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Responses of phosphine susceptible and resistant strains of five stored-product insect species to chlorine dioxide

Xinyi E, Bhadriraju Subramanyam*, Beibei Li

Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA

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ABSTRACT

Adults of phosphine susceptible laboratory strains and phosphine resistant field strains of five stored-product insect species were exposed in vials with 0 or 10 g of wheat for different time periods to 0.54 g/m^3 (200 ppm) of chlorine dioxide gas. After exposure, adult mortality was determined 5 d later at 28 °C and 65% r.h. The 5-d mortality was 100% in laboratory and field strains of the red flour beetle, *Tribolium castaneum* (Herbst); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); lesser grain borer, *Rhyzopertha dominica* (F.); maize weevil, *Sitophilus zeamais* Motschulsky; and rice weevil, *Sitophilus oryzae* (L.) that were exposed in vials with 10 g of wheat to chlorine dioxide for 26, 16, 24–34, 18–24, and 15–18 h, respectively. Corresponding exposure durations for these species and strains in vials without wheat were 15, 3, 18–20, 7–15, and 5–7 h, respectively. Dosages of chlorine dioxide producing 99% mortality (LD_{99}) of *T. castaneum*, *O. surinamensis*, *R. dominica*, *S. zeamais*, and *S. oryzae* strains in vials with wheat ranged from 14.79–22.57, 8.20–8.41, 15.79–21.60, 10.66–14.53, and 7.67–12.20 g-h/m^3, respectively. In vials without wheat, corresponding LD_{99} values for *T. castaneum*, *R. dominica*, and *S. zeamais* strains were 6.51–8.66, 11.46–23.17, and 5.79–10.26 g-h/m^3, respectively. LD_{99} values for *O. surinamensis* and *S. oryzae* could not be computed, because of 100% mortality after a 3–5 h exposure to chlorine dioxide. No adult progeny production of *T. castaneum* and *O. surinamensis* was observed after 8 weeks in control and chlorine dioxide-exposed samples. Adult progeny production of *Sitophilus* spp. was found only in the control samples. The dosage for 99% adult progeny reduction relative to control for *R. dominica* strains ranged from 10.07 to 18.11 g-h/m^3. Chlorine dioxide gas is effective in killing phosphine susceptible and resistant strains of five stored-product insect species and suppressing adult progeny production of three out of the five species.

1. Introduction

Chlorine dioxide gas was discovered in early 1800s, and was initially used as a bleaching agent in the paper industry for pulp bleaching (Simpson, 2005). In 1950s, chlorine dioxide was used for treating drinking water to remove microorganisms and off-odors. Chlorine dioxide gas easily dissolves in water and stays as dissolved gas instead of being hydrolyzed. Once it separates from the solution, the gas tends to decompose into chlorine and oxygen when exposed to sunlight, high temperatures, electric sparks, or high pressure (Simpson, 2005).

A few researchers investigated the possibility of using chlorine dioxide as fumigant to control stored-product insect species. The efficacy of chlorine dioxide against four life stages of the red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* Jacquelin du Val, was reported by Channaiah et al. (2012). Channaiah et al. (2012) exposed eggs, young larvae, old larvae, and adults of *T. castaneum* and *T. confusum* without food in vials to chlorine dioxide concentrations of 380.1, 685.6, 745.0, and 834.4 g-h/m^3. The exposure times varied only from 1.53 to 2.07 h. Mortality was greatest at the highest dosage. The mortality of eggs, young larvae, old larvae, and adults of *T. castaneum* was 9.3, 100, 18.8, and 100%, respectively, after exposure to 834.4 g-h/m^3 chlorine dioxide. Similarly for *T. confusum*, the mortality of the four life stages was 11.1, 100, 31.3, and 100%, respectively, when exposed to a chlorine dioxide concentration of 834.4 g-h/m^3. In the presence of 5 g of flour in vials, only the mortality of adults of both species was 100% at the highest dosage, whereas mortality of eggs, young larvae, and old larvae ranged from 4 to 37%. Channaiah et al. (2012) hypothesized that longer than 2 h exposures may be needed for

* Corresponding author.
E-mail address: sbhadrir@k-state.edu (B. Subramanyam).

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effective control of all life stages. Kumar et al. (2015) exposed late instars of the Indian meal moth, *Plodia interpunctella* (Hübner), to a chlorine dioxide concentration of 0.54 g/m^3 for various time periods, and complete mortality was observed after 24 h. Kim et al. (2015) reported 100% mortality of both larvae (6-7th instars) and adults of *T. castaneum* after exposure to 0.54 g/m^3 concentration of chlorine dioxide for 24 h. They also investigated the mode of action of chlorine dioxide against insects by tracking changes in the quantity of reactive oxygen species and levels of two antioxidant enzymes (superoxide dismutase and thioredoxin-peroxidase) in the larvae of *T. castaneum* before and after chlorine dioxide exposure. After exposure to chlorine dioxide the production of the two antioxidant enzymes failed to keep up with the production of reactive oxygen species, and the authors inferred that this oxidative stress may have likely led to cellular damage and mortality of larvae (Kim et al., 2015).

Chlorine dioxide gas can be produced chemically or electrochemically. Most studies using chlorine dioxide for food applications followed the chemical method, which included using sachets containing sodium chlorite and an acid or an acid precursor (ferric chloride), or occurred in cartridges packed with sodium chlorite fed with chlorine gas (Sy et al., 2005; Trinetta et al., 2013). Another patented method to generate chlorine-free chlorine dioxide is to run sodium chlorite solution through a set of electrolytic cells where chlorite ion is electrochemically oxidized to chlorine dioxide gas (Cawlfield and Kaczur, 1990). In this study, the latter means of chlorine dioxide production was used.

