0.7, and 5% respectively). Similarly, larvae and pupae were less affected when exposed in the deeper flour dishes. Results show accumulated flour residues during aerosol application can compromise the effectiveness of treatments. Reduced efficacy in the deeper flour levels also suggests lower penetration ability of pyrethrin aerosols. Results also emphasize the importance of sanitation and cleaning to remove spillage and extraneous material prior to aerosol application.

Keywords: Synergized pyrethrin aerosol, flour mill, *Tribolium confusum*, sanitation

Introduction

Bagged and processed commodities stored inside flour mills are at risk of infestation by stored product insects. However, finished or processed commodities usually have no or low tolerance for insect damage, especially when products are being directly transferred to consumers (Campbell and Arbogast 2004; Beckett et al. 2007). Many commercial milling facilities, therefore, design and implement calendar-based pest control measures to limit insect infestations and meet marketing standards. Synergized pyrethrin aerosol can be an important component of pest management programs. It is generally safer to workers and bystanders compared to fumigants, and is safe to use in food premises (Keane 1998; Arthur 2010; Sutton et al. 2011). Additionally, pyrethrin is effective against a variety of storage pests (Bernhard and Bennett 1981). The minimum re-entry period after pyrethrin aerosol treatments in a facility can range from 30 min to 2 h, depending on the formulation. This is very short shut-down period compared to the time required for fumigants, which makes aerosol treatment economically appealing to the commercial milling facilities. Aerosols are dispensed as particle sizes ranging from 5-50 microns (Peckman and Arthur 2005) and deposited as liquid droplets, and hence they may primarily affect insects that are directly exposed to the aerosol spray (Bernhard and Bennett 1981; Campbell et al. 2004).

In milling facilities, flour dust and spillage can be generated during various milling operations; nevertheless, the degree of accumulation may vary within a facility. Dense residues may accumulate underneath and adjacent to milling equipment, and in other less disturbed areas such as cracks, crevices, and corners. The accumulated food residues provide excellent harborage and breeding sites to storage insects and can be source of continuous infestations. The confused flour beetle, *Tribolium confusum* Jacquelin du Val, is one of the most common species infesting flour mills (Trematerra and Sciarretta 2004; Trematerra et al. 2007). This species and the related *Tribolium castaneum* (Herbst), the red flour beetle, generally oviposits in flour residues and complete development can occur within refugia (Stanley and Grundmann 1965; Sokoloff 1972; Toews et al. 2010). In addition, when a pest control measure is being applied, the food residue may serve as barrier and limit direct exposure of *T. confusum* to insecticides particles, thereby reducing the effectiviness of the control measure.

Toews et al. (2010) exposed *T. castaneum* to pyrethrin aerosol in Petri dishes that contained 2 g of flour. They reported that mortality never exceeded 60% in any of the exposed developmental stages (eggs, 3rd instars larvae, pupae or adults) of the beetle. Similarly, Arthur and Campbell (2008), exposed *T. confusum* adults to a CO_2 based pyrethrin aerosol in Petri dishes containing 0-2 g of flour and reported increased recovery of adults in the presence of food. These studies show how flour dust and spillages can potentially reduce the effectiveness of pyrethrin aerosols. Integrating sanitation with aerosol could help to exploit their pest control potential. However, there are very few documented scientific studies on the integration of synergized pyrethrin aerosol with sanitation. Hence, the objectives of this study were to determine: 1) effects of different degrees of flour accumulation on the efficacy of synergized pyrethrin aerosol applied to developmental stages of *T. confusum*; and 2) the influence of accumulated flour on adult recovery after exposure to the aerosol.

Materials and Methods

This study was conducted in four experimental sheds at USDA-ARS Center for Grain and Animal Health Research (CGAHR) in Manhattan, KS. The sheds measured 6, 2.9and 2.6 m in length, width, and height, respectively. The temperature and relative humidity (RH) in each shed was maintained by a heating and cooling unit set at 27 °C and 70%, respectively, and were monitored with HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA). Life stages of the test species were obtained from colonies maintained at CGAHR and reared on a diet of wheat flour supplemented with 5% brewer's yeast inside incubators set at 27 °C and 70% RH. The life stages used in the study were 4-week old larvae, 5-week old pupae, and 1-week-old adults. The insecticide used was Entech Fog 10 (EPA Reg. No. 40391-10) which consists of 1.0% pyrethrin, 2.0% piperonyl butoxide (PBO) (technical), 3.33% N-octyl bicycloheptane dicarboximide, and 93.67% other ingredients (Entech Systems, Kenner, LA, USA).

Two of the sheds were used as the untreated control and two sheds for exposure to the insecticide. Inside each shed, a 0.70 m x 0.52 m area was marked with a measuring tape. This grid was about 1.2 m from the end wall and 1.1 m from the side walls, and was divided into 5 equal columns and 3 rows. Glass Petri dishes (90 mm diameter) were prepared with 0, 0.1, 1, 5 or 10 g of wheat flour to simulate different levels of sanitation or food accumulation in a flour mill. Approximately 2 h before the aerosol application, 20 insects of each life stage of *T. confusum* were added to separate Petri dishes for each of the flour accumulations. A total of 15 dishes were exposed per shed (5 flour levels with 3 life stages). The dishes were placed randomly in a 5 x 3 layout. A line was marked for the aerosol spray position at approximately 1.5 m from the door and 2.8 m from the grid where the dishes were set.

The required volume of insecticide per shed (47 mL) was calculated based on the label of Entech Fog 10 (1.04 mL/m^3). The insecticide was delivered using a hand-held aerosol applicator (Fogmaster jr 5330, Fogmaster Corporation, Deerfield Beach, FL, USA) with a delivery capacity of about 27 g/min. Since 1 mL of Entech Fog 10 weighs 0.76 g, a total of 20.8 mL Entech Fog 10 could be delivered per min. Hence, total time required to deliver 47 mL of insecticide was 2 min and 16 sec. The aerosol was applied by standing at the aerosol spray position described above and holding the Fogmaster at about 3 m above the floor and pivoting the unit slowly from side to side. The insects were held in the sheds for 2 h post aerosol application, and then removed. About 1 g of flour was added to each dish, and although, the new flour levels were 1, 1.1, 2, 6, and 11 g, they will be referred to by their original levels during exposure (0, 0.1, 1, 5, and 10 g) to avoid confusion. After the flour was added, the dishes were placed in an incubator set at 27 °C and 70% RH. This entire process was repeated four times at weekly intervals.

For each replicate, adults were assessed at 2, 5, 8, and 15-d post-exposure and classified as live, moribund (on their backs but still moving) and dead (not responding when probed or prodded). Untreated controls were classified in the same manner. To observe the effects of different treatments on adults, the dead and moribund individuals were totaled (affected adults). Exposed pupae and larvae were held in the incubator for 21 and 28-d respectively, to assess adult emergence from the exposed immatures.

Statistical analyses were conducted using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS 9.2 software, SAS Institute, Cary, NC, USA). The number of affected adults, adult emergence from immatures, and responses of adults with time in each treatment were assessed and converted to percentage values, and then and were transformed by arcsine square root to perform statistical analyses. However, the data presented in figures and text are all untransformed means (percentage values). Treatment means for percentage affected adults among different flour levels were separated using the Waller-Duncan *k*-ratio *t*-test. For immatures, the mean adult emergence was compared between stages among flour levels. Differences among means were considered significant at $\alpha = 0.05$ level.

Results

Mortality of untreated controls at 15-d after aerosol application was less than 2% for adult exposure, while the adult emergence for pupal and larval stages was at least 96% at 28 and 21-d, respectively. Hence, no corrections or adjustments were necessary for treatment data and untreated controls were eliminated from the statistical analysis.

The percentage of affected adult of *T. confusum* varied greatly among flour levels at the 15-d post exposure assessment ($F = 33.30$, df = 4, 35, $P < 0.01$). At least 95% affected adults were observed when adults were exposed with 0, 0.1, or 1 g of flour. Exposing beetles with 5 g and 10 g of flour reduced the affected adults to 85% and 53%, respectively (Fig. 1). Similarly, for the pupal and larval stage exposure, the percentage of normal adult emergence at 21 and 28-d, respectively was significantly different among flour levels ($F = 45.51$, df = 4, 35, $P < 0.01$; $F = 20.86$, df = 4, 35, $P < 0.01$, respectively). In dishes with no flour or 0.1 or 1 g of flour, the percentage of adult emergence from exposed pupal and larval stages were less than 2% and 7%, respectively. Adult emergence from pupae and larvae exposed with 5 g of flour was 22% and 15%, respectively, while adult emergence of pupae and larvae exposed with 10 g of flour increased to 45% and 52%, respectively (Fig. 2). However, the two stages were not different in terms of susceptibility to the aerosol ($F = 0.14$, df = 1, 240, $P = 0.72$). Adults that were moribund after they were exposed recovered with time. At 2-d, at least 70% of the adults were moribund in the 1, 5, and 10 g flour dishes, and almost 80% of the adults in the 0 and 0.1 g flour dishes were dead. No mobile adults were observed in any of the treatment dishes at this observation. At 5-d, there was sharp reduction in the percentage of moribund adults. They were either dead or were able to recover as normal adults. The process continued slowly until the last date assessment at 15-d. More adults recovered in the dishes with 5 and 10 g of flour (15 and 10% respectively) compared to the dishes with 0, 0.1, or 1 g of flour 0.7, and 5% respectively (Fig. 3).

Discussion

Our results showed that pyrethrin aerosol produced quick effects because the exposed *T. confusum* adult were either dead or moribund when observed at 2-d post aerosol application. This immediate efficacy of synergized pyrethrin on adults of *T. castaneum* has been previously reported by Toews et al. (2010). After 5-d, the moribund adults began to recover. One possible explanation for the reduced efficacy of pyrethrin aerosol in the deeper flour dishes is that the flour could have limited direct exposure to aerosol during the application. Similar results have been reported by Arthur (2000) with the contact insecticide cyfluthrin, in which adults of *T. castaneum* were exposed on concrete covered with flour and treated with cyfluthrin. Adult recovery was higher in the concrete that had greater area coverage with flour, indicating possible absorption of the insecticide particles, leading to reduced efficacy. In a separate study, Arthur (2000) exposed *T. castaneum* adults for 2 h on concrete treated with cyfluthrin, then transferred the adults to Petri dishes containing either 1 g of flour, pine sawdust, or wheat kernels. Survival was greater in Petri dishes containing flour or sawdust than in dishes with wheat kernels, indicating that flour or extraneous dust may also enable insects to physically remove insecticides particles from their body.

Conversely, the overtime recovery of adult *T. confusum* in treated flour indicates little residual activity of pyrethrin aerosol. The pyrethrin formulation that we used for this study consisted of 1% pyrethrin and 2% PBO, and this formulation did not increase residual activity of pyrethrin as opposed to results obtained by previous researchers (Casida 1980; Keane 1998; Cox 2002). The 1:2 ratio of pyrethrin and PBO may not have been sufficient enough to increase potency of the pyrethrin. McDonald (1968) treated adults of *T. confusum*, adults of *Lasioderma serricorne* L., the cigarette beetle, and larvae of *Attagenus megatoma* F., the black carpet beetle, by topical application of ratios of pyrethrin plus PBO. The result showed that a 1:10 ratio was significantly more potent than the lower ratios.

In addition, larvae and pupae exposed with 5 and 10 g flour were better able to emerge as normal adults than larvae and pupae exposed with 1 g flour, while larvae exposed with 0 and 0.1 g flour did not emerge as adults. These results are consistent with those of Wijayaratne et al. (2012), in which larvae of *T. castaneum* were exposed to methoprene on unsealed concrete and on varnished wood. The efficacy of methoprene was reduced due to the presence of flour on concrete but not on varnished wood. According to Wijayaratne et al. (2012), methoprene was absorbed by the flour on the varnished wood, while in concrete, methoprene was absorbed by flour and also lost due to degradation. Therefore, the larvae on varnished wood ingested methoprene from flour and were affected in the same way as the larvae in varnished wood without flour. Although pyrethrin and methoprene have different modes of action, these results suggest that flour could absorb insecticides and influence its efficacy against immature stages in a similar manner as has been reported for adults.

