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EVALUATION OF CHLORINE DIOXIDE GAS AGAINST EGGS, LARVAE, AND ADULTS OF *TRIBOILUM CASTANEUM* AND *TRIBOLIUM CONFUSUM*

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ABSTRACT

The susceptibility of eggs, young larvae, old larvae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* (Jacquelin du Val), to chlorine dioxide gas was determined in the laboratory. Insect stages were exposed to gas concentrations of 248.4, 331.2, 413.9, and 496.6 g/m^3 for 1.53, 2.07, 1.80, and 1.68 h, respectively, in a specially constructed enclosure with and without food (5 g of wheat flour). These four concentrations provided gas dosages (ct products) of 380.1, 685.6, 745.0, and 834.4 gh/m^3. Wheat flour was used to simulate unsanitary conditions found in food processing facilities. Young larvae of both species succumbed to chlorine dioxide gas only at the highest dosage exposed. The presence of flour enhanced larval survival. Old larvae of both species showed 0 to 31% mortality in the presence or absence of flour. All adults of *T. confusum* died when exposed to chlorine dioxide gas dose of 745.0 and 834.4 gh/m^3. At these dosages all *T. castaneum* adults died only in the absence of flour. In general, *T. confusum* tended to be more sensitive to chlorine dioxide gas than *T. castaneum*. The presence of flour protected insect life stages from the lethal effects of the gas. These preliminary tests show chlorine dioxide gas to have potential in controlling certain life stages of these two species. Additional tests are needed under laboratory and field conditions to establish baseline dosages that provide effective control of life stages of *Tribolium* spp. and other stored-product insect species commonly associated with food processing facilities.

Keywords: Chlorine dioxide, *Tribolium castaneum*, *Tribolium confusum*, life stages, efficacy, novel fumigant

INTRODUCTION

The phase out of the fumigant methyl bromide (MB) in 2005 in the United States, except for certain critical uses, has created a huge challenge for millers, food-processors and fumigators to find effective and economical fumigant alternatives. Two fumigant alternatives to MB include phosphine as ECO$_2$Fume, and sulfuryl fluoride (SF). Phosphine in the United States is commonly used for fumigating bulk grains, and use by the food industry for whole structure treatment is limited because phosphine is corrosive to metals (Bond et al., 1984). SF, registered as ProFume™, is less effective at temperatures below 26.7°C (80°F), especially on eggs of stored-product insects and effective kill of eggs requires higher doses or longer exposure times (Bell and Savvidou, 1999; Hartzer et al., 2010). Concerns have been raised about the global warming potential of SF (Anderson et al., 2009). More recently, there is a debate about revoking tolerances of SF because of its contribution to fluorine residues in food and the adverse effects associated with it (http://www.fluoridealert.org/sf/index.html; http://www.fluoridealert.org/ sf/nov-2006.pdf). The US-EPA is currently reviewing data and comments from end users to decide on its future fate. A non-fumigant MB alternative is the use of elevated temperatures (Brijwani et al., 2012). Heat treatment is not suitable for all facilities, and it is more expensive than MB and may cause adverse effects to structural components of a facility if temperatures exceed 60°C. Hence, there is an urgent need to look for an alternative fumigant source to control stored product insects in food processing facilities.

Chlorine dioxide (ClO_2) is a powerful oxidizing agent being used increasingly to control microbiological growth in a number of different industries (Han et al., 2001, 2003; Du et al., 2002, 2003; Singh et al., 2002; Huang et al., 2006; Isomoto et al., 2006; Mahamoud et al., 2007). This gas has not been evaluated against stored product insects. Like MB a chemical that is effective against microorganisms as well as insects would be an ideal replacement for MB and SF. The present laboratory study was designed to evaluate the efficacy of ClO_2 gas against eggs, young larvae, old larvae, and adults of the red flour beetle *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* (Jacquelin du Val).

MATERIALS AND METHODS

The ClO_2 gas, which is a mixture of sodium hypochlorite and hydrochloric acid, was provided by Sterling Bridges, Palatine, Illinois, USA. Insect life stages of *Tribolium* spp. were exposed in a specially designed enclosure (113.3 m^3) made of polycarbonate plastic (Secador® Techni-Dome® 360 vacuum cabinet). The ClO_2 gas was generated by adding water to the mixture (2 ml/g of chemical), and gas concentrations were recorded with a ClO_2 sensor (Optek AF26, Optek-Danulat GmbH, Emscherbruchallee-Essen, Germany).

Eggs (1 to 2 d old), young larvae (first instars), old larvae (sixth to seventh instars), and adults of *T. castaneum* and *T. confusum*, reared on bleached wheat flour plus yeast (5% by wt) diet, at 28°C and 65% r.h. in a growth chamber (Model I-36 VL; Percival Scientific, Perry, Iowa, USA), were obtained following procedures described by Mahroof et al. (2003) for *T. castaneum* and Boina and Subramanyam (2004) for *T. confusum*. Unsexed adults of mixed ages of each species were directly collected from cultures whereas other stages were reared to a specific age in 150 ml plastic containers holding 20 g of the insect diet.