In the present investigation, responses of phosphine susceptible and resistant strains of five common stored-product insects were evaluated by exposing them to a chlorine dioxide concentration of 0.54 g/m^3 for different durations. The efficacy of chlorine dioxide was evaluated in the presence and absence of wheat. The effect of chlorine dioxide on adult progeny production was also studied.

2. Materials and methods

2.1. Insects

Cultures of *T. castaneum* were reared on organic wheat flour (Heartland Mills, Marienthal, Kansas, USA) fortified with 5% (by wt) brewer's yeast (Lesaffre Yeast Corporation, Milwaukee, Wisconsin, USA). The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), was reared on organic rolled oats (Heartland Mills) plus 5% brewer's yeast diet. Cultures of the lesser grain borer, *Rhyzopertha dominica* (F.), and rice weevil, *Sitophilus oryzae* (L.), were reared on organic hard red winter wheat (Heartland Mills). The maize weevil, *Sitophilus zeamais* Motschulsky, was reared on organic corn (Heartland Mills). Laboratory strains of all species have been in rearing since 1999. Field strains of *T. castaneum*, *R. dominica*, and *O. surinamensis* were collected during 2011 from farm-stored grain in Kansas, USA, whereas field strains of the *S. zeamais* and *S. oryzae* were collected from farm-stored grain in Texas, USA (Table 1).

Table 1
Sites and years of collection of field strains of five stored-product insect species.

Species	County, state	Commodity	Strain	Collection year
T. castaneum	Dickinson, Kansas	Wheat	AB1	2011
	Minneapolis, Kansas	Wheat	MN	2011
O. surinamensis	Abilene, Kansas	Wheat	AB2	2011
R. dominica	Chase, Kansas	Wheat	CS	2011
	Riley, Kansas	Flour	RL	2007
S. zeamais	Texas[a]	Corn	TX	2011
S. oryzae	Texas[a]	Corn	TX	2011

[a] County unknown.

Unsexed adults used in all bioassays were 1- to 4-week old.

Phosphine susceptibility or resistance in laboratory and field strains of five insect species was verified following discriminating dose tests (Champ and Dyte, 1976). In the discriminating dose tests, phosphine concentrations used for *T. castaneum, O. surinamensis*, *R. dominica,* and *Sitophilus* spp., were, 0.042, 0.052, 0.028, and 0.042 g/m^3 (30.0, 37.5, 20.0, and 30.0 ppm), respectively. Fifty unsexed adults of each strain were exposed to phosphine in triplicate. Adults of each strain were exposed to phosphine for 20 h, and mortality was assessed after 14 d to score insects as susceptible, weakly resistant, or strongly resistant to phosphine. All laboratory strains of the five species were susceptible to phosphine whereas the field strains of the species were resistant to phosphine (Table 2).

Cultures of all insect species were reared in 0.95-L glass jars with approximately 250 g of diet at 28 °C and 65% r.h. in environmental growth chambers (model I-36 VL; Percival Scientific, Perry, Iowa, USA). All jars had metal lids fitted with wire mesh screens and filter papers. Adults for use in bioassays were collected directly from culture jars after sifting the culture through an 841-μm opening round-holed sieve (Fisher Scientific Company, Hampton, New Hampshire, USA).

2.2. Bioassays

Bioassays were carried out in snap cap vials (23 mm in diameter and 55 mm in height) that had mesh bottoms (250 μm openings) and perforated plastic caps covered with 250 μm opening sieve to ensure diffusion of chlorine dioxide through the vials, and also to prevent insects from escaping. Chlorine dioxide treatments were conducted in an air-tight polymethyl methacrylate (PMMA) chamber (0.6 m × 0.6 m × 1.0 m). Chlorine dioxide gas was produced by a customized chlorine dioxide generator donated by PureLine Treatment Systems, LLC (Bensenville, Illinois, USA), housed inside a trailer. The trailer was located on the north campus next to the O.H. Kruse Feed Technology Innovation Center, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA. Chlorine dioxide gas was produced from sodium chlorite (31% solution) via two electrochemical reactions:

Anode (oxidation): $ClO_2^- \rightarrow ClO_2 + e^-$

Cathode (reduction): $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$

Chlorine dioxide gas was then admixed with ambient air prior to entering the PMMA chamber where bioassays were held. Chlorine dioxide concentrations were adjusted by mixing different amounts

Table 2
Survival of laboratory and field strains of five stored-product insect species exposed to discriminating doses of phosphine.

Species	Strain	% Survival (mean ± SE)	Resistance classification
T. castaneum[a]	Lab	0	Susceptible
	AB1	43.0	Weak
	MN	98.0	Strong
O. surinamensis	Lab	0	Susceptible
	AB2	1.3 ± 1.3	Weak
R. dominica	Lab	0	Susceptible
	CS	64.4 ± 2.9	Weak
	RL	27.8 ± 1.8	Weak
S. zeamais	Lab	0	Susceptible
	TX	6.7 ± 1.8	Weak
S. oryzae	Lab	0	Susceptible
	TX	9.3 ± 2.4	Weak

[a] Each mean is based on $n = 3$. The test was conducted by a scientist in the Department of Entomology, Kansas State University, and the original values were not supplied to compute a SE.