In conclusion, pyrethrin aerosol gave good control against adult and immature stages of *T. confusum* when exposed without flour or with a low amount of flour, but as the level of flour increased, the aerosol was less effective. Therefore, the presence of flour could reduce the efficacy of pyrethrin aerosol by limiting insects' exposure and providing nutrition to treated insects. Also, the reduced efficacy in deeper flour levels may be due to lower penetration ability of pyrethrin aerosols. Hence, this study emphasizes the importance of thorough cleaning of a facility prior to aerosol application. Attention should be given to the area where maximum accumulation of flour and dusts can occur such as cracks and crevices, dead spaces on floors, and underneath the machinery. In addition, precise targeting of insecticides based on diligent monitoring of populations rather than calendar-based spray schedules could lead to more effective pest management.

References

Arthur, F.H., 2000. Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder. J. Stored Prod. Res. 36: 15–23.

Arthur, F.H., 2010. Residual efficacy of aerosols to control *Tribolium castaneum* and *Tribolium confusum*, pp. 789-792. *In* M.O. Carvalho, C.S. Fields Alder, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Nvarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, N.D.G. White (eds.), Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal. Julius-Kühn-Archiv, 425.

Arthur, F.H., and J.F. Campbell. 2008. Distribution and efficacy of pyrethrin aerosol to control *Tribolium confusum* (Coleoptera: Tenebrionidae) in food storage facilities. J. Stored Prod. Res. 44: 58-64.

Beckett, S.J., P.G. Fields, and B. Subramanyam. 2007. Disinfestations of stored products and associated structures using heat, pp. 182-236. *In* Heat treatments for postharvest pest control: theory and practice. CAB International, Oxon, UK.

Bernhard, K.M., and G.W. Bennett. 1981. Ultra-low-volume applications of synergized pyrethrins for stored-product insect control. J. Econ. Entomol. 74: 572-576.

Campbell, J.F., and R.T Arbogast. 2004. Stored product insects in a flour mill: population dynamics and response to fumigation treatments. Entomol. Exp. Appl. 112: 217-225.

Campbell, J.F., F.H. Arthur, and M.A. Mullen. 2004. Insect management in food processing facilities. Adv Food Nutr Res. 48: 239–295.

Casida, J. E. 1980. Pyrethrum flowers and pyrethroid insecticides. Environ. Health Perspect. 34:189.

Cotton, R.T. 1958. Insect control in flour mills. U. S. Department of Agriculture, Agricultural Marketing Service, Marketing Research Division, Washington, D. C., US.

Cox, C. 2002. Insecticide synergist fact sheet: piperonyl butoxide. http://www.pesticide.org/get-the-facts/pesticide-factsheets/factsheets/piperonylbutoxide (Accessed, Jan, 23, 2013).

Keane, P. 1998. The use of piperonyl butoxide in formulations for the control of pests of humans, domestic pets and food animals, pp. 289-300. *In* D.G. Jones (ed.), Piperonyl butoxide: the insecticide synergist. Academic Press, London, UK.

McDonald, L. L. 1968. Relative effectiveness of tropital and piperonyl butoxide as synergists for pyrethrins against stored product insects. J. Econ. Entomol. 61: 1645-1646.

Peckman, P.S., and F.H. Arthur. 2005. Insecticide space treatments in food plants, pp. 175-182. *In* J.W. Heaps (ed), Insect management for food storage and processing. AACC International, St. Paul, MN, USA.

Sokoloff, A. 1974. The biology of tribolium*:* with special emphasis on genetic aspects. Oxford University Press.

Stanley, M.S.M., and A.W. Grundmann. 1965. Observations on the morphology and sexual behavior of *Tribolium confusum* Duval. J. Kans. Entomol. Soc. 38: 10-18.

Sutton, A.E., F.H. Arthur, K.Y. Zhu, J.F. Campbell, and L.W. Murray. 2011. Residual efficacy of synergized pyrethrin and methoprene aerosol against larvae of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 47: 399-406.

Toews, M.D., J.F. Campbell, and F.H. Arthur. 2010. The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle, *Tribolium castaneum.* J. Insect Sci. 10: 1-14.

Trematerra, P., and A. Sciarretta. 2004. Spatial distribution of some beetles infesting a feed mill with spatio-temporal dynamics of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum*. J. Stored Prod. Res. 40: 363–377.

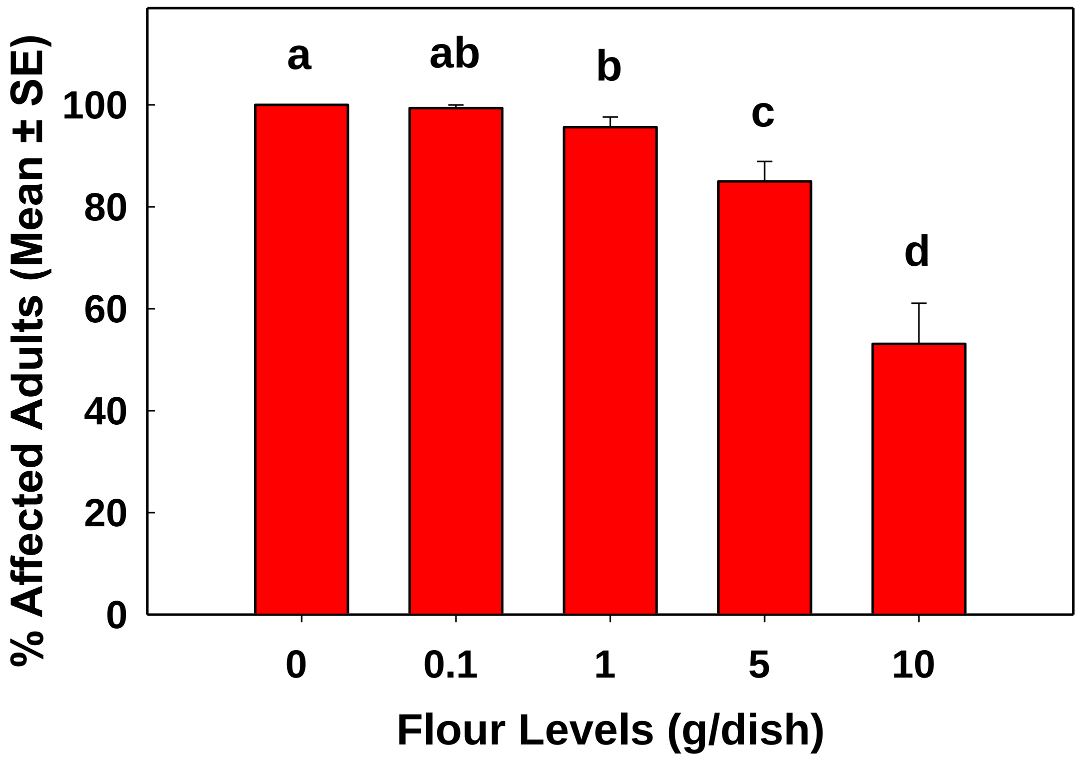
Trematerra, P., P. Gentile, A. Brunetti, L.E. Collins, and J. Chambers. 2007. Spatio temporal analysis of trap catches of *Tribolium confusum* du Val in a semolina mill with a comparison of female and male distributions. J. Stored Prod. Res. 43: 315–322.

Wijayaratne, L.K., P.G. Fields, and F.H. Arthur. 2012. Residual efficacy of methoprene for control of *Tribolium castaneum* (Coleoptera: Tenebrionidae) larvae at different temperatures on varnished wood, concrete, and wheat. J. Econ. Entomol. 105: 718-725.

Figure caption

Fig.1. Percent affected adult of *T. confusum* observed in the Petri dishes with different flour levels at 15-d after aerosol application.

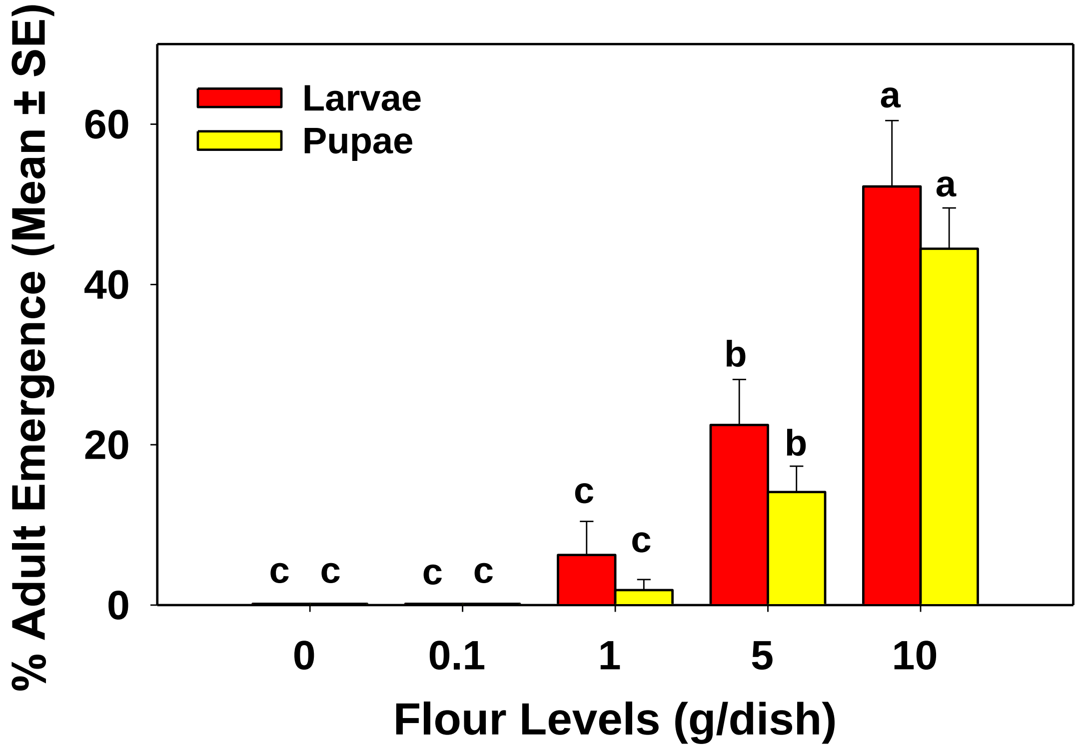
Mortality among flour levels within stage with the same letter not significantly different ($P \geq 0.05$, $n = 8$, Waller-Duncan k-ratio t-test).



a
ab
b
c
d
100
80
60
40
20
0
0
0.1
1
5
10
% Affected Adults (Mean ± SE)
Flour Levels (g/dish)