Fifty insects of each stage were exposed to ClO_2 gas dosages of 380.1, 685.6, 745.0, and 834.4 gh/m^3. Insects were exposed with and without 5 g of flour to examine the impact of food on efficacy against insects. All the fumigation experiments were carried out under the room temperatures (25 to 30°C). Life stages of *Tribolium* spp. handled similarly at 28°C and 65% r.h. but not exposed to ClO_2 served as the control treatment. Each dosage, species, and life stage combination, including the control treatment, was replicated three times. Adult mortality was assessed 24 h after exposure based on number of dead adults of the total exposed and the mortality was expressed as a percentage. The mortality of immature stages was based on rearing them to adulthood. Mortality of insects exposed to ClO_2 was corrected for mortality of insects observed in the control treatment. The mean ± SE of corrected insect mortality responses of each species and life stage exposed to the four ClO_2 dosages were summarized in a table.

RESULTS AND DISCUSSION

Table 1 shows the mortality of *T. castaneum* and *T. confusum* life stages exposed to ClO_2 in the presence and absence of food. Mortality of *T. castaneum* and *T. confusum* life stages increased with an increase in ClO_2 dosage especially in the absence of food, and commercial kill was observed with young larvae and adults at 745.0 and 834.4 gh/m^3. Presence of food significantly decreased activity perhaps due to binding of the ClO_2 gas by food. Also, adults were more susceptible than the other stages.

Table 1. Responses of life stages of *T. casteneum* and *T. confusum* exposed to four dosages of chlorine dioxide gas.

Species	Stage	Dosage (gh/m^3)	Mean ± SE (n = 3) mortality (%)	
			Without flour	With 5 g flour
T. castaneum	Eggs	380.1	1.9 ± 0.9	2.7 ± 0.9
		685.6	2.8 ± 0.9	3.6 ± 0.9
		745.0	4.7 ± 1.6	3.7 ± 0.0
		834.4	9.3 ± 0.9	5.5 ± 0.9
	Young larvae	380.1	40.1 ± 2.7	6.8 ± 1.8
		685.6	60.1 ± 0.7	9.6 ± 2.4
		745.0	72.5 ± 1.3	13.7 ± 1.2
		834.4	100.0 ± 0.0	18.9 ± 1.2
	Old larvae	380.1	0.4 ± 0.4	0.7 ± 0.7
		685.6	6.0 ± 1.8	0.4 ± 0.4
		745.0	9.3 ± 1.8	0.0 ± 0.0
		834.4	18.8 ± 0.7	4.0 ± 1.2
	Adults	380.1	2.7 ± 0.7	0.0 ± 0.0
		685.6	18.7 ± 2.9	0.0 ± 0.0
		745.0	100.0 ± 0.0	77.3 ± 2.7
		834.4	100.0 ± 0.0	100.0 ± 0.0
T. confusum	Eggs	380.1	4.6 ± 0.9	1.9 ± 0.9
		685.6	7.4 ± 0.9	1.9 ± 0.9
		745.0	9.3 ± 2.4	1.9 ± 0.9
		834.4	11.1 ± 1.6	5.6 ± 1.6
	Young larvae	380.1	43.5 ± 2.7	16.3 ± 2.4
		685.6	87.1 ± 1.4	29.5 ± 1.4
		745.0	90.0 ± 0.0	38.8 ± 1.2
		834.4	100.0 ± 0.0	37.2 ± 2.3
	Old larvae	380.1	0.7 ± 0.7	0.0 ± 0.0
		685.6	12.7 ± 1.3	0.9 ± 0.4
		745.0	26.8 ± 0.7	6.7 ± 1.3
		834.4	31.3 ± 0.7	14.7 ± 1.3
	Adults	380.1	4.0 ± 1.2	0.0 ± 0.0
		685.6	29.3 ± 0.7	2.0 ± 1.2
		745.0	100.0 ± 0.0	100.0 ± 0.0
		834.4	100.0 ± 0.0	100.0 ± 0.0

Our results clearly demonstrate that the ClO_2 gas at 834.4 gh/m^3 can be used to kill 100% of young larvae and adults of *Tribolium* spp. The eggs and old larvae were most difficult to kill at the tested dosages. Exposing life stages to the same dosages over 24 h rather than 1.53 to 2.07 h may make the gas more effective. Eggs of stored product insects are the most difficult to kill using fumigants such as SF (Bell and Savvidou, 1999; Hartzer et al., 2010), and with heat (Yu et al., 2011). Additional laboratory tests with longer exposures and pilot scale field tests in a food processing facility are needed to determine viability of ClO_2 fumigant as an insect management tool.

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