Figure caption

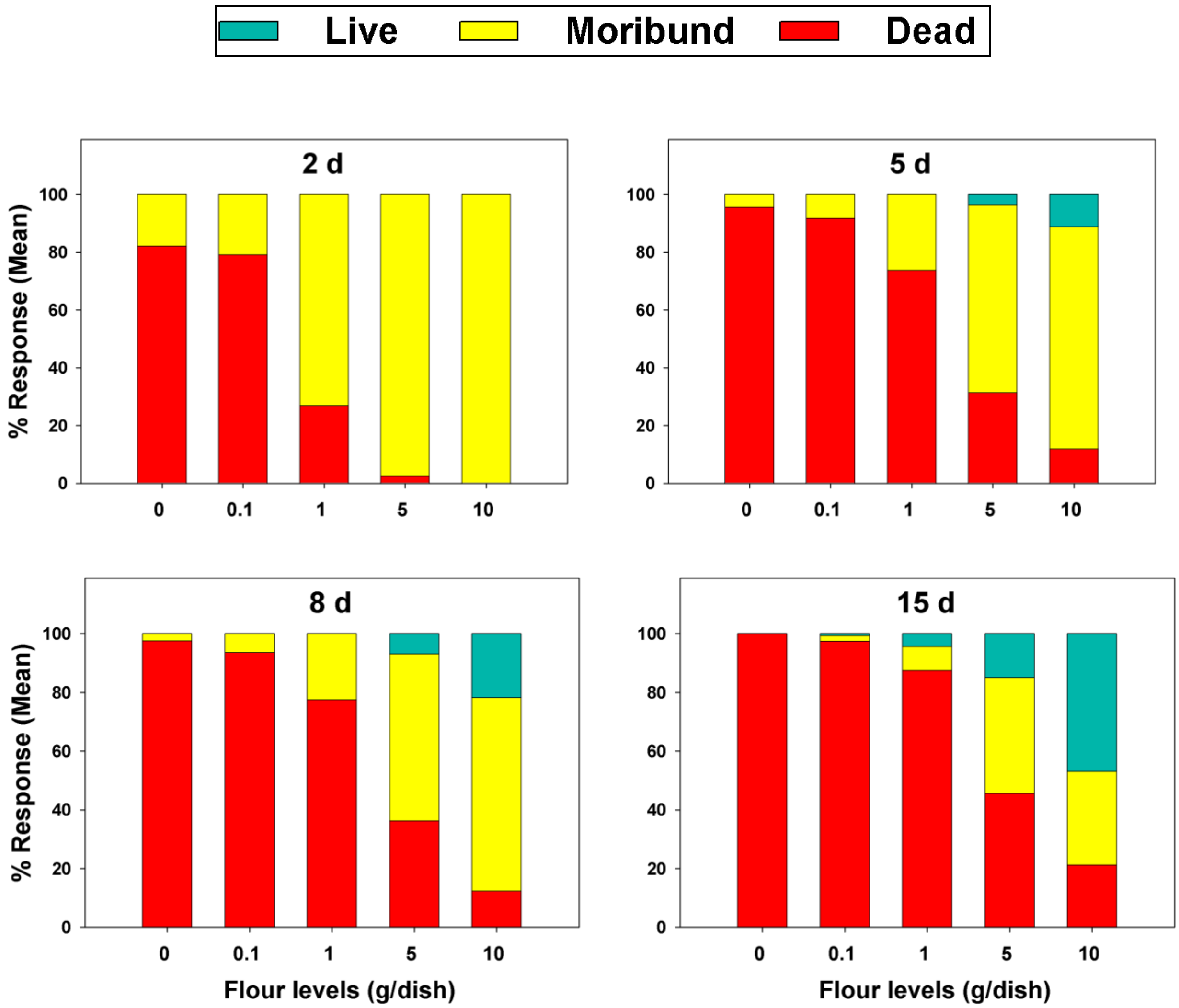
Fig.2. Percent live normal adult of T. confusum observed after exposure of larvae and pupae in the Petri dish with different flour levels. Adult emergence for pupae and larvae stages exposed was observed at 21 and 28-d, respectively, after aerosol application. Adult emergence among flour levels within stage with the same letter are not significantly different ($P \geq 0.05$, $n = 8$, Waller-Duncan *k*-ratio *t*-test).



% Adult Emergence (Mean ± SE)
Larvae
Pupae
0
20
40
60
c
c
c
c
c
c
b
b
a
a
0
0.1
1
5
10
Flour Levels (g/dish)

Figure caption

Fig. 3. Percent response of adult *T. confusum* in each flour levels at different exposure intervals: 2, 5, 8 and 15-d after aerosol application


Live
Moribund
Dead
2 d
5 d
8 d
15 d
% Response (Mean)
0
20
40
60
80
100
0
0.1
1
5
10
Flour levels (g/dish)

Chapter 3 - Influence of seasonal temperature variations and barriers on the efficacy of pyrethrin aerosol in the flour mills

Abstract

Flour mills in the United States are utilizing synergized pyrethrin aerosol for management of stored product insects. However, the dispersal of the aerosol within a facility may be hampered by barriers created from machinery and other equipment that block dispersal. Additionally, seasonal temperature variations may influence the dispersion and toxicity of synergized pyrethrin. A study was conducted to evaluate the influence of barriers and temperatures on the efficacy of synergized pyrethrin aerosol against adults and pupae of *T. confusum*. Insects were exposed to aerosol inside experimental sheds maintained at target temperatures of 22, 27, and 32 °C. Wooden boxes 1 m in length, 20 cm in width, and 5, 10, or 20 cm in height were used for creating different open and concealed areas for exposing insects. Results showed that exposing adults of *T. confusum* in the open produced > 76% mortality at all test temperatures, while mortality was significantly reduced underneath the boxes. Similarly, less than 2% of pupae exposed in the open areas emerged as adults, but as the distance increased from the front to the back of the box adult emergence increased. The recovery of moribund adults increased the as the distance of exposure position increased underneath the boxes. More aerosols dispersed under the box height of 20 cm compared to 5 and 10 cm. Results show pyrethrin aerosol can provide good control of *T confusum* and is less affected by seasonal temperature variations, but areas aerosol dispersion into obstructed or concealed areas may be limited inside flour mills.

Keywords: Pyrethrin aerosols, *Tribolium confusum*, flour mills, barriers, seasonal temperatures

Introduction

Methyl bromide (MB), an important fumigant of the food industry, was scheduled to be phased out within the United States (US) beginning in January 2005 as the result of an international environmental agreement, the Montreal Protocol (Fields and White 2002). Currently MB is being used under critical use exemptions (CUE). However, the amount approved for use is declining each year. In 2011, 2012, 2013, and 2014, the amount approved for use were 8.1, 4.0, 2.2, and 1.7% of 1991 baseline levels (25,500 metric tons), respectively (USEPA 2013). The phase out of MB has shifted the reliance for insect control in flour mills to alternative fumigants such as sulfuryl fluoride, cylinderized phosphine, or on heat treatments. However, heat treatment may not be suitable for many older facilities and can be cost-intensive, while fumigants require several facility shutdowns, and may cause potential negative impacts on equipment and structural components.

In the recent years, synergized pyrethrin with piperonyl butoxide (PBO) is considered as a technically and economically feasible alternative to whole structure treatments. They can be used to target a portion of a facility, which makes them cost effective and desirable option for many commercial operations (Boina and Subramanyam 2012). However, in an active flour mill, the dispersal of the aerosol within a treated area may be impacted by barriers such as milling machinery, equipment and other accessories. Aerosol insecticides are deposited as liquid droplets, primarily in vertical downward directions, therefore, their horizontal movement underneath the concealed and obstructed areas and resulting particle deposition is reduced compared to more open areas (Boina and Subramanyam 2012). Bernhard and Bennett (1981) reported lower penetration of synergized pyrethrin aerosol in open and partly open cabinets, and in closed area compared to completely exposed areas. Similarly, Toews et al. (2010) reported less than 75% mortality in all life stages of the red flour beetle, *Tribolium castaneum* in the Petri dishes exposed under wooden pallets as opposite to at least 80% mortality in open positions.

Additionally, the ambient temperature inside a facility during aerosol application may influence insecticidal efficacy. Campbell et al. (2010) conducted a study in which they monitored temperature within a flour mill and a wheat processing facility. They reported average daily temperature of the facilities to be about 24 and 30 °C during cool and warm seasons, respectively. Although pyrethrin insecticide is known for its negative temperature coefficient, the addition of the synergist PBO increases its toxicity at higher temperatures (Blum and Kearns 1956). Also, high temperature may facilitate diffusion of aerosols into hidden and obstructed areas. However, there is a lack of published data regarding the distribution and efficacy of PBO based pyrethrin at different temperatures.

The confused flour beetle, *Tribolium confusum* Jacquelin du Val, is one of the important pests of flour mills (Campbell and Runnion 2003; Arthur and Campbell 2008; Toews et al. 2009). The constant availability of flour patches and the structural complexity created by different types of processing equipment makes the flour mills an excellent setting for their development (Cotton 1958). This beetle species has been reported to be more tolerant to pyrethrin than its close relative *T castaneum* (Arthur 2008). Also, *T. castaneum* can fly at temperature higher than 30 °C whereas *T. confusum* do not fly (Rees 2004). Therefore, *T. confusum* can be a model species for the study of temperature effects on aerosol efficacy inside a closed environmental setting. Thus, this study was conducted to: 1) evaluate the efficacy of pyrethrin arososol at three different temperatures: 22, 27 and 32 °C against *T. confusum* adults and pupae (the most tolerant and active stage vs. susceptible and immobile life stage of *T. confusum*) and 2) dispersion of aerosol insecticide at different open and obstructed positions.

Materials and Methods

This study was conducted in six experimental sheds at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS, during August to October 2012. The experimental sheds were 6 m long, 2.9 m wide and 2.6 m high. The target temperatures for the study were 22, 27, or 32 °C and each temperature was maintained in an aerosol treatment shed and a control shed. Electrical heaters were used to obtain the required temperatures and the temperatures were monitored with HOBO data loggers (Onset Computer Corporation, Bourne, MA). Colonies *T. confusum* were obtained from CGAHR, and reared on a diet of wheat flour supplemented with 5% brewer's yeast at 27 °C, 70% RH. 5-weeks old pupae and mixed aged adults were used for the study. The formulation of synergized pyrethrin insecticide used in this test was Entech Fog 10 (EPA Reg. No. 40391-10). It was comprised of 1.0% pyrethrins, 2.0% piperonyl butoxide (PBO) (technical), 3.33% N-octyl bicycloheptane dicarboximide, and 93.67% other ingredients (Entech Systems Corporation, Kenner, LA). One of the sheds under each temperature category was used as the untreated control and other for exposure to the aerosol.

Inside each shed three 1 m x 0.20 m areas were marked on the floor at a distance of 0.30 m from one another. These areas were about 0.60 m from the end wall and 0.85 m from the side walls. Three wooden boxes 1 m in length, 20 cm width, and 5, 10, or 20 cm height were placed randomly in each of the three marked areas. The boxes had only one opening at the front. A line (P1 = open position) was marked outside each box, along with four lines (P2, P3, P4, P5 = concealed position) inside the boxes at about 7.6, 35.5, 63.5, and 91.4 cm, respectively, from the open end. Exposure arenas were prepared using Petri dishes (62 cm^2 in area), with 1 g of wheat flour. Each dish was placed at the positions described above, and contained either 20 adults or 10 pupae of *T. confusum* (Fig. 1). A total of 30 Petri dishes containing *T confusum* adults or pupae were exposed per shed (2 life stages x 5 exposure positions x 3 boxes heights = 30). At about 2.8 m from the open exposure positions, a line was marked as the aerosol spray position.

The required volume of insecticide per shed (47 mL) was calculated on the basis of labeled rate of Entech Fog 10 (1.04 mL/m^3). The insecticide was delivered using a hand-held aerosol applicator, Fogmaster jr 5330 (The Fogmaster Corporation, Deerfield Beach, FL) with delivery capacity of 27 g/min. Since 1 mL of Entech Fog 10 weighs 0.76 g, a total of 20.8 mL Entech Fog 10 could be delivered per min. Hence, total time required to deliver 47 mL of insecticide was 2 min and 16 sec. The aerosol was applied by a person standing at the aerosol spray position and holding the Fogmaster spray unit at about 3 m above the floor and pivoting the unit slowly from side to side. Three sprays were done for each replication. For example, 22 °C shed was sprayed in morning, 27 °C in noon, and 32 °C in afternoon. The insects were held in both treated and control sheds for 2 h post aerosol spray then removed from the sheds and placed in an incubator set at 27 °C and 70% RH. This entire process was repeated six times at weekly intervals.

For each replicate, adults were examined at 2, 8, and 15-d after exposure to the aerosols, and classified as follows: live, moribund (intermediate state), and dead adults. Untreated controls were classified in the same manner. Exposed pupae were held in the incubator for 21-d, to assess the number that could successfully emerge as adults. Statistical analyses were conducted with the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS 9.2 software, SAS Institute, Cary, NC). The number of responses, in each temperature and positions were converted to percentage values and were transformed by arcsine square root to perform statistical analyses. The data presented in figures and text are the untransformed values. To observe the response of adults over time for adult stage exposure, treatment means for percentage response among different exposure position within each observation day were separated using the Waller-Duncan *k*-ratio *t*-test in SAS ($P < 0.05$). Mean adult emergence of exposed pupae was compared between different positions within temperature. Differences among means were considered significant at $P < 0.05$.

Results

Percent adult mortality at 15-d post exposure was significantly different among exposure position for all the box heights at 22, 27, and 32 °C ($F = 303.99$, df = 4, 225, $P < 0.01$). Adult mortality was generally >76% when exposed at the open positions at all three temperatures, while mortality was significantly reduced at the exposure positions inside the boxes. At position P2 inside the box height 5, 10, and 20 cm, mortality was generally <36, <29, and <23% at 22, 27, and 32 °C, respectively. Mortality at positions P3, P4, and P5 was different under the box heights of 5 and 10 cm, while under box height 20 cm, mortality at position P3 was greater compared to positions P4 and P5. Although the main effects of temperatures and box height were not statistically significant ($F = 2.95$, df = 2, 225, $P = 0.054$; $F = 2.04$, df = 2, 225, $P = 0.13$, respectively), we found a clear trend indicating that lower temperature and greater box height produces higher mortality (Fig. 2, A-C).

Adult emergence from exposed pupae was significantly different for exposure positions P1, P2, P3, P4, P5 and box heights, 5, 10, 20 cm ($F = 460.18$, df = 4, 225, $P < 0.01$; $F = 20.11$, df

= 2, 225, $P < 0.01$, respectively). There was virtually 100% suppression of adult emergence from pupae exposed in open positions at 22 and 27 °C and about 2 % adult emergence at 32 °C. Exposing pupae at P2 position under box heights 5, 10, 20 cm produced at least 13%, 14%, and 18% survival at 22, 27, and 32 °C, respectively. Adult emergence of pupae exposed at P3, P4, and P5 under box heights 5 and Positions 5 and 10 cm was at least 86%, while for box height 20 cm, adult emergence at positions P3, P4, and P5 was <76%, >81%, and>91%, respectively (Table 4). Similarly, box height was significant for only positions P3 and P4 at 22 °C ($F = 8.74$, df = 2, 15, $P < 0.05$; $F = 3.63$, df = 2, 15, $P = 0.05$, respectively), and position P4 at 32 °C ($F = 7.40$, df = 2, 15, $P = 0.05$). At those positions, adult emergence was lower at the 20 cm box height compared to the 5 and 10 cm box heights, indicating some increased dispersion as the box opening increased. Adult emergence was not significant at the different temperatures ($F = 1.62$, df = 2, 225, $P = 0.20$).

To calculate the adult recovery over time, percent response was analyzed for each exposure position for each box height and for all temperatures at the different observation periods. The box height and temperatures were not significant, while exposure position was always significant (Table 1, 2, 3). Therefore, data for temperature and box height at each observation date were combined for further analysis.

The percentage of moribund adults at each exposure positions was significantly different at 2, 8, and 15-d ($F = 75.55$, df = 4, 265, $P < 0.01$; $F = 62.23$, df = 4, 265, $P < 0.01$; $F = 51.60$, df = 4, 265, $P < 0.01$, respectively). Generally, there were more moribund adults at positions P1 and P2 than at positions P3, P4 and P5. Also, the number of moribund adults at each position declined with time (Fig. 3, A-C). The number of dead adults at each position was significantly different at 2, 8, and 15-d (F = 121.85, df = 4, 265, P < 0.01; F = 170.83, df = 4, 265, P < 0.01; F = 282.35, df = 4, 265, P < 0.01, respectively). More dead adults were found in the open position compared to the concealed positions under the boxes, and the percentage of dead adults declined as the distance underneath the box increased from the open end. Also, the number of dead adults in each position increased over the observation periods (Fig. 3, D-F). Similarly, the percentage of live adults produced among each exposure positions were significantly different at 2, 8, and 15-d (F = 121.85, df = 4, 265, P < 0.01; F = 170.83, df = 4, 265, P < 0.01; F = 282.35, df = 4, 265, P < 0.01, respectively). No live adults were observed at the open positions, while the number of live adults increased in the concealed position with the increasing distance from the open end. Also, the percentage of live adults increased over time in all concealed positions (Fig. 3, G-I).

Discussion

Our results indicated that when adults of *T. confusum* were exposed to pyrethrin aerosol in an open area without any obstructions, mortality was generally >76% at all three test temperatures of 22, 27, and 32 °C. This is consistent with results reported by Arthur (2008) and Arthur and Campbell (2008) in which mortality of *T. confusum* adults was > 80%. Similarly, none of the pupae exposed in the open positions at 22 and 27 °C emerged as adults, while at 32 °C adult emergence was < 2%. In our study, we exposed *T. confusum* in Petri dishes containing 1 g of wheat flour to provide food during and after exposure, but many studies have documented reduced efficacy of applied insecticides in the presence of food (Arthur 2000, 2008; Arthur and Campbell 2008; Toews et al. 2010). Food or extraneous material may limit exposure during aerosol application, enable insects to physically remove insecticides particles from their body, and provide nutritional support for recovery (Arthur 2000). We presume that adult mortality would have been higher if they were exposed without flour.

Additionally, adult mortality was reduced significantly when they were exposed underneath the boxes. Some effects were seen at position P2 which was located at a distance of 7.5 cm from open end of boxes, but mortality decreased with increased distance inside the box. Also, the pupae exposed underneath the boxes were able to emerge as adults. This is consistent with recent results reported by Toews et al. (2010), in which eggs, larvae, pupae and adults of *T. castaneum* were exposed to pyrethrin aerosol. Mortality was reduced in the dishes positioned under pallets compared to those exposed in the open areas. This could indicate that aerosol particles were not distributed horizontally underneath the obstructions, as stated by previous researchers (Bernhard and Bennett 1981; Toews et al, 2010). However, these results seems to contradict with results reported by Arthur (2008) and Jenson et al. (2010) who documented good dispersal of aerosol particles within a field site and inside experimental sheds of similar construction to those used in our study. Several factors may determine the dispersion and efficacy of aerosols such as types of obstacles, insecticide formulations, insect species and life stages. For example, Arthur (2008) showed no differences in adult mortality of *T. confusum* exposed at open and obstructed positions, but there were differences in total survival and number of adults that were knocked down from exposure but not dead. Also, Arthur (2008) exposed insects at the center of the exposure room and near the side walls instead of underneath pallets. Jenson et al. (2010) created obstacles to aerosol by placing eggs of Indian meal moth, *Plodia interpunctella* (Hübner), underneath equipment in the mill that was raised up off the floor. The formulations of aerosol used in their study were: 1% pyrethrin plus methoprene, 3% pyrethrins, and 3% pyrethrin plus methoprene. They demonstrated no difference in adult emergence from eggs exposed at open and obstructed positions. In our study with the different box heights, the available space through which aerosol particles had to disperse might have influenced the results. Our study indicated some differences due to temperatures in live, moribund, and dead adults after 2 and 8-d post exposure but not at the final assessment at 15-d. Also, temperature did not affect adult emergence from exposed pupae. This was contradictory to our assumption that increased temperature facilities dispersal of aerosol and increases the toxicity of synergized pyrethrin (Blum and Kearns 1956). Hence, it appears that seasonal temperature variation may not affect overall efficacy of the pyrethrin aerosol. Additionally, our study showed increased adult recovery from at the concealed positions under the boxes. This result is consistent with the previous study in which Arthur and Campbell (2008) showed greater recovery in *T. confusum* with increased distance from the aerosol application position.

In conclusion, pyrethrin aerosol can provide good control of *T. confusum*, but dispersal of the aerosol may be limited underneath pallets and milling equipment. However, our study was conducted with a hand-held spray applicator, and results with commercial systems may be different due to the increased dispersal capacity. Therefore, aerosols can be used for precise targeting of storage insects in addition to whole structure treatments. Also, temperatures in the range of 22-32 °C had no significant difference on aerosol efficacy, which increases their capacity to be used throughout most of the year in temperate locations.

References

Arthur, F.H. 2000. Impact of high temperatures on efficacy of cyfluthrin and hydroprene applied to concrete to control *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 39: 193–204.

Arthur, F.H. 2008. Aerosol distribution and efficacy in a commercial food warehouse. Insect Sci. 15: 133-140.

Arthur, F.H., and J.F. Campbell. 2008. Distribution and efficacy of pyrethrin aerosol to control *Tribolium confusum* (Coleoptera: Tenebrionidae) in food storage facilities. J. Stored Prod. Res. 44: 58-64.

Bernhard, K.M., and G.W. Bennett. 1981. Ultra-low-volume applications of synergized pyrethrins for stored-product insect control. J. Econ. Entomol. 74: 572-576.

Blum, M.S., and C.W. Kearns. 1956. Temperature and the action of pyrethrum in the American cockroach. J. Econ. Entomol. 49:862-865.

Boina, D.R., and B. Subramanyam. 2012. Insect management with aerosols in food processing facilities, pp. 195-212. *In* F. Perveen (ed.), Insecticides: advances in integrated pest management. InTech. http://www.intechopen.com (Accessed, Jan, 27, 2013).

Campbell, J.F., and C. Runnion. 2003. Patch exploitation by female red flour beetles, *Tribolium castaneum*. J. Insect Sci., Available online: insectscience.org/3.20

Campbell, J.F., M.D. Toews, F.H. Arthur, and R.T. Arbogast. 2010. Long-term monitoring of *Tribolium castaneum* in two flour mills: seasonal patterns and impact of fumigation. J. Econ. Entomol. 103: 991-1001.

Cotton, R.T. 1958. Insect control in flour mills. *U.S.* Department of Agriculture, Agricultural Marketing Service, Marketing Research Division, Washington, D. C, US.

Fields, P.G., and N.D.G. White. 2002. Alternatives to methyl bromide treatments for stored product and quarantine insects. Annu. Rev. Entomol. 47:331-359.

Jenson, E.A., F.H. Arthur, and J.R. Nechols. 2010. Methoprene and synergized pyrethrins as aerosol treatments to control *Plodia interpunctella* (Hubner), the Indian meal moth (Lepidoptera: Pyralidae). J. Stored Prod. Res. 46: 103-110.

Rees, D. 2004. Insects of stored products. CSIRO Publishing, Collingwood, Victoria, Australia.

Toews M.D., J.F. Campbell, and F.H. Arthur. 2010. The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle, *Tribolium castaneum.* J. Insect Sci. 10: 1-14.

Toews, M.D., F.H. Arthur and J.F. Campbell. 2009. Monitoring *Tribolium castaneum* (Herbst) in pilot-scale warehouses treated with *b*-cyfluthrin: are residual insecticides and trapping compatible? Bull. Entomol. Res. 99: 121–129.

USEPA, 2013. Critical use exemption information. http://www.epa.gov/ozone/mbr/cueinfo.html (Accessed, Feb, 9, 2013).

Table 1. Analysis of variance for effects on percent survival of *T confusum* adults exposed to aerosol at open and obstructed positions under different height boxes at three temperatures levels.

Factors	Num DF	Den DF	F value	P value
Day 2				
Temperatures: 22, 27, 32 °C	2	225	8.60	<0.01
Box height: 5, 10, 20 cm	2	225	48.95	<0.01
Positions	4	225	205.04	<0.01
Day 8				
Temperatures: 22, 27, 32 °C	2	225	2.42	<0.05
Box height: 5, 10, 20 cm	2	225	22.39	<0.01
Positions	4	225	214.40	<0.01
Day 15				
Temperatures: 22, 27, 32 °C	2	225	1.37	0.25
Box height: 5, 10, 20 cm	2	225	17.40	<0.01
Positions	4	225	342.52	<0.01

Table 2. Analysis of variance for effects on percent moribund adults of *T confusum* exposed to aerosol at open and obstructed positions under different height boxes at three temperatures levels

Factors	Num DF	Den DF	F value	P value
Day 2				
Temperatures: 22, 27, 32 °C	2	225	4.26	<0.05
Box height: 5, 10, 20 cm	2	225	38.15	<0.01
Positions	4	225	115.98	<0.01
Day 8				
Temperatures: 22, 27, 32 °C	2	225	0	0.99
Box height: 5, 10, 20 cm	2	225	16.93	<0.01
Positions	4	225	74.18	<0.01
Day 15				
Temperatures: 22, 27, 32 °C	2	225	0.27	0.76
Box height: 5, 10, 20 cm	2	225	13.52	<0.01
Positions	4	225	58.27	<0.01

Table 3. Analysis of variance statistical effects on percent dead adults of *T confusum* exposed to aerosol at open and obstructed positions under different height boxes at three temperatures levels

Factors	Num DF	Den DF	F value	P value
Day 2				
Temperatures: 22, 27, 32 °C	2	225	4.03	<0.05
Box heights: 5, 10, 20 cm	2	225	0.12	0.89
Positions	4	225	53.39	<0.01
Day 8				
Temperatures: 22, 27, 32 °C	2	225	7.37	<0.01
Box height: 5, 10, 20 cm	2	225	0.29	0.749
Positions	4	225	105.29	<0.01
Day 15				
Temperatures: 22, 27, 32 °C	2	225	2.95	0.054
Box height: 5, 10, 20 cm	2	225	2.04	0.132
Positions	4	225	303.99	<0.01

Table 4. Percent live adults (mean ± SE) produced from pupae exposed at different exposure position in and outside of different height box at different temperatures

	Exposure positions	H1	H2	H3
22 °C	P1	0 D	0 C	0 D
	P2	27.1 ± 11.3 Ca	23.5 ± 10.5 Ba	13.3 ± 5.6 Ca
	P3	88.3 ± 4.8 Ba	86.7 ± 4.2 Aa	48.5 ± 10.3 Bb
	P4	98.3 ± 1.7 Aa	93.3 ± 4.2 Aab	81.7 ± 7.5 Ab
	P5	95.0 ± 3.4 ABa	96.8 ± 2.1 Aa	91.7 ± 5.4 Aa
27 °C	P1	0 Ca	0 Ca	0 Da
	P2	36.7 ± 11.7 Ba	26.7 ± 9.9Ba	14.8 ± 4.3 Ca
	P3	87.1 ± 4.6 Aa	90.0 ± 6.3Aa	71.7 ± 7.9 Ba
	P4	93.3 ± 2.1 Aa	96.7 ± 2.1Aa	88.3 ± 4.8 Aa
	P5	95.0 ± 3.4 Aa	96.7 ± 3.3Aa	93.3 ± 3.3 Aa
32 °C	P1	1.7 ± 1.7 Ca	0 Ca	0 Da
	P2	37. 2 ± 8.3 Ba	30.0 ± 11.0 Ba	18.3 ± 9.1 Ca
	P3	90. 0 ± 4.5 Aa	88.3 ± 5.4 Aa	76.7 ± 3.3 Ba
	P4	95. 0 ± 3.4 Aa	98.3 ± 1.7 Aa	86.7 ± 2.1 ABb
	P5	96.7 ± 2.1 Aa	98.3 ± 1.7 Aa	91.7 ± 4.0 Aa

Means within rows (upper case) or between columns (lower case) followed by the same letters are not significantly different ($P \geq 0.05$, Waller-Duncan *k* ratio *t*- test).

P1 = open position; P2, P3, P4, P5 = concealed position: 7.6, 35.5, 63.5, and 91.4 cm, respectively from the open end of the box

Box heights: H1 = 5cm, H2 = 10cm, H3 = 20 cm

Figure captions

Fig. 1. Exposure of T confusum adults and pupae held in Petri dishes at open and concealed positions in and outside of the box (open position = P1, concealed positions = P2, P3, P4, and P5). The exposure boxes used in the test were 1 m long, 20 cm wide with heights 5, 10 and 20 cm

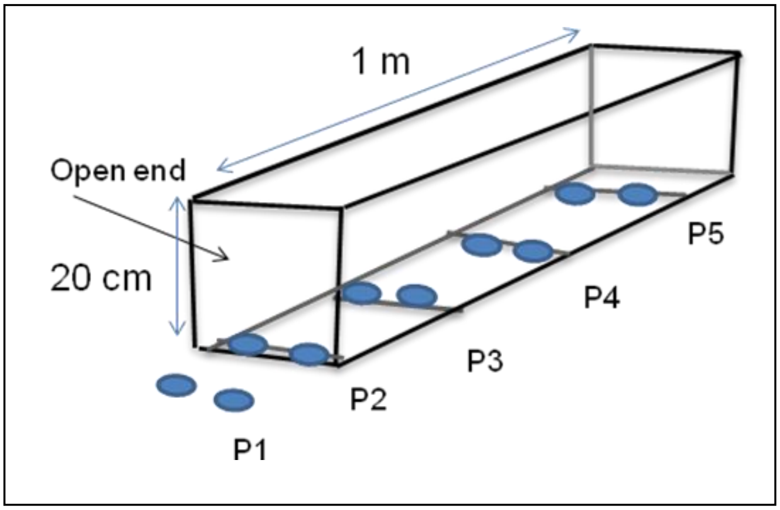

1 m
Open end
20 cm
P1
P2
P3
P4
P5

Figure captions

Fig. 2. Percent mortality of adult T. confusum after 15-d post exposure to aerosol at different exposure position in and outside of different height boxes at different temperature; A: 22 ^{0}C, B: 27 ^{0}C, and C: 32 ^{0}C

P1 = open position; P2, P3, P4, P5 = concealed position: 7.6, 35.5, 63.5, and 91.4 cm, respectively from the open end of the box

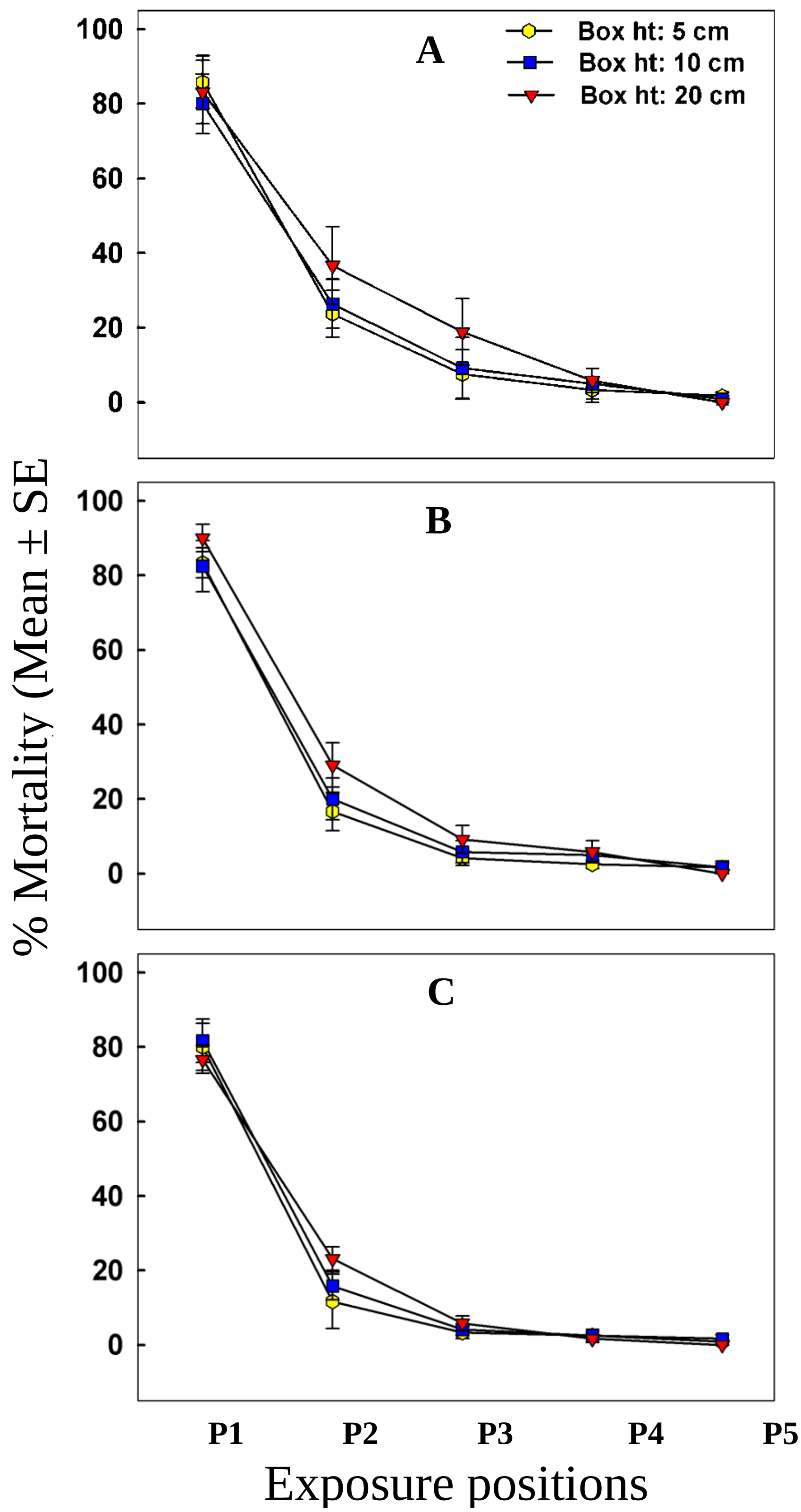
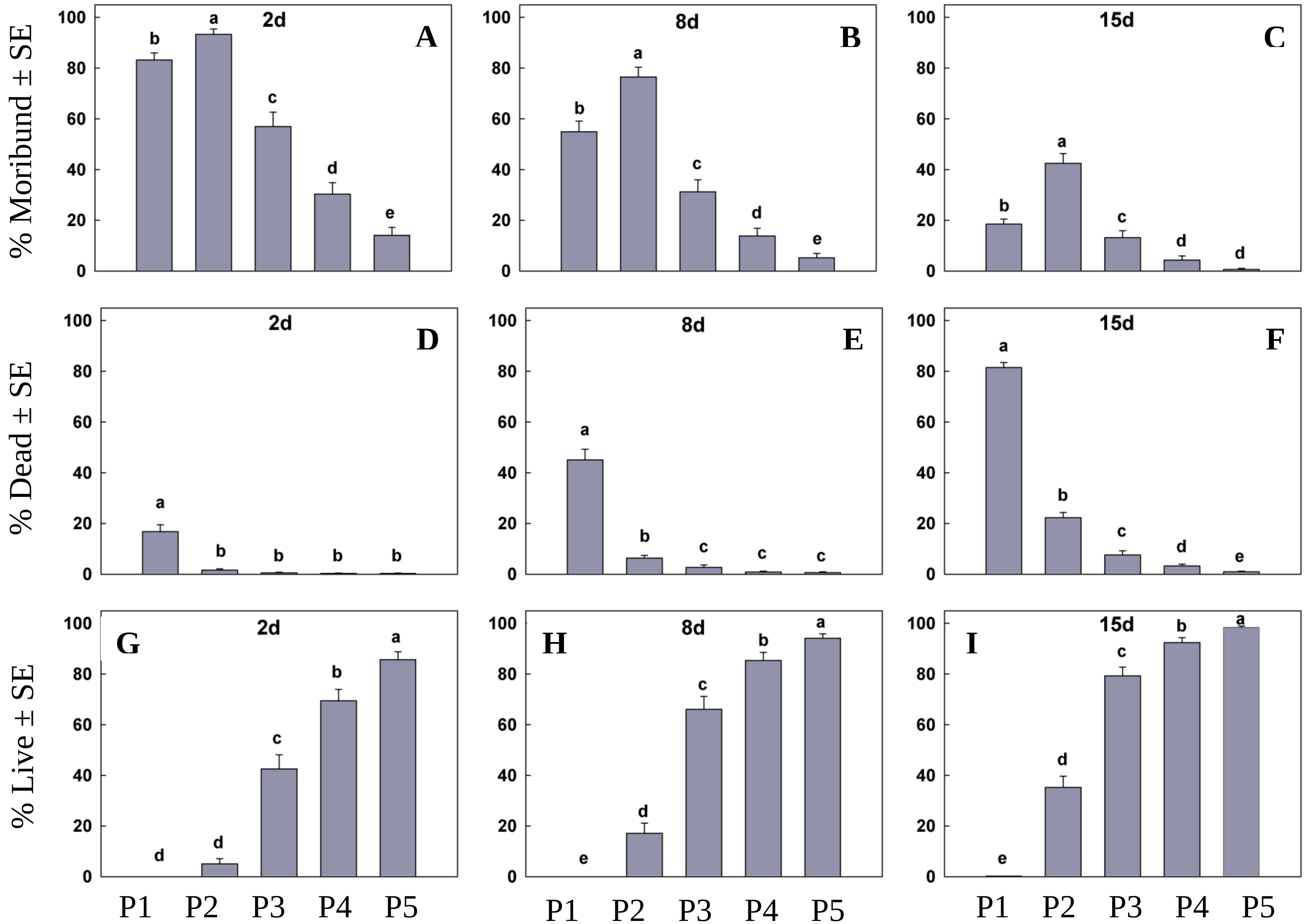

A
Box ht: 5 cm
Box ht: 10 cm
Box ht: 20 cm
B
C
100
80
60
40
20
0
% Mortality (Mean ± SE
P1
P2
P3
P4
P5
Exposure positions

Figure caption

Fig. 3. Percent response of adult T. confusum among different exposure positions, P1, P2, P3, P4, and P5, at 2, 8 and 15-d after aerosol application; A-C. Percent moribund; D-F. Percent dead adults; G-I. Percent live adults

Where, P1 = open position; P2, P3, P4, P5 = concealed position: 7.6, 35.5, 63.5, and 91.4 cm, respectively from the open end of the box

Means among positions within observation day with same letter are not significantly different ($P \geq 0.05$, $n = 6$, Waller-Duncan *k*-ratio *t*-test)


% Moribund ± SE
% Dead ± SE
% Live ± SE
2d
8d
15d
A
B
C
D
E
F
G
H
I
P1
P2
P3
P4
P5

Summary

The complex structural setting of flour mills and the constant availability of whole grains and milled products provide excellent feeding and oviposition sites for stored product insects. *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jacquelin du Val, are two most important pests in the United States. They can cause economic loss by feeding and also through contamination of finished products. Flour mills have historically depended on whole structure treatments, such as fumigants and some use of heat, for managing insect pests. However, whole structure treatment methods pose many technical and economical challenges to a commercial operation, including cost, safety considerations, and shut-down time associated with the treatment. Pyrethrin insecticides formulated with the synergist PBO is a more targeted pest control method that can be a potential alternative to whole structure treatments. This study was conducted to assess the efficacy of synergized pyrethrin aerosol against *T. castaneum* and *T. confusum* under the environmental conditions encountered in a typical flour milling facility.

In the studies presented in Chapter 1, we evaluated the efficacy of synergized pyrethrin aerosol at different direct and indirect exposure conditions, compared the susceptibility between *T. castaneum* and *T. confusum*; and compared the susceptibility of different life stages of the two beetle species. Two day-old eggs, late-stage larvae, pupae, and one-week-old adults of each of the two species were used for the test. Our results indicated that direct exposure to pyrethrin aerosol was effective on all developmental stages of the two species, whereas efficacy was significantly reduced when various developmental stages were either treated together with flour or untreated insects were transferred to treated flour. Additionally, the moribund adults initially observed in indirect exposure treatments were able to recover with time. In addition, this study

indicated that larvae and adults (mobile and feeding stages) were more tolerant to pyrethrin aerosol than pupae and eggs (immobile and non-feeding stages).

In the studies described in Chapter 2, we evaluated effects of different degrees of flour accumulation on the efficacy of synergized pyrethrin aerosol applied on developmental stages of *T. confusum*. The life stages used in this experiment were 4-week-old larvae, pupae, and 1-week-old adults. Insects were exposed inside the experimental sheds in glass Petri dishes (90 mm diameter) containing 0, 0.1, 1, 5 or 10 g of wheat flour to simulate different levels of sanitation or food accumulation in a flour mill. Our results indicated that pyrethrin aerosol gave good control against adult and immature stages of *T. confusum* when exposed without flour or with a low amount of flour, but as the level of flour increased, the aerosol was less effective. Also, recovery of moribund adults increased as the amount of flour increased in the Petri dishes.

In the studies described in Chapter 3, we evaluated the efficacy of pyrethrin aerosol on adults and pupae of *T. confusum* positioned at open and obstructed areas inside experimental sheds maintained at target temperatures of 22, 27 and 32 °C. Our results indicated that exposing adults of *T. confusum* and pupae at open areas resulted in greater efficacy compared to conceal areas at all test temperatures. However, temperature had no significant effect on efficacy of pyrethrin aerosol toward adult mortality. Recovery from knockdown increased as the distance of exposure location increased underneath the boxes.

In conclusion, pyrethrin aerosol can provide good control of *Tribolium spp*, and is not affected by seasonal temperature variations, thus, could be used throughout most of the year. Nevertheless, the aerosol may not provide control in more concealed areas such as inside flour residues, underneath pallets, and open spaces between milling equipment. These results emphasize the importance of hygienic and sanitary measures in milling facilities in conjunction with insecticide applications. Integrating sanitation with aerosols could facilitate more direct exposure to aerosols by reducing available food material. In addition, precise targeting of insecticides based on diligent monitoring of populations rather than calendar-based spray schedules could lead to more effective pest management. Recovery of moribund adults suggests little residual activity of synergized pyrethrin. Therefore, removal of moribund insects through cleaning shortly after aerosol treatment can be an important practice.

Bibliography

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Andersen, M. P.S., D. R. Blake, F. S. Rowland, M. D. Hurley, and T. J. Wallington. 2009. Atmospheric chemistry of sulfuryl fluoride: reaction with OH radicals, Cl atoms and O_3, atmospheric lifetime, IR spectrum, and global warming potential. Environ. Sci. Technol. 43: 1067-1070.

Arthur, F.H. 1998. Residual toxicity of cyfluthrin wettable powder against *Tribolium confusum* (Coleoptera: Tenebrionidae) exposed for short time intervals on concrete. J. Stored Prod. Res. 34: 19-25.

Arthur, F.H. 2000a. Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder. J. Stored Prod. Res. 36: 15–23.

Arthur, F.H. 2000b. Impact of high temperatures on efficacy of cyfluthrin and hydroprene applied to concrete to control *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 39: 193–204.

Arthur, F.H. 2000c. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. J. Econ. Entom. 93: 526-532.

Arthur, F.H. 2001. Susceptibility of last instar red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) to hydroprene. J. Econ. Entom. 94: 772-779.

Arthur, F.H. 2008. Aerosol distribution and efficacy in a commercial food warehouse. Insect Sci. 15: 133-140.

Arthur, F.H. 2010. Residual efficacy of aerosols to control *Tribolium castaneum* and *Tribolium confusum*, pp. 789-792. *In* M.O. Carvalho, C.S. Fields Alder, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Nvarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, N.D.G. White (eds.), Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal. Julius-Kühn-Archiv, 425.

Arthur, F.H. 2012. Aerosols and contact insecticides as alternatives to methyl bromide in flour mills, food production facilities, and food warehouses. J. Pest Sci. 85:323–329.

Arthur, F.H., and J.F. Campbell. 2008. Distribution and efficacy of pyrethrin aerosol to control *Tribolium confusum* (Coleoptera: Tenebrionidae) in food storage facilities. J. Stored Prod. Res. 44: 58-64.

Arthur, F.H., J.F. Campbell, E.A. Fontenot, and M.D. Toews. 2013. Assessing effects of esfenvalerate aerosol applications on resident populations of *Tribolium castaneum* (Herbst), the red flour beetle, through direct and indirect sampling. J. Stored Prod. Res.53: 1-6.

Arthur, F.H., S. Liu, B. Zhao, and T.W, Phillips. 2009. Residual efficacy of pyriproxyfen and hydroprene applied to wood, metal and concrete for control of stored-product insects. Pest Manag. Sci. 65: 791-797.

Barson, G. 1991. Laboratory assessment of the residual toxicity of commercial formulations of insecticides to adult *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) exposed for short time intervals. J. Stored Prod. Res. 27: 205-211.

Beckett, S.J., P.G. Fields, and B. Subramanyam. 2007. Disinfestations of stored products and associated structures using heat, pp. 182-236. *In* Heat treatments for postharvest pest control: theory and practice. CAB International, Oxon, UK.

Beeman, R.W., and S. Haas, and K. Friesen. 2012. Beetle wrangling tips: an introduction to the care and handling of *Tribolium castaneum*. http://www.ars.usda.gov/Research/docs.htm?docid=12892 (Accessed, Feb, 19, 2013).

Bell, C.H. 2000. Fumigation in the 21st century. Crop Prot. 19: 563-569.

Bell, C.H., and N. Savvidou. 1999. The toxicity of Vikane (sulfuryl fluoride) to age groups of eggs of the Mediterranean flour moth (*Ephestia kuehniella*). J. Stored Prod. Res. 35: 233-247.

Benhalima, H., M.Q. Chaudhry, K.A. Mills, and N.R. Price. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stor. Prod. Res. 40: 241-249.

Bernhard, K.M., and G.W. Bennett. 1981. Ultra-low-volume applications of synergized pyrethrins for stored-product insect control. J. Econ. Entomol. 74: 572-576.

Blum, M.S., and C.W. Kearns. 1956. Temperature and the action of pyrethrum in the American cockroach. J. Econ. Entomol. 49:862-865.

Boina, D.R., and B. Subramanyam. 2012. Insect management with aerosols in food processing facilities, pp. 195-212. *In* F. Perveen (ed.), Insecticides: advances in integrated pest management. InTech. http://www.intechopen.com (Accessed, Jan, 2, 2013).

Bond, E.J., H.A.U. Monro, T. Dumas, J. Benazet, and E.E. Turtle. 1972. Control of insects in empty cargo ships with dichlorvos. J. Stored Prod. Res. 8: 11-18.

Bonjour E.L, T.W. Phillips, J.T. Pitts. 2006. Spinosad provides long-term protection for stored wheat, pp 1189–1193. *In* I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J.P. Santos, J.D. Biagi, J.C. Celaro, L.R.D.A Faroni, L.O.F. Bortolini, M.R. Sartori, M.C. Elias, R.N.C. Guedes, R.G. Fonseca, and V.M. Scussel (eds.), Proceedings of the 9th

International Working Conference on Stored Product Protection. ABRAPOS, Campinas, Brazil.

Brijwani, M., B. Subramanyam, M. Hartzer, D. E. Maier, W. Chayaprasert, S. Savoldelli, P. Flinn, and J. Campbell. 2009. Efficacy of structural heat treatments against *Tribolium castaneum* life stages. Presentation at methyl bromide alternatives outlook conference, San Diego, CA., November 2009. Available at: http://mbao.org (Accessed, Nov, 15, 2012).

Brijwani, M., B. Subramanyam, P.W. Flinn. 2012. Impact of varying levels of sanitation on mortality of *Tribolium castaneum* eggs and adults during heat treatment of a pilot flour mill. J. Econ. Entomol. 105: 703-708.

Brooke, J.P. 1958. The stabilization of pyrethrins by piperonyl butoxide in water, with special reference to their use as mosquito larvicides. Ann. Appl. Biol. 46:254.

Bry, R.E., and R. Davis. 1985. *Tribolium confusum* and *Tribolium castaneum*, 291-293. *In* P. Singh and P.W. Moore (eds.), Handbook of insect rearing, Vol 1. Elsevier, NY, USA.

Campbell, J.F., and C. Runnion. 2003. Patch exploitation by female red flour beetles, *Tribolium castaneum*. J. Insect Sci., Available online: insectscience.org/3.20

Campbell, J.F., and D.W. Hagstrum D.W. 2002. Patch exploitation by *Tribolium castaneum*: movement patterns, distribution and oviposition. J. Stored Prod. Res. 38: 55-68.

Campbell, J.F., and R.T Arbogast. 2004. Stored product insects in a flour mill: population dynamics and response to fumigation treatments. Entomol. Exp. Appl. 112: 217-225.

Campbell, J.F., F.H. Arthur, and M.A. Mullen. 2004. Insect management in food processing facilities. Adv Food Nutr Res. 48: 239–295.

Campbell, J.F., M.A. Mullen, and A.K. Dowdy. 2002. Monitoring stored product pests in food processing plants with pheromone trapping, contour mapping, and mark recapture. J. Econ. Entomol. 95: 1089- 1101.

Campbell, J.F., M.D. Toews, F.H. Arthur, and R.T. Arbogast. 2010a. Long-term monitoring of *Tribolium castaneum* in two flour mills: seasonal patterns and impact of fumigation. J. Econ. Entomol. 103: 991-1001.

Campbell, J.F., M.D. Toews, F.H. Arthur, and R.T. Arbogast. 2010b. Long term monitoring of *Tribolium castaneum* populations in two flour mills: rebound after fumigation. J. Econ. Entomol. 103: 1002-1011.

Casida, J. E. 1980. Pyrethrum flowers and pyrethroid insecticides. Environ. Health Perspect. 34:189.

Chattopadhyay, D.P., S.K. Dighe, D.K. Dube, and Purnanand, 1982. Changes in toxicity of DDVP, DFP, and parathion in rats under cold environment. Bull. Environ. Contam. Toxicol. 29: 605-610.

CNMA, 2007. Comparative evaluation of integrated pest management, heat treatments and fumigants as alternatives to methyl bromide for control of stored product pests in canadian flour mills. Canadian National Millers Association, Ottawa, Canada. http://www.canadianmillers.ca/english/pdf/issues/ComparativeEvaluation_ March2007.pdf (Accessed, Aug, 5, 2012).

Cotton, R.T. 1958. *Insect control in flour mills.* U. S. *Department of Agriculture,* Agricultural Marketing Service, Marketing Research Division, Washington, D. C., USA.

Cox, C. 2002. Insecticide synergist fact sheet: piperonyl butoxide. http://www.pesticide.org/get-the-facts/pesticide-factsheets/factsheets/piperonylbutoxide (Accessed, Jan, 23, 2013).

Cramer, M.M. 2006. Food plant sanitation: design, maintenance, and good manufacturing practices, pp 1-29. CRC Press, Boca Raton, FL, USA.

Derrick, M.R., H.D. Burgess, M.T. Baker, and N.E. Binnie. 1990. Sulfuryl fluoride (Vikane): a review of its use as a fumigant. J. Am. Inst. Conserv. 29: 77-90.

Dosland, O., B. Subramanyam, K. Sheppard, and R. Mahroof. 2006. Temperature modification for insect control, pp. 89-103. *In* J.W. Heaps (ed), Insect management for food storage and processing. AACC International, St. Paul, MN, USA.

Dowdy, A.K., and P.G. Fields. 2002. Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill. J. Stored Prod. Res. 38: 11-22.

Emekci, M. 2010. Fumigation, modified atmospheres and hermetic storage, pp. 303-313. *In* M.O. Carvalho, C.S. Fields Alder, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Nvarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, N.D.G. White (eds.), Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal. Julius-Kühn-Archiv, 425.

Fedina, T.Y., and S.M. Lewis. 2008. An integrative view of sexual selection in *Tribolium* flour beetles. Biol. Rev. 83: 151-171.

Fields, P.G., and N.D.G. White. 2002. Alternatives to methyl bromide treatments for stored product and quarantine insects. Annu. Rev. Entomol. 47:331-359.

Gist, G.L., and C.D. Pless 1985. Synergistic activity of piperonyl butoxide with nine synthetic pyrethroids against the fall armyworm, *Spodoptera frugiperda*. Fla. Entomol. 68: 316-320.

Goel A., and P. Aggarwal. 2007. Pesticide poisoning. Natl. Med. J. India. 20:182–191.

Good, N.E. 1937. Insects found in the milling streams of flour mills in the southwestern milling area. J. Kans. Entomol. Soc. 10: 135-148.

Gunasekara, A.S. 2004. Environmental Fate of Pyrethrins. California Environmental Protection Agency (Cal/EPA), Department of Pesticide Regulation. Sacramento, CA, USA.

Guthrie, F.E. 1950. Effect of temperature on toxicity of certain organic insecticides. J Econ Entomol. 43:559-560.

Hagstrum, D.W., and B. Subramanyam. 2000. Monitoring and decision tools, pp. 1-28. *In* B. Subramanyam and D.W. Hagstrum (eds.), Alternatives to pesticides in stored product IPM. Kluwer Academic Publishers, Norwell, MA, USA.

Harein, P.K., H.B. Gillenwater, and E.G. Jay. 1970. Dichlorvos: methods of dispensing, estimates of concentration in air, and toxicity to stored product insects. J. Econ. Entomol. 63:1263-1268.

Hawkin, K. 2008. Monitoring populations of the flour beetles *Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst) in flour mills and in laboratory settings. M.S. thesis. University of Manitoba, Winnipeg, Manitoba, Canada.

Hill, D.S. 2002. Pests of stored foodstuffs and their control, pp 135-316. Kluwer Academic Publishers, Dordrecht, Netherlands.

Hinks, C. F. 1985. The influence of temperature on the efficacy of three pyrethroid insecticides against the grasshopper, Melanoplus sanguinipes (fab.)(Orthoptera: Acrididae), under laboratory conditions. Can. Entomol. 117:1007-1012.

Hulasare, R., B. Subramanyam, P.G. Fields, and A.Y. Abdelghany. 2010. Heat treatment: a viable methyl bromide alternative for managing stored-product insects in food-processing facilities, pp. 661-667. *In* M.O. Carvalho, C.S. Fields Alder, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Nvarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, N.D.G. White (eds.), Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal. Julius-Kühn-Archiv, 425.

Isikber, A.A., S. Navarro, S. Finkelman, M. Rindner, A. Azrieli, and R. Dias. 2004. Toxicity of propylene oxide at low pressure against life stages of four species of stored product insects. J. Econ. Entomol. 97:281-285.

Jenson, E.A., F.H. Arthur, and J.R. Nechols. 2009. Efficacy of methoprene applied at different temperatures and rates on surface substrates to control eggs and fifth instars of *Plodia interpunctella*. J. Econ. Entomol. 102: 1992-2002.

Jenson, E.A., F.H. Arthur, and J.R. Nechols. 2010. Methoprene and synergized pyrethrins as aerosol treatments to control *Plodia interpunctella* (Hubner), the Indian meal moth (Lepidoptera: Pyralidae). J. Stored Prod. Res. 46: 103-110.

Kane, J., F.B. Fishwick, A.A. Green, and P.R. Warre. 1977. Automatic dispensing of dichlorvos to control *Ephestia kuehniella*Zell. (Lepidoptera: Phycitidae) in flour mills. J. Stored Prod. Res. 13:59-64.

Keane, P. 1998. The use of Piperonyl butoxide in formulations for the control of pests of humans, domestic pets and food animals, pp. 289-300. *In* D.G. Jones (ed.), Piperonyl butoxide: the insecticide synergist. Academic Press, London.

Lellinger, D.B. 1972. Dichlorovos and lindane as herbarium insecticides. Taxon 21:91-95.

Lis, L.B., T. Bakuła, M. Baranowski, and A. Czarnewicz. 2011. The carcinogenic effects of benzoquinones produced by the flour beetle. Pol. J. Vet. Sci. 14: 159-164.

Mahroof, R., B. Subramanyam, and D. Eustace. 2003. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. J. Stor. Prod. Res. 39: 555-569.

Mahroof, R., B. Subramanyam, and P. Flinn. 2005. Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minimum heat treatment temperature as pupae and adults. J. Econ. Entom. 98: 626-633.

Mason, L. 2003. Insects and mites, pp. 293-315. *In* Y.H. Hui, B.L. Bruinsma, J.R.Gorham, W.K. Nip, P.S. Tong, and P. Ventresca (eds.), Food plant sanitation. Marcel Dekker, Inc., NY, USA.

McDonald, L. L. 1968. Relative effectiveness of tropital and piperonyl butoxide as synergists for pyrethrins against stored product Insects. J. Econ. Entomol. 61: 1645-1646.

Ming Q.L., and C. Cheng. 2012. Influence of nutrition on male development and reproduction in *Tribolium castaneum.* J. Econ. Entomol. 105: 1471-1476.

Mukerji, D., and R.N. Sinha. 1953. Effect of food on the life history of the flour beetle, *Tribolium castaneum* Herbst. J. Kans. Entomol. Soc. 26: 118-124.

Neethirajan, S., C. Karunakaran, D. S. Jayas, and N. D. G. White. 2007. Detection techniques for stored-product insects in grain. Food Control 18:157-162.

Opit, G.P., T.W. Phillips, M.J. Aikins, M.M. Hasan. 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. J. Econ. Entomol. 105: 1107–1114.

Pappas, P.W., and S.E. Morrison. 1995. Benzoquinones of the beetles, *Tribolium castaneum* and *Tribolium confusum*. Prep Biochem. 3:155-68.

Park, T. 1934. Observations on the general biology of the flour beetle, *Tribolium confusum*. Q. Rev. Biol. 9: 36-54.

Peckman, P.S., and F.H. Arthur. 2005. Insecticide space treatments in food plants, pp. 175-182. *In* J.W. Heaps (ed), Insect management for food storage and processing. AACC International, St. Paul, MN, USA.

Pike, V. 1994. Laboratory assessment of the efficacy of phosphine and methyl bromide fumigation against all life stages of *Liposcelis entomophilus* (Enderlein). Crop Prot. 13: 141–145.

Prabhakaran, S. 2006. Commercial performance and global development status of ProFume gas fumigant, pp. 635-641 *In* I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J.P. Santos, J.D. Biagi, J.C. Celaro, L.R.D.A Faroni, L.O.F. Bortolini, M.R. Sartori, M.C. Elias, R.N.C. Guedes, R.G. Fonseca, and V.M. Scussel (eds.), Proceedings of the 9th International Working Conference on Stored Product Protection. ABRAPOS, Campinas, Brazil.

Rajendran, S. 1992. Selection for resistance to phosphine or methyl bromide in *Tribolium castaneum* (Coleoptera: Tenebrionidae). Bull. Entomol. Res. 82: 119-124.

Rathore, H.S., and S. Ullah. 2009. Simple and affordable methods: spectrophotometric, thin-layer chromatographic and volumetric determination of pesticide residues, pp. 343-380. In L.M.L. Nollet and H.S. Rathore (eds.), Handbook of Pesticides: Methods of Pesticide Residues Analysis, CRC Press, Boca Raton, FL, USA.

Rees, D. 2004. Insects of stored products, p 192. CSIRO Publishing, Collingwood, Victoria, Australia.

Roesli, R., B. Subramanyam, F.J. Fairchild, K.C. Behnke. 2003. Trap catches of stored-product insects before and after heat treatment in a pilot feed mill. J. Stor. Prod. Res. 39: 521–540.

Roesli, R., B. Subramanyam, J.F. Campbell, and K. Kemp. 2003. Impact of selected IPM practices on insect populations in retail pet stores, pp. 410-419. *In* P.F. Credland, D.M. Armitage, C.H. Bell, P.M. Cogan, and E. Highley E(eds.), Proceedings, 8th International Working Conference on Stored Product Protection, 22-26 July 2002. CAB International, York, UK.

Sallam, M.N. 1999. Insect damage: damage on post-harvest. http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_Pests-Insects.pdf (Accessed, Aug, 17, 2013).

SAS Institute, 2002-2008. SAS 9.2 for Windows. SAS Institute, Cary, NC, USA.

Sokoloff, A. 1974. The biology of tribolium*:* with special emphasis on genetic aspects. Oxford University Press.

Sonleitner, F.J., and J. Gutherie. 1991. Factors affecting oviposition rate in the flour beetle *Tribolium castaneum* and the origin of the population regulation mechanism. Res.Popul.Ecol. 33: 1-11.

Srivastava, C., S.R. Sinha, and S.N. Sinha. 2000. Occurrence of insecticide resistance in lesser grain borer, *Rhyzopertha dominica* (Fab.). Ann. Agr. Res. 21: 93-95.

Stanley, M.S.M., and A.W. Grundmann. 1965. Observations on the morphology and sexual behavior of *Tribolium confusum* Duval. J. Kans. Entomol. Soc. 38: 10-18.

Staunton, I., J. Gerozisis, and P. Hadlington. 2008. Insect pests of commerce, industry, accommodation and recreation, pp. 148-207. *In* Urban pest management in Australia. The University of New South Gales Press Ltd., Sydney, Australia.

Subramanyam, B., and D.W. Hagstrum. 1995. Resistance measurement and management, pp 331-397. *In* B. Subramanyam and D. Hagstrum (eds.), Integrated management of insects in stored products. Marcel Dekker Inc, NY, USA.

Subramanyam, B., R. Mahroof, and M. Brijwani. 2011. Heat treatment of grain-processing facilities for insect management: a historical overview and recent advances Stewart Postharvest Review. 7:1-11.

Sutton, A.E., F.H. Arthur, K.Y. Zhu, J.F. Campbell, and L.W. Murray. 2011. Residual efficacy of synergized pyrethrin and methoprene aerosol against larvae of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 47: 399-406.

Tenhet, J.N., C.O. Bare, and D.P. Childs. 1958. Further studies on the control of the cigarette beetle with DDVP. Tob Sci. 147: 106-10.

Toews M.D., J.F. Campbell, and F.H. Arthur. 2010. The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle, *Tribolium castaneum.* J. Insect Sci. 10: 1-14.

Toews, M. D., B. Subramanyam, and J. M. Rowan. 2003. Knockdown and mortality of adults of eight species of stored-product beetles exposed to four surfaces treated with spinosad. J. Econ. Entomol. 96: 1967-1973.

Toews, M.D., F.H. Arthur and J.F. Campbell. 2009. Monitoring *Tribolium castaneum* (Herbst) in pilot-scale warehouses treated with *b*-cyfluthrin: are residual insecticides and trapping compatible? Bull. Entomol. Res. 99: 121–129.

Toews, M.D., F.H. Arthur and J.F. Campbell. 2009. Monitoring *Tribolium castaneum* (Herbst) in pilot-scale warehouses treated with *b*-cyfluthrin: are residual insecticides and trapping compatible? Bull. Entomol. Res. 99: 121–129.

Toews, M.D., J.F. Campbell, and F.H. Arthur. 2010. The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle, *Tribolium castaneum.* J. Insect Sci. 10: 1-14.

Toews, M.D., J.F. Campbell, F.H. Arthur, and M. West. 2005. Monitoring *Tribolium castaneum* (Coleoptera: Tenebrionidae) in pilot-scale warehouses treated with residual applications of (S)-hydroprene and cyfluthrin. J. Econ. Entomol. 98: 1391-1398.

Trematerra, P., and A. Sciarretta. 2004. Spatial distribution of some beetles infesting a feed mill with spatio-temporal dynamics of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum*. J. Stored Prod. Res. 40: 363–377.

Trematerra, P., P. Gentile, A. Brunetti, L.E. Collins, and J. Chambers. 2007. Spatio temporal analysis of trap catches of *Tribolium confusum* du Val in a semolina mill with a comparison of female and male distributions. J. Stored Prod. Res. 43: 315–322.

Tsai, W.T., L.J. Mason, W. Chayaprasert, D.E. Maier, and K.E. Ileleji. 2011. Investigation of fumigant efficacy in flour mills under real-world fumigation conditions. J. Stored Prod. Res. 179-184.

USEPA, 2012. EPA Proposes to withdraw sulfuryl fluoride tolerances. http://www.epa.gov/pesticides/sulfuryl-fluoride/evaluations.html (Accessed, March, 5, 2013).

USEPA, 2013. Critical use exemption information. http://www.epa.gov/ozone/mbr/cueinfo.html (Accessed, Feb, 9, 2013).

Wang, J.J., W.X. Cheng, W. Ding, and Z.M. Zhao. 2004. The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, *Liposcelis bostrychophila* and *L. entomophila*. J. Insect Sci. 4: 1-5.

Watters, F. L. 1968. Pyrethrins-piperonyl butoxide applied as a fog in an empty grain bin. J. Econ. Entomol. 61:1313- 1316.

White, N.D.G., and J.G. Leesch. 1995. Chemical control, pp 287-330. *In* B. Subramanyam and D. Hagstrum (eds.), Integrated management of insects in stored products. Marcel Dekker Inc, NY, USA.

Wijayaratne L.K., P.G. Fields, and F.H. Arthur. 2012. Residual efficacy of methoprene for control of *Tribolium castaneum* (Coleoptera: Tenebrionidae) larvae at different temperatures on varnished wood, concrete, and wheat. J. Econ. Entomol. 105: 718-725.

Williams, G.D., and K.A. Rosentrater. 2007. Design considerations for the construction and operation of flour milling facilities. part i: planning, structural, and life safety considerations. Paper presented at the Annual International Meeting of *American Society of Agricultural and Biological Engineers.* ASABE Paper No. 074116, 17 - 20 June 2007, Minneapolis Convention Center, Minneapolis, MN.

Williams, S.L., Walsh, E.A., 1989. Behavior of DDVP in storage cases. Curator: The Museum Journal. 32: 41–49.

Yezerski, A., T.P Gilmor, and L. Stevens. 2004. Genetic analysis of benzoquinone production in *Tribolium confusum*. J. Chem. Ecol. 30: 1035-1044.

Zettler, J.L., and F.H. Arthur. 2000. Chemical control of stored product insects with fumigants and residual treatments. Crop Prot. 19: 577-582.

Zettler, J.L., J. Larry, J.G. Leesch, R.F. Gill, and B.E. Mackey. 1997. Toxicity of carbonyl sulfide to stored product insects. J. Econ. Entomol. 90: 832-836.

Zhu, K.Y., and J.R. Gao. 1999. Increased activity associated with reduced sensitivity of acetylcholinesterase in organophosphate-resistant greenbug, *schizaphis graminum* (homoptera: aphididae). Pestic. Sci. 55: 11-17.

Appendix A - Chapter 1: Effects of Direct Exposure

Fig. 1. Larvae exposed directly to synergized pyrethrin aerosol and transformed to untreated flour post aerosol treatment. No adult emergence from this exposure. Left: *T. castaneum*; Right *T. confusum*

Fig. 2. Pupae exposed directly to synergized pyrethrin aerosol and transformed to untreated flour post aerosol treatment. No adult emergence from this exposure. Left: *T. castaneum*; Right *T. confusum*

Appendix B - Chapter 2: Flour Amounts used to Simulate Different Sanitation Levels in Flour Mills

a

b

c

d

e

Fig. 3. *T confusum* adults exposed at different flour levels in 90 mm Petri dishes: a. 0 g; b. 0.1 g; c. 1 g; d. 5 g; e. 10 g.

Appendix C - Chapter 2: Effects of Flour Amounts on the Efficacy of Pyrethrin Aerosol

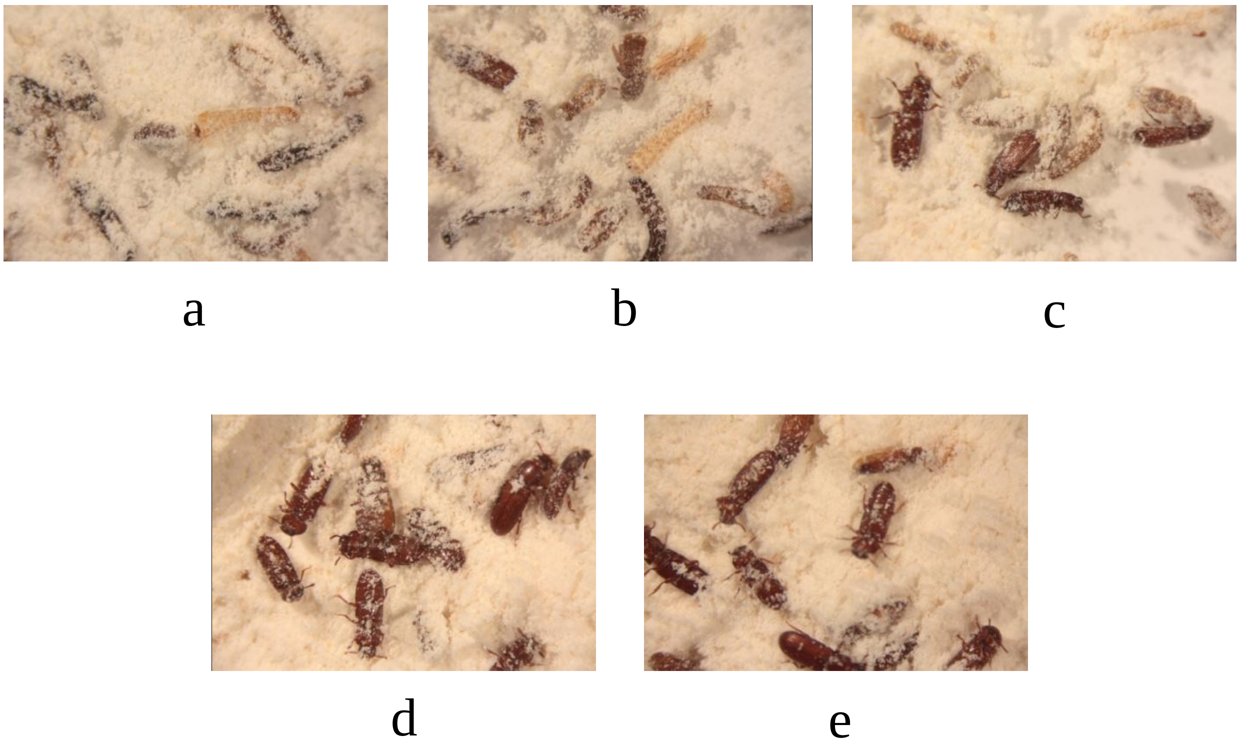


Fig. 4. *T confusum* larvae exposed at different flour levels in 90 mm Petri dishes: a. 0 g; b. 0.1 g; c. 1 g; d. 5 g; e. 10 g. After exposure to aerosol, 1 g of flour was added to each dish to sustain life. Pictures were taken 28-d after aerosol application. The larvae exposed with 5 and 10 g flour were better able to emerge as normal adults than larvae exposed with 1 g flour, while larvae exposed with 0 and 0.1 g flour did not emerge as adults.

Appendix D - Chapter 3: Effects of Barriers on the Efficacy of Aerosol Spray

Fig. 5. Exposure of *T confusum* adults and pupae held in Petri dishes at open and concealed positions in and outside of the box. The exposure boxes used in the test were 1 m long, 20 cm wide with heights 5, 10 and 20 cm. The boxes were randomly located within three marked area inside the sheds